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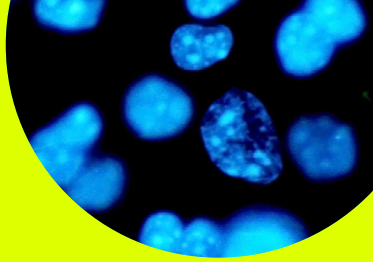

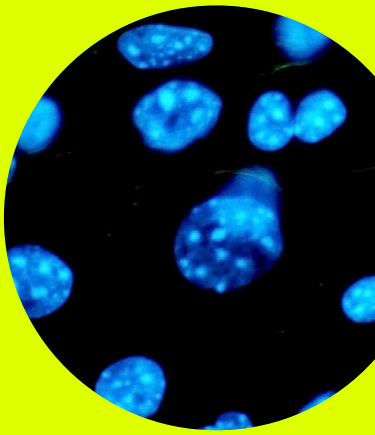

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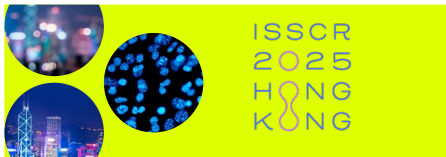

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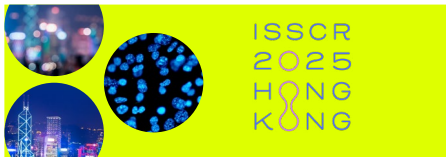

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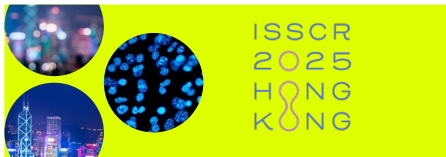

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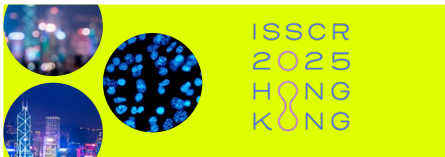

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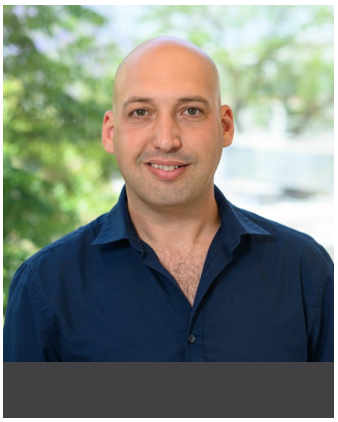

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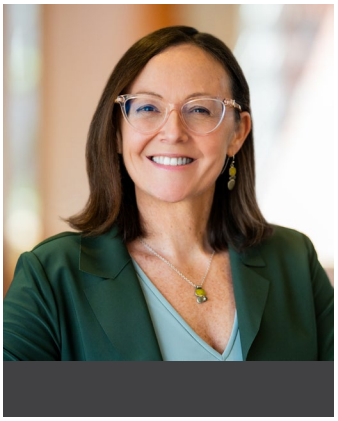

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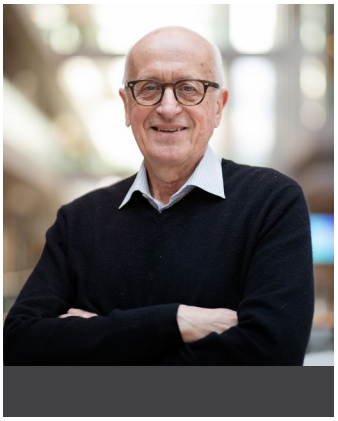

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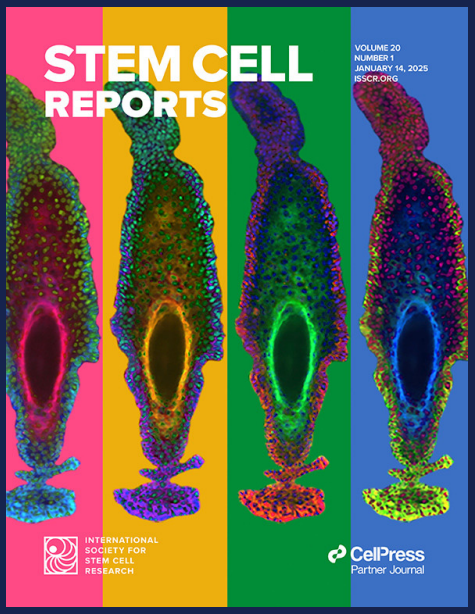

Your support of *Stem Cell Reports* strengthens the ISSCR's global mission and outreach to promote, educate and advocate for stem cell research and its applications to human health.

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THE STEM CELL REPORT PODCAST

Join editor-in-chief of *Stem Cell Reports,* Janet Rossant, on The Stem Cell Report Podcast as authors go "beyond the paper" and share new insights on their breakthroughs and explore questions about the future of the field.

PODCAST GUESTS INCLUDE:


Hans Clevers, MD, PhD


Douglas Melton, PhD


Masayo Takahashi, MD, PhD


Hideyuki Okano, MD, PhD


Fiona Watt, DPhil


Magdalena Zernicka-Goetz, PhD

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PROGRAM AT A GLANCE

**all times are listed in Hong Kong Time (HKT)*

WEDNESDAY, 11 JUNE

8:30 AM – 10:00 AM	**Science Spotlight Sessions** **Clinical Trial Updates Session**
10:00 AM – 10:30 AM	**Refreshment Break**
10:30 AM – 12:15 PM	**Plenary I: Presidential Symposium**
12:00 PM – 6:00 PM	**Exhibit & Poster Hall Open**
12:30 PM – 1:30 PM	**Innovation Showcases**
2:00 PM – 3:30 PM	**Focus Sessions** **Scientific Publishing Strategies Session**
4:00 PM – 6:00 PM	**Welcome Reception & Poster Session I** *Supported by: LUXA Biotechnology, Danaher Corporation, Cell Regeneration, ThinkCyte, PentaHibe by Pharmacosmos* **Exhibit Hall Theater Talks** **Meet-Up Hubs** **One-on-One Partnering Appointments**
6:00 PM – 6:30 PM	**Innovation Showcases**

THURSDAY, 12 JUNE

8:30 AM – 10:00 AM	**Concurrent Track Sessions**
10:00 AM – 10:30 AM	**Refreshment Break**
10:30 AM – 12:00 PM	**Plenary II** *Sponsored by: Stem Cell Reports*
12:00 PM – 6:00 PM	**Exhibit & Poster Hall Open**
12:30 PM – 1:30 PM	**Innovation Showcases**
2:00 PM – 3:30 PM	**Concurrent Track Sessions**
3:00 PM – 6:00 PM	**Exhibit Hall Pub Crawl**
3:30 PM – 5:30 PM	**Refreshment Break**
4:00 PM – 6:00 PM	**Poster Session II** **Exhibit Hall Theater Talks** **Meet-Up Hubs** **One-on-One Partnering Appointments**
6:00 PM – 6:30 PM	**Innovation Showcases**

FRIDAY, 13 JUNE

8:30 AM – 10:00 AM	**Concurrent Track Sessions** **Career Panel**
10:00 AM – 10:30 AM	**Refreshment Break**
10:30 AM – 12:00 PM	**Plenary III** *Sponsored by: Roche IHB*
12:00 PM – 6:00 PM	**Exhibit & Poster Hall Open**
12:30 PM – 1:30 PM	**Innovation Showcases**
2:00 PM – 3:30 PM	**Concurrent Track Sessions**
3:30 PM – 5:30 PM	**Refreshment Break**
4:00 PM – 6:00 PM	**Poster Session III** **Exhibit Hall Theater Talks** **Meet-Up Hubs** **One-on-One Partnering Appointments** **Career Exploration Presentations**

SATURDAY, 14 JUNE

9:30 AM – 11:00 AM	**Plenary IV** *Sponsored by: BlueRock Therapeutics*
11:00 AM – 11:30 AM	**Refreshment Break**
11:30 AM – 12:30 PM	**Plenary V** *Sponsored by: The Burroughs Wellcome Fund*
1:30 PM – 3:00 PM	**Plenary VI: Awards & Keynote Session**

Public Symposium

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The Power Of Stem Cells For Regenerative Medicine & Biotechnology

10 JUNE 2025

10:00 am - 5:00 pm

Charles K. Kao Auditorium, Hong Kong Science and Technology Park, Shatin, HK

INVITED SPEAKERS

Jose Silva Guangzhou Laboratory

Anna Blocki The Chinese University of Hong Kong

Lijian Hui Chinese Academy of Sciences

Stephanie Ma The University of Hong Kong

Arial Zeng Chinese Academy of Sciences

Yufang Shi Soochow University

Hongkui Deng Peking University

Martin Cheung The University of Hong Kong

Pentao Liu The University of Hong Kong

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CN 31-2197/Q2
EISSN 2045-9769 ISSN 2097-3411

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A fully Open Access journal officially affiliated with the Chinese Society for Cell Biology (CSCB), aims to provide a worldwide platform for **research on stem cells and regenerative biology to develop basic science and to foster its clinical translation.**

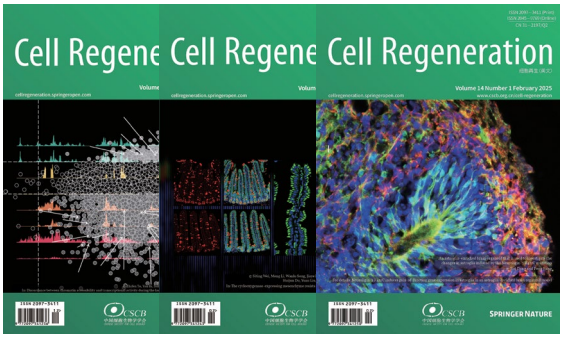

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EXHIBIT HALL PUB CRAWL

THURSDAY, 12 JUNE | 3:00 PM – 6:00 PM

The ISSCR will host an Exhibit Hall Pub Crawl from 3:00 PM – 6:00 PM on Thursday, 12 June in the Exhibit & Poster Hall. Enjoy a complimentary beverage and/or snack from participating exhibitors as you connect with exhibitors, colleagues, and friends!

Participating Exhibitors:

- Axion BioSystems at Booth #1503
- BioLamina at Booth #1003
- Bio-Techne China at Booth #2107
- Rohto Advanced Research Hong Kong at Booth #912

EXHIBIT HALL THEATER

The ISSCR 2025 Exhibit Hall Theater is located toward the center of Exhibit & Poster Hall, 3BCDE, Level 3 in the Hong Kong Convention and Exhibition Center (HKCEC).

WEDNESDAY, 11 JUNE

4:00 PM – 4:15 PM

FUNCTIONAL CHARACTERIZATION OF COMPLEX IPSC-DERIVED NEURAL MODELS: NEW TOOLS FOR ORGANOIDS, SPHEROIDS, AND ORGAN-ON-CHIP PLATFORMS

Presented by: Axion BioSystems, Inc.

Complex human iPSC-derived cell models (e.g., spheroids, organoids, and organ-on-chip devices) are drastically changing neurological disorder research and therapeutic development. Compared to traditional methods, these advanced three-dimensional (3D) models are more physiologically relevant and provide a closer representation of the human brain. To characterize the functional activity of these models, scientists are increasingly relying on live-cell analysis platforms such as high-throughput Maestro MEA and AI-driven Omni imaging. These powerful tools provide deep insights into human biology, across various stages and applications—from generating cell lines and 3D models to modeling diseases and screening potential therapeutics. This talk will provide an overview of how these technologies work together through Axion's Connected Lab, as well as new solutions tailored to label-free functional assessment of advanced iPSC-derived models.

PRESENTER:
Austin Passaro, *Axion BioSystems, Inc, USA*

4:20 PM – 4:35 PM

FATEVIEW™: AI-POWERED, NON-DESTRUCTIVE 'LIVE' CELL ANALYTICS AND CELL FATE FORECASTING

Presented by: CellVoyant Technologies Ltd

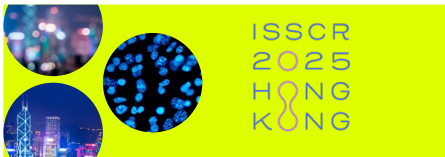
Visualizing and characterizing cell proliferation and differentiation dynamics is paramount to unravel developmental biology mechanisms and design efficient strategies for cell therapy development. Methods like immunohistochemistry or single-cell 'omics provide only endpoint cell state data without real-time insights. Conversely, genetically-encoded fluorescent reporters yield real-time data but are complex and lengthy to implement and restricted in applicability. FateView™ is a novel online App designed specifically for biologists. It combines live-cell imaging with advanced computer vision and artificial intelligence to track, predict and quantitate cell characteristics and differentiation dynamics non-invasively at single-cell resolution. Researchers can upload microscopy images into FateView™'s intuitive interface and access interactive, predictive, quantitative readouts quickly, effortlessly and without interrupting experiments or sacrificing samples, saving time, cost, and speed of decisions in cell selection, differentiation, drug screening and more.

FateView™ empowers biologists to:

- Obtain complex cell characterization currently achievable only by multiple molecular assays
- Measure critical quality attributes and differentiation variability across cell lines
- Predict future cell characteristics and obtain insights to improve target cell derivation

We will showcase these features using hPSCs, cardiac progenitors and RPE cells as exemplars. Accelerate your discoveries and cell therapy development. Experience the future of cell fate analytics — try FateView™ today!

PRESENTERS:
Rafael Carazo Salas, *CellVoyant Technologies Ltd, UK*
Adel Benamara, *CellVoyant Technologies Ltd, UK*



4:40 PM – 4:55 PM

HIGH-THROUGHPUT APPROACH TO IMAGING AND SORTING 3D CELL CULTURES, SPHEROIDS, AND ORGANOIDS

Presented by: Union Biometrica, Inc

Large particle flow cytometers from Union Biometrica provide automation for the analysis and dispensing of intact cell clusters. Cells growing in clusters communicate with each other and behave differently than cells grown as monolayers or in suspension. Many cell types will naturally form cell clusters when given the opportunity. These organoids and spheroids are believed to more closely recapitulate a normal physiological state. Research using stem cell clusters, organoids, tumor spheres and other types of 3D cultures are important biological systems for the discovery of signals responsible for normal development as well as the abnormal disease states. The COPAS Vision instrument can characterize these sample types with measurements typical of flow cytometry, like size, optical density, and fluorescence. In addition, the COPAS Vision collects brightfield images of the cell clusters during analysis. The COPAS Vision instrument provides automation for unbiased analysis, handling of large numbers of cell clusters, and dispensing of these sample types in a multiwell plate format. This approach can be used to characterize populations of organoids and organoid bodies of various types. Dispensing to wells of multiwell plates provides an approach to using these 3D cultures in large scale biological assays and screens.

PRESENTER:
Rock Pulak, *Union Biometrica, Inc., USA*

4:00 PM – 4:15 PM

HIGHLY EFFICIENT AND AUTOMATED SINGLE-CELL CLONING OF HIPSCS UTILIZING THE POWER OF AUTOMATION & AI

Presented by: iotaSciences

Gene-editing in combination with induced pluripotent stem cells (iPSCs) has provided a powerful opportunity to accelerate human disease modelling and drug discovery. However, among gene-editing workflows, single-cell cloning remains the most challenging bottleneck according to ~50% of researchers in a recent survey. While existing high-throughput cell sorting and isolation techniques offer the benefit of automated single-cell seeding, they are often too harsh on cells and do not offer comprehensive automation, such as cell feeding and harvesting of clones. iotaSciences' Cloning Platform XT offers a complete single-cell cloning solution. The system utilizes platform-specific miniature culture chambers, requiring <1 microlitre of culture medium to grow single cells into verified clonal cultures. Ultra-gentle cell and fluid handling maximizes cell viability and achieves market-leading cloning efficiencies, while AI-powered monoclonality assessment and image capture capabilities provide a fully documented workflow.

PRESENTER:
Jonathan Whitchurch, *iotaSciences, UK*

4:20 PM – 4:35 PM

A SERUM-FREE, CHEMICALLY DEFIED WORKFLOW SOLUTION FOR THE DEVELOPMENT & MANUFACTURING OF CELL-BASED THERAPEUTIC PRODUCTS

Presented by: BioLegend

Cell therapy has emerged as a promising approach of leveraging the potential of living cells to treat a variety of diseases such as Cancer, Autoimmune diseases, and Neurological disorders. Expansion and/or differentiation of cells with the right therapeutic profiles, in a consistent and safe manner, requires the use of defined cell isolation, culture and handling reagents manufactured under GMP conditions. One major cause of variability is serum or serum-derived component, with its undefined composition. The presence of undefined reagents can lead to the inability to reproduce results between labs and stop the progression from discoveries to clinical applications. Here we introduce critical GMP-grade, serum-free ancillary reagents such as magnetic bead-based cell isolation kits, xeno-free media for both immune (T & NK) & stem/progenitor cells as well as chemically defined cryopreservation solution specially designed for therapeutic development & manufacturing. Our development work has allowed us to gain in depth knowledge of the impact of each component in primary cell biology, allowing us at BioLegend to provide custom tailored solutions. The development of defined reagents provides a smooth transition and reproducibility between research and clinical development.

PRESENTER:
Jessie Ni, *BioLegend, USA*

4:40 PM – 4:55 PM

THE ROAD TO SCALABLE MANUFACTURING: CHALLENGES AND INNOVATIONS

Presented by: InnoCellular Tech, a subsidiary of Applitech Biological Technology Co., Ltd.

Mesenchymal stem cells (MSCs) hold immense promise in regenerative medicine due to their immunomodulatory and therapeutic potential. However, transitioning from laboratory research to large-scale manufacturing presents significant challenges, including maintaining cell quality, consistency, and scalability. Stirred-tank bioreactors (STRs), such as those developed by Applitech, provide a scalable and controlled platform for MSC expansion, addressing these challenges while ensuring reproducibility and efficiency. This talk will highlight the use of Applitech's STR systems for MSC manufacturing, focusing on key process parameters such as media optimization using InnoCellular's proprietary MesenPlify™ media and dynamic control of culture conditions. These strategies ensure the preservation of MSC functionality and therapeutic properties during scale-up. By leveraging the advantages of STR technology and optimized media formulations, this approach enables efficient and reproducible production of high-quality cells. This advancement aims to meet the growing demands of clinical applications, paving the way for scalable and cost-effective cell-based therapies.

PRESENTER:
Sam Tsz Wing, *InnoCellular Tech, a subsidiary of Applitech Biological Technology Co., Ltd., Singapore*

5:00 PM – 5:15 PM

MYTOS IDEM: A FLASK-BASED AUTOMATION PLATFORM, TO MOVE FAST FROM LAB TO MANUFACTURING

Presented by: Mytos

PSC-derived therapies face existential scaling challenges: manual cell production is too expensive, and requires 100s of trained scientists to get to enough patients. But teams waste years trying to get their manual 2D process working in bioreactors - usually resulting in failure. Mytos iDEM enables existing 2D processes to scale with 10x less labour, and 2.5x less Grade B cleanroom space. CCO Ignacio Willats will share how regmed teams can solve scalability early, by rapidly transitioning their flask-based process to Mytos iDEM within a handful of runs.

PRESENTER:
Ignacio Willats, *Mytos, UK*

5:20 PM – 5:35 PM

FATEVIEW™: AI-POWERED, NON-DESTRUCTIVE 'LIVE' CELL ANALYTICS AND CELL FATE FORECASTING

Presented by: CellVoyant Technologies Ltd

Visualizing and characterizing cell proliferation and differentiation dynamics is paramount to unravel developmental biology mechanisms and design efficient strategies for cell therapy development. Methods like immunohistochemistry or single-cell 'omics provide only endpoint cell state data without real-time insights. Conversely, genetically-encoded fluorescent reporters yield real-time data but are complex and lengthy to implement and restricted in applicability. FateView™ is a novel online App designed specifically for biologists. It combines live-cell imaging with advanced computer vision and artificial intelligence to track, predict and quantitate cell characteristics and differentiation dynamics non-invasively at single-cell resolution. Researchers can upload microscopy images into FateView™'s intuitive interface and access interactive, predictive, quantitative readouts quickly, effortlessly and without interrupting experiments or sacrificing samples, saving time, cost, and speed of decisions in cell selection, differentiation, drug screening and more.

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PRESENTERS:
Rafael Carazo Salas, *CellVoyant Technologies Ltd, UK*
Adel Benamara, *CellVoyant Technologies Ltd, UK*

CAREER EXPLORATION PRESENTATIONS

FRIDAY, 13 JUNE

Career Exploration presentations will take place in the Exhibit Hall Theater and will feature short, 5-15 min presentations from companies seeking to recruit from ISSCR's talented community.

5:20 PM – 5:25 PM **CAREER EXPLORATION WITH NUWACELL BIOTECH & SHOWNIN BIOTECH**

5:30 PM – 5:35 PM **CAREER EXPLORATION WITH SCHOOL OF LIFE SCIENCE AND TECHNOLOGY, SHANGHAITECH UNIVERSITY**

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Published in association with Center for Excellence in Molecular Cell Science, Chinese Academy of Sciences and the Chinese Society for Cell Biology

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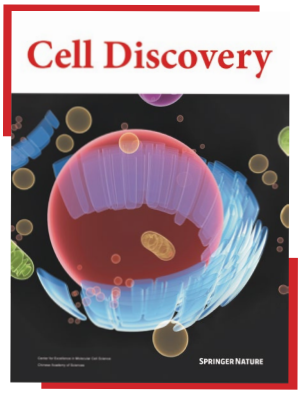

Featured Papers

Donor MHC-specific thymus vaccination allows for immunocompatible allotransplantation
Cell Research (2025) 35:132–144; https://doi.org/10.1038/s41422-024-01049-5

Reducing functionally defective old HSCs alleviates aging-related phenotypes in old recipient mice
Cell Research (2025) 35:45–58; https://doi.org/10.1038/s41422-024-01057-5

GeneCompass: deciphering universal gene regulatory mechanisms with a knowledge-informed cross-species foundation model
Cell Research (2024) 34:830–845; https://doi.org/10.1038/s41422-024-01034-y

Past, present, and future of cell replacement therapy for parkinson's disease: a novel emphasis on host immune responses
Cell Research (2024) 34:479–492; https://doi.org/10.1038/s41422-024-00971-y

NR5A2 connects zygotic genome activation to the first lineage segregation in totipotent embryos
Cell Research (2023) 33:952-966; https://doi.org/10.1038/s41422-023-00887-z

SPRINGER NATURE

FOCUS SESSIONS

WEDNESDAY, 11 JUNE | 2:00 PM – 3:30 PM

TOOLS FOR BASIC AND APPLIED STEM CELL BIOLOGY

Organized by: Stem Cell COREdinates

Supported by: STEMCELL Technologies and Thermo Fisher Scientific

Hall 3G, Level 3

Stem Cell COREdinates (*www.COREdinates.org*) is an international consortium of human pluripotent stem cell-focused cores that share expertise with protocols, reagents, and technological advancements to establish "best practices". We work in coordination with the European Network for Stem Cell Core Facilities (*https://coreustem.eu/*). In our Focus Session, we have selected presentations covering a number of different areas of expertise including organoids, gene editing, disease modeling, GMP manufacturing and biobanking.

2:00 PM – 2:05 PM **Wenli Yang,** *University of Pennsylvania, USA*
WELCOME AND OVERVIEW

2:05 PM – 2:19 PM **Chong Li,** *Chinese Institute for Brain Research (CIBR), China*
DECODING NEURODEVELOPMENTAL DISORDERS THROUGH SINGLE-CELL FUNCTIONAL GENOMICS IN BRAIN ORGANOIDS

2:19 PM – 2:33 PM **Stefan Semrau,** *New York Stem Cell Foundation, USA*
USING THE NYSCF BIOBANK TO DISCOVER GENETIC LOCI ASSOCIATED WITH EMBRYOGENESIS

2:33 PM – 2:47 PM **Kimberly Snyder,** *STEMCELL Technologies Inc, Canada*
LEVERAGING AUTOMATION FOR LONG-TERM SINGLE CELL PASSAGING TO EVALUATE THE IMPACT OF eTeSR™ MEDIUM ON GENETIC STABILITY IN HUMAN PLURIPOTENT STEM CELLS

2:47 PM – 3:01 PM **Dhruv Sareen,** *Cedars-Sinai Biomanufacturing Center, USA*
ENHANCING ASSAY DEVELOPMENT & CELL BANK CHARACTERIZATION FOR GMP IPSC MANUFACTURING

3:01 PM – 3:15 PM **Zhe Zhang,** *Shenzhen Bay Laboratory/Thermo Fisher Scientific, China*
HIGH THROUGHPUT IMAGING OF CARDIAC ORGANOID IN DRUG TESTING

3:15 PM – 3:29 PM **Xiaoxia Cui,** *Washington University in St. Louis, USA*
UNBIASED, WHOLE-GENOME DETECTION OF MULTI-KILOBASE DONOR INTEGRATIONS BY LOCK-SEQ

3:29 PM – 3:30 PM **Xiaoxia Cui,** *Washington University in St. Louis, USA*
CLOSING REMARKS

ADVANCING CELL-REPLACEMENT THERAPY FOR NEUROLOGICAL DISORDERS: OVERCOMING BARRIERS AND EXPLORING OPPORTUNITIES

Organized by: Novo Nordisk

Theater 1, Level 1

Research into cell therapy treatment options for neurological disorders has progressed greatly in recent years, such that we now have multiple first-in-human trials across a range of conditions. There is an opportunity to use the experiences and knowledge from these trials to further inform the trials of the future. In this focus session we will visit the current state of clinical trials in cell therapy for neurological disorders, identify learnings from past trials, and explore future prospects. An expert faculty will discuss advances and learnings from cell-replacement therapy trials in amyotrophic lateral sclerosis, Parkinson's disease and spinal cord injury, and discuss considerations for future trials. The session will culminate in a faculty panel discussion with an audience Q&A session.

2:00 PM – 2:05 PM **Clive Svendsen,** *Cedars-Sinai, USA*
WELCOME AND INTRODUCTION

2:05 PM – 2:25 PM **Clive Svendsen,** *Cedars-Sinai, USA*
THE STATE OF CURRENT CLINICAL TRIALS IN CELL THERAPY FOR NEUROLOGICAL DISORDERS AND LEARNINGS FROM THE PAST

2:25 PM – 3:00 PM **Malin Parmer,** *Wallenberg Neuroscience Center and Lund Stem Cell Center Lund University, Sweden*
INSIGHTS FROM TRANSEURO AND STEM-PD: GUIDANCE FOR FUTURE RESEARCH AND TRIAL STRATEGIES

3:00 PM – 3:20 PM **Hideyuki Okano,** *Keio University, Japan*
STEM CELL THERAPY FOR SPINAL CORD INJURY: BARRIERS AND CHALLENGES

3:20 PM – 3:30 PM **Clive Svendsen,** *Cedars-Sinai, USA*
Malin Parmer, *Wallenberg Neuroscience Center and Lund Stem Cell Center Lund University, Sweden*
Hideyuki Okano, *Keio University, Japan*
EXPERT DISCUSSION AND Q&A

LEVERAGING OPEN-SOURCE IMAGE-BASED EXPERIMENTAL AND COMPUTATIONAL TOOLS TO INVESTIGATE DYNAMIC STEM CELL STATES

Organized by: Allen Institute for Cell Science

Hall B, Level 1

The Allen Institute for Cell Science aims to understand the ways multicellular systems organize, behave, and transition between cell states. We integrate experimental and computational approaches across multiple levels—from molecules to tissues—to develop models that explain and predict cell behavior in human induced pluripotent stem cell (hiPSC) systems. All of the resources and tools generated in pursuit of this mission are shared openly with the scientific community. During this session, Allen Institute team members will present ongoing scientific projects using these tools to investigate changes in state of hiPSC derived endothelial cells exposed to shear stress, changes of state during the formation of 2D vs 3D structures in hiPSCs, and the use of neural networks to extract meaningful features in 3D microscopy data. In addition, Soah Lee from Sungkyunkwan University will present the use of our recent set of hiPSC derived disease cell line collections for cardiomyopathy. Finally, Sadao Ota from the University of Toyko will share the application of a recent Representation Learning framework to quantify interpretable measurements or representations of intracellular structures. The Allen Institute for Cell Science tools and resources shared in this session are openly available at our website allencell.org.

2:00 PM – 2:10 PM **Ru Gunawardane,** *Allen Institute for Cell Science, USA*
WELCOME AND INTRODUCTION TO THE ALLEN INSTITUTE FOR CELL SCIENCE

2:10 PM – 2:25 PM **Soah Lee,** *Sungkyunkwan University, South Korea*
MODELING HUMAN CARDIAC DEVELOPMENT AND DISEASE IN VITRO: INSIGHTS FROM IPSCS, ENGINEERING, AND COLLABORATION

2:25 PM – 2:40 PM **Becky Zaunbrecher,** *Allen Institute for Cell Science, USA*
LIVE-CELL MICROSCOPY & QUANTIFICATION TO STUDY CELL STATE TRANSITIONS IN HIPSC-DERIVED ENDOTHELIAL CELLS

2:40 PM – 2:55 PM **Leigh Harris,** *Allen Institute for Cell Science, USA*
ADHERENT HIPSC LUMENOIDS: A ROBUST 3D PLATFORM FOR BRIDGING CELLULAR DYNAMICS AND MULTICELLULAR ORGANIZATION

2:55 PM – 3:05 PM **Gideon Dunster,** *Allen Institute for Cell Science, USA*
INTERPRETABLE REPRESENTATION LEARNING FOR 3D MULTI-PIECE INTRACELLULAR STRUCTURES USING POINT CLOUDS

3:05 PM – 3:20 PM **Sadao Ota,** *The University of Tokyo, Japan*
3D IMAGE ANALYSIS OF POOLED MULTICELLULAR SYSTEMS.

3:20 PM – 3:30 PM **QUESTIONS**

THE FUTURE OF CELL THERAPIES IN PARKINSON'S DISEASE

Organized by: BlueRock Therapeutics and Bayer Pharmaceuticals

Theater 2, Level 1

Parkinson's disease affects more than 8.5 million people worldwide and is the second most common neurodegenerative disease, after Alzheimer's disease. Current pharmacotherapeutic strategies for treating Parkinson's disease motor symptoms include augmenting dopamine levels in the brain through dopamine agonists, enhancing dopamine bioavailability, or limiting levodopa degradation. However, these treatments are associated with motor complications, including the development of dyskinesias and narrowing of the therapeutic window, and adverse effects such as exacerbation of non-motor symptoms. Further, these therapies do not address the loss of dopaminergic neurons; thus, there remains a critical need for novel therapies for Parkinson's disease. The transplantation of dopaminergic neuronal cells into the putamen is an innovative strategy for restoring dopaminergic function in the brains of people with Parkinson's disease. In this session, we will discuss the advancements in surgical techniques involved in the delivery of cell therapies in the brain. We will also discuss emerging data from the first-in-human phase 1 trial of bemdaneprocel, an hESC derived allogenic, dopaminergic progenitor cell therapy.

Amit Rakhit, *BlueRock Therapeutics, USA*
WELCOME AND INTRODUCTIONS

Andrew Evans, *The Royal Melbourne Hospital, Australia*
THE UNMET NEED IN PARKINSON'S DISEASE: WHERE WE ARE TODAY

Lorenz Studer, *Memorial Sloan Kettering Cancer Institute, USA*
ON THE HORIZON: CLINICAL ADVANCES IN CELL THERAPY FOR PARKINSON'S PATIENTS

Q&A AND CLOSING REMARKS

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INNOVATION SHOWCASES

WEDNESDAY, 11 JUNE

12:30 PM – 1:00 PM
ADVANCING NEUROLOGICAL AND RETINAL RESEARCH WITH GENETICALLY DIVERSE HUMAN IPSC-DERIVED CELLULAR MODELS
Presented by: STEMCELL Technologies Inc.

Hall 3F, Level 3

Human induced pluripotent stem cell (hiPSC)-derived models provide physiologically relevant, reproducible platforms for modeling neurological and retinal diseases and advancing drug discovery. STEMCELL Technologies has developed a panel of diverse, genetically characterized, diverse hiPSC lines manufactured under ISSCR research standards, including disease-specific variants such as APOE ε4/ε4, APP KM670/671NL (Alzheimer's disease), and ABCA4 knockout (Stargardt disease). This session presents characterization data for differentiated models derived from our healthy control hiPSC lines, including forebrain neuron precursor cells (demonstrating electrophysiological activity, synaptic connectivity, and region-specific marker expression), astrocytes (exhibiting mature functional marker profiles), and retinal pigment epithelial (RPE) cells (demonstrating cellular polarization, barrier integrity, and phagocytic activity). Learn how these differentiated cells and organoids, generated using optimized STEMdiff™ protocols, accelerate research by providing physiologically relevant, ready-to-use models with diverse genetic backgrounds. Discover how STEMCELL's platforms enable precise disease modeling, efficient drug screening, and detailed mechanistic studies.

PRESENTER:
Andrew Gaffney, *STEMCELL Technologies Inc., Canada*

1:05 PM – 1:30 PM
RAPIDLY GENERATING FUNCTIONAL FOREBRAIN NEURONS FROM HUMAN PLURIPOTENT STEM CELLS USING AN NGN2 mRNA-LNP PLATFORM
Presented by: STEMCELL Technologies Inc.

Hall 3F, Level 3

Differentiating human pluripotent stem cells (hPSCs) into neuronal cells is a crucial step in neurological research. While lentiviral delivery of the transcription factor neurogenin-2 (NGN2) is widely used to accelerate this process, it comes with challenges such as genomic integration risks, variability, and labor-intensive steps. In this talk, we introduce an integration-free, mRNA-lipid nanoparticle (LNP) system designed for the efficient and reproducible generation of highly pure neurons: STEMdiff™-TF Forebrain Induced Neuron Differentiation Kit. Attendees will learn how this method enables rapid neuron differentiation with high purity and functional maturation, promotes sustained synaptic activity, simplifies workflows, and reduces common challenges associated with

viral methods. With functional characterization confirming this system's capability to generate physiologically relevant forebrain neurons, researchers can access a reliable platform for drug discovery. Join us to discover how STEMdiff™-TF can accelerate your neuronal research while providing a user-friendly approach to hPSC differentiation.

PRESENTER:
Robert Judson, *STEMCELL Technologies Inc., Canada*

12:30 PM – 12:55 PM
BREAKTHROUGH STRATEGIES USING NOVEL REAGENTS IN MASS PRODUCTION WITH IPSC EXPANSION & DIFFERENTIATION
Presented by: FUJIFILM

Hall 3G, Level 3

The optimized protocols for expansion and differentiation of iPS cells on a small laboratory scale are subject to extreme degradation against target cell yield and its stability between when scaled up to full-scale production. It is desirable to develop methodology for equivalent performance in mass production with scale-up. In particular, cell death in the early phase of iPS expansion (after cell seeding to initiation of cell division) and in the early induction phase of differentiation (first step of induction from undifferentiated state) are considered to impact the yield and its batch-to-batch variability of the target differentiated cells. Here, I will present breakthrough strategies using novel reagents that improve yield and stability in mass cell production.

PRESENTER:
Masahiro Kino-oka, *Osaka University, Japan*

1:00 PM – 1:30 PM
TECHNOLOGICAL STRATEGY FOR REALIZING THE PRODUCTION REVOLUTION OF DIFFERENTIATED CELLS DERIVED FROM IPSCS USING BOTULINUM HEMAGGLUTININ
Presented by: FUJIFILM

Hall 3G, Level 3

Although the potential of iPSCs to differentiate into various cell types has shown great application promise in regenerative medicine, variation in differentiation efficiency and low reproducibility significantly limit their practical application. Here, I will introduce a novel culture strategy for generating synchronous iPSC-derived differentiated cells using botulinum hemagglutinin (HA). This approach is not only a simple and efficient way to increase iPSC homogeneity, but also a synchronous differentiation method to produce target cells during multistep differentiation. HA may play an important role as a tool for culture stabilization because it can improve the robustness of the differentiation process of various differentiated cell types such as hepatocytes, pancreatic progenitors, cardiomyocytes, neural progenitors, and retinal pigment epithelial cells.

PRESENTER:
Mee-Hae Kim, *Osaka University, Japan*

12:30 PM – 1:30 PM
SCALABLE FEEDER-FREE IN VITRO DIFFERENTIATION OF STEM CELL-DERIVED NK CELLS
Presented by: ACROBiosystems

Theater 2, Level 1

Immuno-oncology cell therapies have gained significant attention, with natural killer (NK) cells emerging as a promising candidate due to their innate ability to target tumors through cytotoxicity and immune activation. NK cells uniquely infiltrate solid tumor microenvironments, destroy malignant cells, and recruit other immune components. Despite this potential, clinical adoption remains limited by challenges in reliably sourcing and expanding functional NK cells. Peripheral blood-derived NK cells constitute only 10-15% of lymphocytes and are difficult to isolate at therapeutic scales, while existing NK cell lines lack critical Fc receptors, necessitating alternative sources like pluripotent stem cells (PSCs). Traditional PSC differentiation methods, however, face variability in expansion rates, purity, and functionality, compounded by reliance on feeder cells that introduce contamination risks and regulatory hurdles. To address these limitations, this study establishes a serum- and feeder-free platform for scalable NK cell production from PSCs. By employing GMP-compliant reagents and stage-specific cytokine regimens, the protocol generated 96.33% CD3-CD56+ NK cells within 35 days. Rigorous quality control at each differentiation phase ensured consistent cell phenotype and function, with final products demonstrating robust tumor-killing activity. The elimination of animal-derived components and feeder layers not only enhances safety but also standardizes manufacturing, enabling large-scale production. This closed-system approach provides a blueprint for automated, GMP-ready workflows, overcoming critical bottlenecks in off-the-shelf NK cell therapy development for cancer immunotherapy.

PRESENTER:
Tianfu Zhang, *ACROBiosystems, China*

12:30 PM – 1:30 PM
SUCCESSFUL 2D-TO-3D TRANSITION IN MSC BIOPROCESSING: BRIDGING BENCHTOP SCALE TO CLINICAL MANUFACTURING
Presented by: Sartorius - Advanced Therapies Solutions

Hall B, Level 1

Mesenchymal Stem/Stromal Cells (MSCs) have significant therapeutic potential but require large-scale manufacturing for clinical applications. Traditional 2D static culture methods are unsustainable at scale and require manual operations. Successful transition from 2D to 3D microcarrier-based Stirred Tank Reactor (STR) expansion is an important step toward fulfilling the commercial demand of clinical grade MSCs. In this study, we demonstrate how a small-scale static culture was transitioned to a suspension-based culture at 2L and then subsequently scaled up to 30L. Join this presentation to learn:

- Transition from 2D static culture to 3D suspension-based culture using a xeno-free media
- Successful scale-up of the expansion process to 30L scale using an STR
- Maintain consistent phenotypic characterization, immunosuppressive function, and karyotypic stability of cells across different scales

PRESENTER:

Rukmini Ladi, *Sartorius, USA*

6:00 PM – 7:00 PM
THE STEM CELL PODCAST PRESENTS: ADVANCING REGENERATIVE MEDICINE WITH CHEMICALLY INDUCED PLURIPOTENT STEM CELLS
Presented by: The Stem Cell Podcast

Hall 3F, Level 3

Join the Stem Cell Podcast for a special live episode at ISSCR 2025 featuring Dr. Hongkui Deng, a leader in the field of cellular reprogramming. Dr. Deng will discuss his groundbreaking work on chemically induced pluripotent stem cells (CiPSCs) and their potential in regenerative medicine. The conversation will highlight a recent first-in-human study involving the transplantation of CiPSC-derived islets into a patient with type 1 diabetes, marking a major milestone for stem cell-based therapies. Attend the show to explore the science behind CiPSCs, the challenges of bringing this approach to the clinic, and what the future holds for personalized regenerative treatments.

PRESENTERS:
Daylon James, *Weill Cornell Medical College, USA*
Arun Sharma, *Cedars-Sinai Medical Center, USA*
Hongkui Deng, *Peking University, China*
Candice S.Y. Liew, *Reprogenix Bioscience, China*

6:00 PM – 6:30 PM
PRECLINICAL EVALUATION OF AUTOLOGOUS IPSC-DERIVED MIDBRAIN DOPAMINERGIC PROGENITORS FOR PARKINSON'S DISEASE, UX-DA001
Presented by: Shanghai Unixell Biotechnology Co., Ltd

Hall C, Level 1

UniXell has developed an autologous iPSC-derived midbrain dopaminergic progenitors (mDAPs) for Parkinson's disease, UX-DA001, which have been cleared for FIH trial by the National Medical Products Administration in China and FDA. Here the preclinical data will be presented, including establishment of clinical-grade induced pluripotent stem cells (iPSCs) from patients, manufacturing of mDAPs, GLP-compliant toxicity study, biodistribution and tumorigenicity evaluation.Notably, we achieved high in vivo mDA neuron yields across batches derived from multiple patients, with more than 50% of the human cells in the graft positive for TH (DA neuron marker), accounting for more than 20% of the number of transplanted cells, at 6 months post-grafting. Most of these DA neurons are positive for EN1, a classical midbrain marker. These in vivo outcomes demonstrate the high efficiency and robustness of our new differentiation protocol, bolstering the feasibility of personalized cell therapy for numerous patients with PD. An efficacy study confirmed that the transplanted cells mediated full dopamine level restoration in the grafted striatum (by microdialysis coupled with HPLC) and behavioral recovery in a mouse model of PD. Furthermore, the parkinsonian non-human primates receiving mDAP transplantation exhibited behavioral improvements accompanied with strong DA activity in positron emission tomography.

PRESENTER:

Shanzheng Yang, *Shanghai Unixell Biotechnology Co., Ltd, China*

6:00 PM – 6:30 PM
THE TRANSITION TO CHEMICALLY DEFINED, ANIMAL-ORIGIN-FREE MEDIA FOR SAFE AND SUSTAINABLE CELL THERAPY MANUFACTURING
Presented by: Xcell Therapeutics Inc.

Hall 3G, Level 3

In modern cell therapy manufacturing, the transition from serum-based culture systems to chemically defined, animal-origin-free (AOF) media has become essential to ensure enhanced safety, reproducibility, and regulatory compliance. Conventional use of fetal bovine serum (FBS) presents significant challenges, including the risk of xenoantigen exposure, heightened immunogenicity, and ethical and environmental concerns. Specifically, FBS introduces foreign proteins such as Neu5Gc and extracellular vesicle (EV) contaminants, which may lead to immune rejection and compromise therapeutic efficacy. Technological advancements now enable the use of chemically defined media that achieve equivalent or superior cell proliferation without reliance on animal-derived components, thereby minimizing variability and contamination risks. Xcell Therapeutics' CellCor CD AOF media exemplifies these advances, demonstrating superior consistency, reduced immunogenicity, and suitability for clinical and industrial applications. It supports not only traditional two-dimensional cultures but also emerging technologies such as 3D cell culture, organoid development, extracellular vesicle production, and bioprinting. Transitioning to AOF, chemically defined systems is no longer an option but a necessity to advance safe, ethical, and sustainable regenerative medicine. Through collaborative innovation, we aim to lead the future of cell and gene therapies by providing reliable, high-quality media solutions tailored to evolving scientific and clinical needs.

PRESENTER:
Hyungtaek Jeon, *Xcell Therapeutics Inc., South Korea*

6:00 PM – 6:30 PM
PLURIPOTENCY FOR THE PLANET: A GLOBAL CALL TO ADVANCE STEM CELL TECHNOLOGY FOR BIODIVERSITY
Presented by: Revive & Restore

Hall A, Level 1

With the sixth mass extinction crisis looming, Revive & Restore is assembling a network of passionate stem cell scientists and reproductive biotechnologists to advance stem cell technology for wild species. Following on from a catalytic meeting in 2023, this collective issued a global call to the research community to prioritize the development of pluripotent stem cell technology across the evolutionary tree. In late 2024, the Applied Stem Cell Conservation Fund launched an international request for proposals, seeking bold breakthroughs in reprogramming, in vitro gametogenesis, embryo models, disease mitigation, and innovations in biobanking. The response exceeded expectations—spanning six continents and over 100 species, demonstrating that this field is primed and poised to grow. Revive & Restore now invites ISSCR 2025 Hong Kong participants to learn about the outcome of this landmark award, and to discover our strategy for catalyzing stem cell technology to save species from extinction.

PRESENTER:
Ashlee Hutchinson, *Revive & Restore, Australia*

6:00 PM – 6:30 PM
EPIGENETICALLY CORRECTED REPROGRAMMING FOR IPS CELL-DERIVED THERAPIES AND DISEASE MODELING
Presented by: iCamuno Biotherapeutics Pty Ltd

Hall B, Level 1

iCamuno Biotherapeutics is an innovative biotechnology company advancing the frontier of induced pluripotent stem (iPS) cell therapies, focusing on immunology and regenerative medicine. We harness proprietary epigenetically corrected cell reprogramming technologies, AI-driven differentiation platforms, precise gene editing tools, and GMP-compliant cell manufacturing capabilities to deliver groundbreaking therapeutic solutions. Our sophisticated target identification and disease modeling platforms enhance the precision and effectiveness of treatment strategies by providing deeper insights into complex diseases. Driven by multidisciplinary expertise and a commitment to scientific excellence, iCamuno accelerates the development and translation of cutting-edge stem cell-based therapies, effectively addressing significant clinical challenges and improving patient outcomes in immunology and regenerative medicine.

PRESENTER:
Matthew McCormack, *iCamuno Biotherapeutics Pty Ltd, Australia*

THURSDAY, 12 JUNE

12:30 PM – 1:30 PM
ROBUST PROTOCOL FOR DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO IMMUNE CELLS TO SUPPORT THE DEVELOPMENT OF CELL THERAPIES
Presented by: STEMCELL Technologies Inc.

Hall 3F, Level 3

Allogeneic stem cell-based immunotherapy manufacturing requires reliable large-scale protocols to produce high-quality immune cells totaling up to 1.5 x 10^9 cells per dose. The high cost of optimization and manufacturing challenges often hampers the development of such protocols In this talk, we present a robust suspension culture protocol for scalable differentiation of human pluripotent stem cells (hPSCs) to CD34+ hematopoietic stem and progenitor cells (HSPCs). With high-volume culture in mind, the seed, feed, and harvest steps were optimized in 2 mL cultures and then scaled up to 100 mL, where multipotent HSPCs were generated at more than 1.0 x 10^6 CD34+ cells per 1 mL culture volume. HSPCs were then differentiated to T, NK, and B cells using specialized differentiation culture systems. This protocol provides a path to scalable manufacturing of high-quality hPSC-derived immune cells.

PRESENTER:
Diana Golubeva, *STEMCELL Technologies Inc., Canada*

12:30 PM – 1:30 PM
ADVANCING IPSC WORKFLOWS TO PAVE THE WAY FOR AUTOLOGOUS MANUFACTURING IN THE CLOSED SYSTEM CLINIMACS PRODIGY®
Presented by:Miltenyi Biotec B.V. & Co. KG

Hall 3G, Level 3

In the first part of the talk, Dr. Sebastian Knöbel from Miltenyi Biotec will present solutions to enhance iPSC workflows from patient material to iPS-derived differentiated cells using the closed CliniMACS Prodigy Adherent Cell Culture process. He will highlight applications for dopaminergic cells, endothelial cells, MSCs, and MSC-derived EVs, along with tools for cell culture and characterization. In the second part, Prof. Yoshida from the CiRA Foundation will discuss advancements in iPSC production for clinical applications. The foundation has generated and supplied 27 HLA homozygous, 3 HLA genome-edited, and 2 Sendai virus vector-produced iPSC lines for clinical use, with several trials underway or planned globally. He will review their development, originally using manual culture methods, and their status nearly a decade after release. The focus will then shift to autologous iPSCs, which minimize immune rejection, but manual production presents challenges such as high costs, long production times, donor variability, and quality control. To address these, the team is automating the iPSC production process in a closed system. Using the CliniMACS Prodigy system, they have developed a process to generate approximately 1×10^7 iPSCs from 20 mL of blood in three weeks. The talk will highlight progress toward fully automated iPSC manufacturing.

PRESENTERS:
Sebastian Knöbel, *Miltenyi Biotec, Germany*
Shinsuke Yoshida, *CiRA Foundation, Japan*

12:30 PM – 1:30 PM
REDEFINING NEUROPSYCHIATRIC DISEASE MODELS WITH STEM CELLS AND NEXT-GENERATION ELECTROPHYSIOLOGY
Presented by: MaxWell Biosystems

Hall A, Level 1

Neurological and psychiatric disorders remain the leading cause of illness and disability worldwide, posing a major challenge to global health. In response, the field is rapidly evolving driven by transformative tools such as induced pluripotent stem cell (iPSC)-derived 2D and 3D models, which offer unprecedented opportunities to study disease mechanisms and identify therapeutic targets. Next-generation high-density microelectrode arrays (HD-MEAs), as provided by the MaxOne and MaxTwo platforms, offer a powerful, non-invasive method for investigating neural morphology, maturation, connectivity, and functional dynamics in both healthy and disease-specific iPSCs-derived models. In this Innovation Showcase, we will be joined by leading researchers to spotlight the power of next-generation electrophysiology in advancing neuropsychiatric disease modeling. Together, we will explore how HD-MEAs are enabling detailed functional characterization of diverse neuron subtypes providing further insights into signaling pathways driving neuronal fate. In addition,

novel cutting-edge electrophysiological strategies to study gene dysfunctions linked to psychiatric conditions will be presented.
These approaches are not only reshaping how we model complex brain disorders, but also accelerating progress toward more precise therapies.

PRESENTERS:
Jen Pan, *Broad Institute of MIT and Harvard, USA*
Hsiu-Chuan Lin, *ETH Zurich, Switzerland*
Marie Obien, *MaxWell BioSystems, Switzerland*
Zhuoliang Li, *MaxWell BioSystems, Switzerland*

12:30 PM – 1:30 PM
EXPLORING THE APPLICATION OF DETERMINISTICALLY PROGRAMMED HIPSC-DERIVED CELLS TO ADVANCE EARLY-STAGE DRUG DISCOVERY
Presented by: bit.bio

Hall C, Level 1

In drug discovery, researchers need reliable, ready-to-use human cell models that deliver reproducible results at every stage of the workflow. Join experts as they present data on the application of bit.bio's deterministically programmed human iPSC-derived ioCells across key drug discovery stages. Through case studies and real-world applications, you will learn how these cells and the associated protocols support workflows in target identification, assay development, disease modelling, and toxicology.
Key learning points:

- Discover quick and easy generation of gene knockouts and CRISPR screens for target ID and validation in human iPSC-derived neurons and microglia using CRISPR-Ready ioCells
- Learn about assay development in neuroinflammation and demyelinating diseases using neuronal and glial co-cultures
- Explore rapidly maturing, consistent human iPSC-derived disease models for neurodegenerative disorders such as Huntington's and Alzheimer's disease
- See data for predicting drug-induced liver injury in toxicology studies with new human iPSC-derived liver models

PRESENTERS:
Ann Bryne, bit.bio, *UK*
Gianmarco Mastrogiovanni, bit.bio, *UK*

12:30 PM – 1:30 PM
FROM RESEARCH TO REALITY: TRANSFORMING REGENERATIVE MEDICINE AND FERTILITY
Presented by: BioLamina

Hall B, Level 1

How do advancements in stem cell research shape the future of regenerative medicine, particularly in women's reproductive health? Ensuring the reliability and effectiveness of stem cell-based therapies is key to developing safer applications for both research and clinical use. This session

delves into groundbreaking work in the field from Fredrik Lanner's research on human embryonic stem cells to Gameto's innovations in fertility treatments. Their discoveries highlight how scientific progress in stem cell biology and ovarian function can open new doors for reproductive and regenerative medicine. Addressing challenges such as cellular integrity and function is essential to improving therapeutic outcomes. Join us to explore the latest advancements in stem cell science and fertility research and how they are paving the way for the future of medicine.

PRESENTERS:
Fredrik Lanner, *Karolinska Institute, Sweden*
Christian Kramme, *Gameto, USA*
Bruna Paulsen, *Gameto, USA*
Ferran Barrachina, *Gameto, USA*

6:00 PM – 6:30 PM
GENO-WRITING™: THE IPSC GENOME ENGINEERING ENGINE FOR TOMORROW'S CELL THERAPY
Presented by: Logomix

Hall B, Level 1

Human induced pluripotent stem cells (iPSCs) offer transformative potential for disease modeling and regenerative medicine, but current genome engineering tools lack the scale, speed, and precision required for clinical applications. Our proprietary platform, Geno-Writing™, overcomes these limitations by enabling:

(1) bi-allelic and precise modification of endogenous loci up to 100 kb, and
(2) stable integration and expression of up to 12 transgenes in human iPSCs within two months.

This capability for rapid, large-scale genomic rewriting sets a new benchmark in iPSC engineering. As a proof of concept, we are developing iPSC-based therapies for Type 1 Diabetes, addressing two key challenges:

(1) Immune tolerance — Geno-Writing™ allows selective deletion of HLA genes at the native locus, avoiding the functional drawbacks and immune risks of conventional methods like B2M or CIITA disruption.
(2) β cell differentiation — Using our proprietary gene prioritization algorithm, we identified and precisely knocked out key regulators, significantly improving β cell yield and insulin secretion from iPSCs.

These results demonstrate Geno-Writing™ as a robust and versatile platform for creating next-generation, allogeneic iPSC-derived cell therapies.

PRESENTER:
Yasunori Aizawa, *Logomix Inc., Japan*

FRIDAY, 13 JUNE

12:30 PM – 1:30 PM

ADVANCING ORGANOID MODELS: INTRODUCING NEW CAPABILITIES FOR INTESTINAL AND HEPATIC RESEARCH

Presented by: STEMCELL Technologies Inc.

Hall 3F, Level 3

Epithelial organoid cultures have transformed in vitro modeling, providing predictive, physiologically relevant models for drug discovery and disease research. In this presentation, we introduce innovative media products and tools designed to overcome current limitations and make organoids more practical and informative research models. IntestiCult™ Plus Organoid Growth Medium enables researchers to generate intestinal organoids that contain a balanced cell population of proliferative cells and highly differentiated cells—without sacrificing expansion rates. STEMdiff™ Hepatic Organoid Media provide a robust platform for generating, expanding, and differentiating hepatic organoids from hPSCs, eliminating reliance on primary tissue. Additionally, we present novel culture tools designed to optimize epithelial organoid workflows, improving consistency and imaging capability to maximize the impact and efficiency of your organoid-based research.

PRESENTER:

Riya Sharma, *STEMCELL Technologies Inc., Canada*

SPEAKER ABSTRACTS

Wednesday, June 11, 2025

PLENARY I: PRESIDENTIAL SYMPOSIUM
10:30 AM – 12:15 PM
GRAND HALL, LEVEL 3

THE PRESENT AND FUTURE OF GENE THERAPIES FOR INBORN ERRORS OF IMMUNITY
Booth, Claire, *Infection, Immunity and Inflammation Department, UCL Great Ormond Street Institute of Child Health, UK*

This session delves into groundbreaking advancements in gene therapies, focusing on the hematopoietic stem cell (HSC) system. Topics include gene addition and editing techniques, including CRISPR/Cas9 and prime editing, and their applications in treating genetic diseases affecting the blood, central nervous system (CNS), skeletal systems, and more. This session will also cover emerging in vivo gene therapy strategies targeting HSCs and immune cells, as well as the regulatory, accessibility, and economic challenges associated with these advanced therapies.

MONOAMINES, MITOCHONDIA, AND MOOD
Vaidya, Vidita A., *Biological Sciences, Tata Institute of Fundamental Research, India*

Monoamines are phylogenetically ancient molecules that predates the evolution of the nervous system, that were likely co-opted to function as neurotransmitters. Serotonin and norepinephrine retain “pre-nervous” trophic-factor actions influencing development and growth, while exerting pleiotropic neurotransmitter effects on diverse brain functions and behavior. Monoamines are speculated to exert antioxidant-like actions; however, their influence on the energy producing organelle, mitochondria, and on a neuron’s stress buffering capacity remains poorly understood. Mitochondrial function is essential to fulfill substantial neuronal metabolic demands, maintain excitability, and facilitate synaptic transmission. Mitochondria serve as key signaling platforms, coupling metabolic status to mitochondrial dynamics, biogenesis and function, and influence neuronal metabolism, intracellular signaling, and synaptic plasticity. Mitochondrial biogenesis is an adaptive mechanism that responds to cellular energetic demands and oxidative insults, and can promote neuronal viability. We hypothesized that monoamines in keeping with their putative trophic and antioxidant-like actions, may serve as upstream modulators of mitochondria in neurons and thus influence stress buffering. In my talk I will discuss work that demonstrates that serotonin, via the serotonin2A receptor, and norpeinephrine via the Beta2 adrenoceptor enhance mitochondrial biogenesis in rodent cortical and hippocampal neurons respectively, increase mitochondrial function, respiratory capacity, and ATP generation. These intriguing effects arise via recruitment of master modulators of mitochondrial biogenesis, the sirtuin SIRT1, and the transcriptional coactivator PGC-1α, that

are strongly implicated in metabolic control and longevity. This link between monoamines, bioenergetics, and neuronal survival provides a new framework for how monoaminergic signaling impacts stress responses, both at a cellular and organismal level.

ENSURING GLOBAL ACCESS TO ADVANCED THERAPIES

Pepper, **Michael S.**, *Institute for Cellular and Molecular Medicine, SAMRC Extramural Unit for Stem Cell Research and Therapy, University of Pretoria, South Africa*

Exciting developments in the field of advanced therapies have raised the hopes of many who suffer from serious and incurable diseases, as well as those who deliver these services in the hope of improving the quality of life of those they serve. All elements of the value chain are implicated: from teaching and learning, to basic and clinical research, to the generation of intellectual property and the entrepreneurial activities that ensue. With the patient's wellbeing as the final goal, careful consideration of each element of the value chain and the translation of research findings into services and products will help us to deliver on the hope we have created. However, a transdisciplinary approach is required to navigate the complexities of this field. Sustainable success requires rigorous adherence to ethical principles and careful monitoring of risk versus benefit. The pace of scientific progress frequently exceeds the ability of the law to keep pace, and a lack of harmonization across jurisdictions adds to the complexity of the field. Our ancestry from the African continent reminds us that we share a common heritage. It is therefore incumbent on us to ensure that no-one is left behind or excluded from accessing the exciting possibilities that continue to emerge in the field of advances therapies. Genetic diversity in sub-Saharan Africa is amongst the highest in the world, and this impacts on many facets of healthcare in the region. It also provides unparalleled opportunities for research and development. Importantly, this diversity reminds us that a one-size-fits-all approach may not be appropriate, and that this needs to be factored in to our work if we are to ensure that access is equitable.

Funding Source: South African Medical Research Council; National Research Fund; National Health Laboratory Services Research Trust.

SUSTAINABLE RETINAL CELL THERAPY

Takahashi, **Masayo**, *Vision Care Inc. and Kobe City Eye Hospital, Ritsumeikan University, Japan*

Since the autologous transplantation of iPS cells in 2014, we have attempted to develop a standard treatment for retinal pigment epithelial (RPE) cell transplantation. We changed the formulation of RPE cells from sheets and suspensions to strips to improve safety and efficacy, which was not predicted by animal studies. In 2017, clinical studies of allogeneic transplantation confirmed that immune responses can be controlled with local steroids alone if HLA mismatches are avoided, and this lead to a pipeline using HLA minimal KO iPS cells. After these clinical studies, we will move on to a track called "advanced medicine" in Japan: since RPE transplantation is a surgical treatment, using this system in parallel with clinical trials will allow us to involve expert surgeons at from early stage, allowing us to provide better treatment and avoid unnecessary surgeries. One of the challenges in making a standard cell therapy is cell production and technology transfer to CDMOs. We have transferred implicit technology to a humanoid robot to validate culture techniques and enable stable cell processing. Furthermore,

to provide good treatment, it is necessary to prepare the entire treatment, not just the cell products. We are preparing a patient candidate registration system under the ophthalmology society and a patient selection system through expert meetings. Regarding cost sharing, private insurance is available for advanced medical treatment in Japan. We will discuss strategies for standard “surgical” treatment.

Thursday, June 12, 2025

ANIMAL MODELS OF REGENERATION
8:30 AM – 10:00 AM
HALL C, LEVEL 1

OUT OF THE TANK APPROACHES TO PROMOTE NEURAL REPAIR
Mokalled, **Mayssa H.**, *School of Medicine, Washington University, USA*

The Mokalled Lab aims to elucidate evolutionarily conserved mechanisms of neural regeneration, and to develop zebrafish-inspired interventions to promote neural repair in mammals. Adult zebrafish possess an elevated regenerative capacity and lack the anti-regenerative complications displayed after mammalian nervous system injuries. Our studies aim to uncover pro-regenerative neuronal and glial cell identities and mechanisms in highly regenerative zebrafish, and employs cell fate reprogramming to reconstruct analogous cell states and functions in poorly regenerative mammals.

PRE-EXISTING STEM CELLS REGENERATE SKELETAL MUSCLE DE NOVO IN THE SUPER-HEALING LIZARD TAIL
Almada, **Albert Ernesto**, *Orthopaedic Surgery and Stem Cell Biology and Regenerative Medicine, University of Southern California, USA*
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One of the greatest mysteries in regenerative biology is why certain vertebrate species like fish, amphibians, and reptiles have “Super-Healing” abilities—rapidly rebuilding complex tissues after traumatic injury—whereas humans have more limited regenerative capacity. Here, we discuss our progress towards establishing the green anole lizard (Anolis carolinensis) as a new vertebrate model for studying the super-regenerative properties of skeletal muscle. First, we demonstrate that green anoles regenerate adult skeletal muscle tissue de novo within 3 weeks after tail amputation and that the regenerating myofibers re-grow to ~50% of the original tail muscle after 70 days, highlighting their remarkable ability to rapidly regenerate large amounts of muscle tissue from scratch. Second, using electron microscopy, immunohistochemical (IHC) staining, and single-cell RNA-Sequencing (scRNA-Seq), we identified a pre-existing, PAX7-expressing cell population that resides under the basal lamina of resting myofibers and

expresses a transcriptional signature (including Myf5 and MyoD) that is similar to mammalian adult muscle stem cells (MuSCs). Third, leveraging scRNA-Seq along with Fluorescence Activated Cell Sorting (FACS), we identified a surface marker, Calcitonin receptor (CalcR), that positively selects for a population of lizard muscle progenitor cells that is >50% PAX7-positive and robustly expands in number and efficiently makes myofibers ex vivo. Lastly, using an injury time-course (0, 14, 21, 24, 70 days post amputation) and a newly developed cell transplantation model system to track lizard PAX7+ MuSCs in vivo, we found that PAX7+ cells from the original tail get recruited into the regenerating blastema (Day 14) and then expand and differentiate into newly formed skeletal muscle several weeks later (Day 21-28). Altogether, we discovered a highly regenerative lizard MuSC population that may hold the code for rebuilding skeletal muscle de novo in humans.

RETINOIC ACID REGULATES K+ CHANNEL ACTIVITY VIA RCAN2 TO SCALE THE SIZE OF DEVELOPING AND REGENERATING ZEBRAFISH APPENDAGES

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All organs require the coordinated growth of stem and progenitor cells to scale to the correct proportions with the body. We describe how an electrophysiological mechanism using K+-leak channels is integrated into molecular mechanisms to control the proportions of entire anatomical structures. Using the zebrafish pectoral fin bud as a model for early vertebrate fin/limb development, we observed coordinated decreases in endogenous intracellular K+ levels in all embryonic tissues during bud outgrowth and observed that the K+-leak channel Kcnk5b is expressed in the mesenchyme of the developing fin bud during its outgrowth. Overexpression of Kcnk5b was sufficient to increase the size of the entire anatomical structure by enhancing the transcription of several morphogens (fgf10, fgf8, shh and aldh1a2) as this channel decreased intracellular K+ levels. Because these morphogens are part of an embryonic fin/limb bud developmental program that is conserved from fish to human, these results indicate that this electrophysiological scaling mechanism is conserved among all vertebrates. We also observed that overexpression of Kcnk5b in just a few cells led to broader decreases in intracellular K+ levels in distant cells in the fin bud that lack increased Kcnk5b activity, which indicates that mechanisms that control Kcnk5b activity can broadly decrease intracellular K+ levels throughout the fin bud and coordinately control the growth of cells in the entire structure. Retinoic acid (RA) is a hormone morphogen that can increase limb proportions, and we found that it decreased intracellular K+ in all bud tissues. We subsequently found that RA up-regulated regulator of calcineurin (rcan2) in the buds and that rcan2 overexpression was sufficient to decrease intracellular K+ as well as to enhance growth of the entire buds. Conversely, knockout of rcan2 increased intracellular K+ and decreased scaling. We also found that Rcan2's ability to scale was dependent on Kcnk5b, since the knockout of Kcnk5b prevented Rcan2-induced decreases in intracellular K+ and Rcan2-enhanced proportional growth. We observed similar results in regenerating adult fins. Together, these results provide a previously unknown mechanism for how RA can regulate a specific K+-leak channel via Rcan2 to adjust the size of the fin buds.

Funding Source: ShanghaiTech University and National Science Foundation of China.

MITOCHONDRIAL DYNAMICS GOVERN WHOLE-BODY REGENERATION THROUGH STEM CELL PLURIPOTENCY AND MITONUCLEAR BALANCE

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Pan, Xue*, Westlake University, China*
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Li, Yucong*, Westlake University, China*

Mitochondrial dynamics and metabolism play a pivotal role in development and wound repair. However, their precise roles in large-scale tissue regeneration remain largely unclear. Planarians, with their exceptional regenerative capabilities, provide an invaluable model to investigate this process. Our research has unveiled that the knockdown of the mitochondrial fusion gene, opa1, negatively affects both tissue regeneration and the pluripotency of stem cells. Intriguingly, the regeneration defects caused by opa1 knockdown can be mitigated by concurrently knocking down the mitochondrial fission gene, drp1, which partially restores the balance of mitochondrial dynamics. Moreover, we have uncovered that Mito low stem cells exhibit an enrichment of pluripotency due to their fate choices at earlier stages. Transcriptomic analysis has illuminated the delicate mitonuclear balance in metabolism and mitochondrial proteins in regeneration, controlled by mitochondrial dynamics. These findings, which highlight the significance of maintaining mitochondrial dynamics in the context of large-scale tissue regeneration, have recently been published. While we have made progress in this direction, there are still numerous questions left to explore. We aim to delve deeper into the signals and cellular behaviors within the animals to gain insights that could have implications for the initiation of tissue regeneration. We look forward to presenting our past and ongoing efforts to unravel the complexities of mitochondrial dynamics and metabolism for tissue regeneration in the coming meeting.

INHIBITION OF LYSOZYME2 IN ENDOCARDIAL CELLS PROMOTES RAPID RECOVERY OF NON-REGENERATIVE HEARTS

Zhang, Hui, *Chinese Academy of Medical Sciences, China*

Adult mammalian hearts are incapable of regeneration following injury. Here, we observed aberrantly high expression of Lysozyme 2 (Lyz2) in mouse hearts at both local injury sites and at remote zones, with sustained Lyz2 expression conspicuous in endocardial cells of non-regenerative hearts. We demonstrate that LYZ2 functions as an injury-specific, positive regulator of lysosomal degradation capacity that mediates (pathogenic) degradation of the extracellular matrix. We observed cardioprotection upon disrupting LYZ2/LYZ function in mice and in a human endomyocardium experimental model. Harnessing these insights, we show that both Lyz2 KO and pharmacological inhibition of lysosomal degradation confer rapid functional recovery in injured non-regenerative hearts. Thus, targeting a remote injury response in non-cardiomyocyte cell type rapidly promotes post-MI recovery of non-regenerative hearts.

INSIGHTS INTO SHARED AND DISTINCT MECHANISMS IN SALAMANDER LIMB AND HUMAN FINGERTIP REGENERATION

Sandoval-Guzmán, Tatiana, *Technische Universität Dresden, Germany*

Regeneration has been regarded as a feature limited to a number of species or developmental stage. In mammals, regeneration is attributed to scarless healing during development, and to even more limited regenerative capacity in childhood. This is the case for human fingertip regeneration, inaccurately reported as occurring only in children. In this study, we have analyzed the injured fingertips of 22 patients ranging from infants to old adults. Using a non-occlusive silicone finger cap, wounds fluids were collected at several intervals and analyzed by mass spectrometry. The injuries were clinically assessed revealing four typical clinical phases. Proteomic data correlated to these phases and highlighted processes previously observed in animal models, and also significant differences. A critical question remains, why in mammals only the distal tip of appendages possesses the capacity to regenerate? In salamanders, although capable of regenerating at any axial point of the limb, we have found differences in mechanisms according to the position of the injury; including tissue remodeling, cell cycle length and marker expression. Building a comprehensive understanding of how the site of an injury influences appendage regeneration, will significantly shape our interventions in human healing.

METABOLIC UNDERPINNINGS OF STEM CELLS
8:30 AM – 10:00 AM
HALL B, LEVEL 1

METABOLIC REGULATION OF LIVER REGENERATION
Ding, Qiurong, *Shanghai Institute for Nutrition and Health, Chinese Academy of Sciences, China*

The mammalian liver displays robust regenerative capabilities under healthy conditions, a process compromised in metabolic disorders like fatty liver disease. In an effort to elucidate the underlying mechanisms, we developed an in vivo liver CRISPR screening methodology to uncover novel regulatory factors. Complementing this approach, comprehensive omic studies were conducted on both healthy and fatty livers during the regeneration process. Through our investigations, we identified MIER1 (mesoderm induction early response 1) as a pivotal epigenetic modulator that orchestrates the intricate interplay between lipid dynamics and cell cycle gene expression during liver regeneration, with notable dysregulation observed in fatty liver contexts. Importantly, depletion of MIER1 in hepatocytes significantly enhances regeneration in fatty liver-afflicted animals. Furthermore, our exploration of cell-cell interactions during liver regeneration unveiled aberrant hepatocyte-endothelial cell connections in fatty liver settings during regeneration, exhibiting distinct zonal regulations. Restoring the functional interplay between hepatocytes and endothelial cells also emerges as a promising avenue for enhancing regeneration in fatty liver conditions.

METABOLIC PROGRAMMING OF EMBRYONIC, TROPHOBLASTIC, AND MATERNAL CELLS DURING PREGNANCY
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Our previous studies established that the metabolic state of ESCs, particularly their amino acid and acetyl-CoA levels, can profoundly influence epigenetics and cell fate decisions. Building on

this foundation, we have begun to explore how metabolic-epigenetic coupling mechanisms may operate in the context of placental trophoblast development and maternal adaptations during pregnancy. In human trophoblast stem cells (hTSCs), we found that glucose metabolism is highly active to fuel rapid proliferation and differentiation. However as hTSCs fuse to form syncytiotrophoblasts in the placenta, their glucose metabolism decreases to just the basal levels needed to fuel histone acetylation to activate syncytialization. When trophoblasts experience even brief periods of glucose deficiency during syncytialization, it permanently impairs differentiation and causes inflammation. Remarkably, these deleterious phenotypes can be rescued by acetate supplementation, which restores proper histone acetylation. Building on our insights into hTSC biology, we constructed a metabolome atlas of the placenta and maternal tissues in pregnant monkeys. It revealed widespread metabolic decoupling and rewiring across 23 tissues during primate pregnancy, affecting core pathways such as steroid, fatty acid, and arachidonic acid metabolism. Palmitoyl-carnitine promotes progenitor differentiation in multiple maternal tissues, including muscles. Biosynthesis of the stress-related corticosterone in both the placenta and other tissues, governs TSC differentiation and placental maturation. Disruption of these metabolic regulators could induce preeclampsia-like inflammation. Interestingly, the metabolic stress of pregnancy was found to have paradoxical effects on maternal health. On one hand, it can accelerate aging and functional decline in some organs. On the other hand, pregnancy-induced metabolic programming can activate regenerative responses that reverse age-related impairments in other tissues. Elucidating the mechanisms underlying this metabolic plasticity with spatial metabolomics promises to yield important insights for reproductive biology, developmental programming, and the management of pregnancy-related disorders.

Funding Source: HHMI International Scholar.

RECENT EVOLUTION OF THE DEVELOPING HUMAN SMALL INTESTINAL EPITHELIUM

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Secchia, Stefano, *Institute of Human Biology, Switzerland*
Adam, Lukas, *Institute of Human Biology, Switzerland*
Tsai, Yu-Hwai, *University of Michigan Medical School, USA*
Fauci, Christiana, *Duke University School of Medicine, USA*
Janssens, Jasper, *ETH Zürich, Switzerland*
Childs, Charlie, *University of Michigan Medical School, USA*
Walton, Katherina, *University of Michigan Medical School, USAc*
Sandoval, Ruben, *Institute of Human Biology, Switzerland*
Wu, Angeline, *University of Michigan Medical School, USA*
Bellavista, Marina, *Institute of Human Biology, Switzerland*
Huang, Sha, *University of Michigan Medical School, USA*
Steiner, Calen, *University of Michigan Medical School, USA*
Throm, Yannick, *Institute of Human Biology, Switzerland*
Boyle, Michael, *Yale School of Medicine, USA*
He, Zhisong, *ETH Zürich, Switzerland*
Beumer, Joep, *Institute of Human Biology, Switzerland*
Treutlein, Barabara, *ETH Zürich, Switzerland*
Lowe, Craig, *Duke University School of Medicine, USA*
Spence, Jason, *University of Michigan Medical School, USA*

Camp, Gray J., *Institute of Human Biology, Switzerland*

Diet, microbiota, and other exposures place the intestinal epithelium as a nexus for evolutionary change; however, little is known about genomic changes associated with adaptation to a uniquely-human environment. Here, we interrogate the evolution of cell types in the developing human intestine by comparing tissue and organoids from humans, chimpanzees and mice. We find that recent changes in primates are associated with immune barrier function and lipid/xenobiotic metabolism, and that human-specific genetic features impact these functions. Enhancer assay, genetic deletion, and in silico mutagenesis resolve regulatory components of a distal Lactase (LCT) enhancer. Altogether, we identify enterocytes of the developing intestinal epithelium as a rapidly evolving cell type, and show that great ape organoids provide insight into the human condition.

TGF-BETA-REGULATED PYRUVATE ENTRY INTO THE TCA CYCLE CONTRIBUTES TO HUMAN ENDODERM DIFFERENTIATION
Jiang, Wei, *Medical Research Institute, Wuhan University, China*

Cell fate determination is closely linked to global metabolic changes. The metabolic changes during three germ layers differentiation from human pluripotent stem cells are recently characterized. However, it is largely unclear whether and how the metabolic changes affect differentiation process. Here, we reveal that the metabolic switch with decreased lactate production and increased TCA cycle and oxidative phosphorylation, which is controlled by TGF beta-activated PDHB, is necessary for the definitive endoderm differentiation from human pluripotent stem cells. The inhibition of glucose utilization or pyruvate entry into TCA cycle significantly impairs endoderm differentiation. In contrast, inhibiting lactate production can increase endoderm differentiation efficiency. Mechanistically, inhibiting glucose utilization and TCA cycle during endoderm differentiation leads to a marked reduction in intracellular ATP levels. This decrease adversely affects the function of BAF complex, an important ATP-dependent chromatin remodeling complex, thereby hindering the differentiation process. The BAF complex, centered around the helicase BRG1, promotes the expression of lineage-related genes by opening the local chromatin during endoderm induction. Overall, our findings demonstrate that TGF□-mediated glucose metabolic reprogramming regulates cell fate determination through alterations in ATP levels and the function of BAF complex during human definitive endoderm differentiation.

LYSOSOMAL CATABOLIC ACTIVITY PROMOTES THE EXIT OF TOTIPOTENT STATE BY SILENCING EARLY-EMBRYONIC RETROTRANSPOSONS
Fu, Xudong, *Zhejiang University, China*

During preimplantation development, a subset of retrotransposons/genes are transiently expressed in the totipotent embryos. These transcripts rapidly shut down their expression beyond the totipotent stage of embryos, promoting the embryo to exit from the totipotent stage. However, mechanisms regulating this shutdown remain unclear. Here, we identified that lysosomal catabolism played a role in the exit of the totipotent state. Our results showed that the activation of embryonic lysosomal catabolism promoted the embryo to exit from the totipotent stage and suppressed totipotent transcript expression. Mechanistically, our results indicated that lysosomal catabolism suppressed totipotent transcripts through replenishing

cellular amino acid levels, thereby inactivating transcriptional factors TFE3/TFEB and abolishing their transcriptional activation of totipotent retrotransposons. Collectively, our study identified that lysosomal activity modulated the transcriptomic landscape and development in early embryos and identified an unanticipated layer of transcriptional control on early-embryonic retrotransposons from lysosomal signaling.

METABOLIC REGULATION OF ADULT NEURAL STEM CELLS
Knobloch, Marlen, *University of Lausanne, Switzerland*

Cellular metabolism has emerged as a key player in the regulation of stem cells. This is also true for neural stem/progenitor cells (NSPCs), which form the brain during development, and persist in certain adult brain regions, generating new neurons throughout adulthood. While classically seen as rather glycolytic cells, recent studies have shown that mitochondrial metabolism directly controls NSPC behaviour and influences their quiescence versus activation state. In this talk, I will present the most recent findings of my laboratory to better understand the metabolic requirements of NSPCs and how metabolic alterations affect their behaviour in vitro and in vivo, with a specific focus on lipid metabolism.

NICHE REGULATION IN STEM CELLS AND TISSUE REGENERATION
8:30 AM – 10:00 AM
HALL A, LEVEL 1

HUMAN UMBILICAL CORD MESENCHYMAL STEM CELL-DERIVED EXTRACELLULAR VESICLES AMELIORATE INFLAMMATORY PHENOTYPE OF IMQ INDUCED RECHALLENGED MOUSE MODEL THROUGH PP6
Wang, Honglin, *Shanghai Jiao Tong University School of Medicine, China*

Psoriasis is a chronic inflammatory dermatosis and the hyperproliferation of epidermal KCs is a hallmark of it. Recent studies have demonstrated the therapeutic potential of umbilical cord mesenchymal stem cell-derived extracellular vesicles (UC-MSC-EVs) to psoriasis because of their immunomodulation functions. However, the efficacy and underlying mechanisms are also need more study. Our research demonstrates that PP6 plays a crucial role during the progression of psoriasis especially in keratinocytes. More importantly, we found that PP6 is a component of exosomes, and our results indicated that increasing PP6 expression levels in extracellular vesicles through gene editing can significantly improve its efficacy in psoriasis treatment and alleviate IMQ-induced inflammatory phenotypes in rechallenged mouse models. In summary, our findings provide a theoretical basis for promoting the clinical application of exosomes in treating psoriasis and offer a novel approach to enhance their efficacy.

METABOLIC STRESS AND NICHE INFLAMMAGING IN YOUNG-ONSET COLORECTAL CANCER DEVELOPMENT
Cheng, Chia-Wei, *Department of Genetics and Development, Columbia University, USA*
Ho, Jennifer, *Columbia University, USA*

Cancer has been considered a disease of old age, with chronic inflammation associated with aging acting as a tumor-initiating niche. However, the recent rise in young-onset or early-onset colorectal cancer (EO-CRC) among younger patients raises questions concerning alternative cell origins and niches in CRC development. Despite the strong association between diet-related inflammation and EO-CRC, the mechanistic link between metabolic dysregulation and EO-CRC remains unclear. Here, we demonstrated that tumor-resident stem cells in young (< 50 years old) CRC patients exhibit region-dependent metabolic dysregulation, which can be modeled by perturbating rate-limiting metabolic enzymes in the mouse colon. Using murine models with targeted perturbation of rate-limiting metabolic enzymes in the colon, we reveal that metabolic stress in the distal colon predisposes epithelial cells to inflammatory regenerative states, epithelial-to-mesenchymal transition (EMT), and microbiome dysbiosis. Early-onset tumors arising from this environment predominantly localize to the distal colon and are enriched in bacteria-responsive $Saa1^{+}Areg^{+}Tac1^{+}$ senescent cells, which promote the expansion of $C7^{+}IGF1^{+}IL6^{+}$ cancer-associated fibroblasts (CAFs), closely resembling tumors in human CRC. Using germ-free mice and epithelial-enriched tumor organoids, we demonstrate that metabolic impairment of $Lgr5^{+}$ stem cells directly induces inflammatory and senescent cell states, creating a permissive niche for tumor initiation. However, the progression to early-onset tumorigenesis requires dysbiotic microbiota to activate EMT-dependent CAF expansion, driving tumor growth and regional specificity. These findings establish a definitive mechanistic framework linking metabolic dysregulation in stem cells with microbiota-induced inflammation, EMT-mediated stromal remodeling, and distal colon tumorigenesis in EO-CRC. This integrated model elucidates the metabolic and microbial determinants of early-onset colorectal cancer and highlights potential therapeutic targets for intervention.

INTRAVITAL AND EX VIVO LIVE IMAGING PIPELINES UNCOUPLE STEM CELL MIGRATION AND FATE PHENOTYPES WITHIN THE MYOPATHIC NICHE

Tajbakhsh, Shahragim, *Institut Pasteur, France*
Sarde, Liza, *Institut Pasteur, France*
Letort, Gaëlle, *Institut Pasteur, France*
Varet, Hugo, *Institut Pasteur, France*
Lavilla, Vincent, *Institut Pasteur, France*
Fernandes, Julien, *Institut Pasteur, France*
Evano, Brendan, *Institut Pasteur, France*

Duchenne Muscular Dystrophy (DMD), a fatal neuromuscular disorder affecting ~1/5000 boys, arises from mutations in the Dystrophin gene, compromising myofibre integrity. Advances in palliative care have improved life expectancy, yet a comprehensive understanding of the disease's progression remains elusive, particularly regarding the dynamic behaviors of muscle stem cells (MuSCs) and their interactions with the niche. Here, we developed innovative quantitative live imaging platforms that integrate intravital microscopy with continuous ex vivo assays on isolated myofibres. This allowed us to capture unprecedented spatiotemporal insights into MuSC behaviors during both normal and dystrophic muscle regeneration. Our findings reveal that dystrophic MuSCs exhibit impaired migration kinetics in vivo using multiple readouts including speed and directionality, and this was attributed to extracellular niche dysfunction. Notably, we found that precocious differentiation was driven by cell-autonomous dysregulation of p38 and PI3K signaling pathways, in contrast to the p38-only dependence of healthy MuSCs. Newly devised cross-grafting experiments involving stem/niche cell exchanges further demonstrated that while MuSC symmetric and asymmetric fate decisions are intrinsically

governed, their migration is highly myofibre niche-dependent. These findings resolve long-standing debates on dystrophic MuSC function by delineating the interplay between intrinsic and extrinsic factors. Critically, we observed that MuSC defects manifest before overt myofibre damage, redefining DMD as a "stem cell-opathy" and challenging current therapeutic paradigms. Our study establishes a dynamic framework for understanding MuSC pathophysiology in DMD, with implications extending to stem cell-niche systems in other tissues and organs. By bridging gaps in the understanding of stem cell and niche dynamics, our work opens the door to targeted approaches aimed at restoring stem cell function and improving regenerative outcomes in myopathies and other pathologies.

ESTROGEN PROMOTES BONE FORMATION THROUGH UPREGULATION OF OSTEOLECTIN EXPRESSION IN LEPR+ CELLS

Yang, Min, *National Institute of Biological Sciences (NIBS), China*
Sheng, Bo, *National Institute of Biological Sciences (NIBS), China*
Guo, Jiaming, *National Institute of Biological Sciences (NIBS), China*

Bone homeostasis is a critical equilibrium between bone formation and resorption, which is disrupted in osteoporosis, particularly following menopause due to estrogen depletion. Our study leverages a Tet-on LepR-iCre allele to trace LepR+ bone marrow stromal cells and demonstrates that estrogen directly enhances their osteogenic differentiation into osteoblasts. With the Esr1-TriGFP reporter allele, we detected the most significant enrichment of the estrogen receptor Esr1 in LepR+ cells compared to other bone marrow cell types. Conditional deletion of Esr1 in LepR+ cells, but not osteoblasts and osteocytes, led to early-onset osteoporosis, particularly in females, without affecting hematopoiesis. RNA-seq analysis of LepR+ cells identified Osteolectin, a bone growth factor, which is upregulated by Esr1 and promotes osteogenic differentiation. We explored the therapeutic potential of Osteolectin in estrogen deprivation-induced osteoporosis and found that recombinant Osteolectin administration increased bone formation and mitigated ovariectomy-induced osteoporosis in mice. Serum Osteolectin levels were significantly lower in women experiencing menopause or premature ovarian failure, indicating a role for LepR+ cells and Osteolectin in reduced bone formation during menopausal osteoporosis. To identify compounds that activate Osteolectin expression, we generated an Osteolectin-nanoLuciferase strain and screened over 15,000 compounds using primary LepR+ cells. We identified 87 compounds that reproducibly increased Osteolectin-nanoLuciferase levels, including five FDA-approved statins. Mechanistically, statins promote Osteolectin expression and osteogenic differentiation of LepR+ cells by inhibiting protein prenylation, a process independent of their cholesterol synthesis inhibition. In conclusion, our study establishes LepR+ cells and Osteolectin as key mediators in reduced bone formation during menopausal osteoporosis and potential therapeutic targets. Statins, through their ability to upregulate Osteolectin expression, may provide a beneficial approach to promote bone formation and alleviate osteoporosis in postmenopausal women.

DUAL LINEAGES OF LANGERHANS CELLS COOPERATE FOR IMMUNE BARRIER RECOVERY AFTER SKIN INJURY

Park, Sangbum, *Michigan State University, USA*

Tissue-resident immune cells in the skin provide a first line of defense against infections. Langerhans cells (LCs) in the epidermis act as sentinels by surveilling skin and presenting antigens in lymph nodes. While LCs maintain well-organized spatial distribution within epithelial

stem and progenitor cells in healthy skin, the mechanisms governing their de novo reconstitution following tissue damage along with neighboring stem cells remain elusive. Through longitudinal tracking of LCs and their progenitor cells in live adult mice, we discovered that most activated LCs near wounds (eLCs) stay local and directly contribute to restoring LCs in injured tissue rather than migrating to lymphatics. Simultaneously, monocytes infiltrate the epidermis during wound repair and differentiate into additional LCs (mLCs) that remain long after healing. While the inhibition of Cxcr2 signaling impairs eLCs contribution, mLCs compensate for this deficiency. Our findings reveal fundamental mechanisms of immune barrier recovery through coordinated repopulation by distinct LC lineages in damaged skin.

Funding Source: NIH R01 AR083086.

FROM LOCAL TO SYSTEMIC: HOW BODY-WIDE SIGNALS ORCHESTRATE REGENERATION AND DEFENSE IN THE SKIN
Zhang, Bing, *School of Life Sciences, Westlake University, China*

ABSTRACT NOT AVAILABLE AT TIME OF PUBLISHING

REVOLUTIONIZING THERAPIES FOR CHRONIC DISEASES
8:30 AM – 10:00 AM
THEATER 2, LEVEL 1

“THE REGULATORY SYSTEM IS CAPABLE OF HANDLING MOST OF THE SCARY SCENARIOS THAT PEOPLE THROW UP”: KEY INFORMANT PERSPECTIVES ON PRENATAL GENE EDITING
Isasi, Rosario, *University of Miami, USA*
Bombard, Yvonne, *Institute of Health Policy, University of Toronto, Canada*
Feys, Roel, *Hussman Institute of Human Genomics, University of Miami, USA*
Howard, Heidi, *Lund University, Sweden*
Ormond, Kelly, *ETH Zurich Health Ethics and Policy Lab, Switzerland*

Research on prenatal gene editing is anticipated to occur in a non-distance future, guiding the way for its eventual clinical translation. In this context, developing normative frameworks grounded in collective socio-ethical deliberation is critical. This would ensure that the values of affected/interested groups, including patients, professionals and society at large, are considered, ultimately fostering responsible and ethically sound scientific innovations. To elucidate how individual attitudes could shape the direction of science and policy, the PASAGE study conducted semi-structure key informants’ interviews with professionals (e.g., clinicians, scientists, bioethicists) with experience in policymaking (e.g., laws, professional guidelines) in areas germane to potential prenatal gene editing applications. Our interviews explored attitudes towards scientific innovation, governance, risk tolerance, and socio-ethical/moral values. Findings were then compared against socio-ethical and moral values stated in gene editing and prenatal therapy policies. In this presentation, we provide results of both our key informants interviews and comparative policy study. We also outline a proposed value-based governance framework based on these findings.

Funding Source: Patient Supported Approaches to Gene Editing – PASAGE, National Human Genome Research Institute (Grant R01HG011461).

DEVELOPMENT AND PRECLINICAL VALIDATION OF CELLTHRPE1: A STEM CELL-DERIVED RETINAL THERAPY FOR DRY AGE-RELATED MACULAR DEGENERATION

Saitez, **Sarah Kieler**, *Intervention and Technology (CLINTEC), Karolinska Institutet, Sweden*
Baqué-Vidal, Laura, *Karolinska Institutet, Sweden*
Main, Heather, *Karolinska Institutet, Sweden*
Reilly, Hazel, *Karolinska Institutet, Sweden*
Hedenskog, Mona, *Karolinska Institutet, Sweden*
Beri, Nefeli-Eirini, *Karolinska Institutet, Sweden*
Metzger, Hugo, *Karolinska Institutet, Sweden*
Locri, Filippo, *Karolinska Institutet, Sweden*
Bär, Frederik, *Karolinska Institutet, Sweden*
Muth, Daniel, *St. Erik Eye Hospital, Karolinska Institutet, Sweden*
Plastino, Flavia, *St. Erik Eye Hospital, Karolinska Institutet, Sweden*
Melin, Yesenia, *St. Erik Eye Hospital, Karolinska Institutet, Sweden*
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Markland, Katrin, *Karolinska Cell Therapy Center, Karolinska University Hospital, Sweden*
Kvanta, Anders, *St. Erik Eye Hospital, Karolinska Institutet, Sweden*
Lanner, Fredrik, *Karolinska Institutet, Sweden*

Dry age-related macular degeneration (dAMD) is a leading cause of blindness in the elderly in developed countries. The impairment of retinal pigment epithelium (RPE) cells leads to the progressive degeneration of photoreceptors in the macula, resulting in the irreversible loss of central, high-acuity vision. While recent pharmacological advances have shown promise in halting disease progression, no approved clinical interventions currently exist to cure dAMD. Pluripotent stem cell-based replacement therapy offers an attractive strategy to prevent vision loss. To this end, we have developed a Good Manufacturing Practice (GMP)-compliant protocol for the large-scale production of the cryopreserved allogeneic embryonic stem cell (ESC)-derived RPE drug product, CellThRPE1. We have established and validated methodologies to ensure the product's viability, purity, and absence of lingering pluripotent cells, including FACS-based purity assays, targeted genetic analyses to detect tumorigenic or pathogenic variants, and functional assays to assess epithelial integrity. Toxicological preclinical and biodistribution studies were performed with Charles River and Pharamaron in nude rats. Efficacy studies conducted by Pharmaron in Royal College of Surgeons (RCS) rats demonstrated functional visual improvement, increased retinal outer nuclear layer (ONL) thickness, and enhanced optokinetic reflex and electroretinography responses. Additionally, we have confirmed 36 months of stability for our clinical batches and validated a 6-hour shelf-life for the drug substance at room temperature, facilitating transport from the formulation facility to the operating room (OR) for administration. These data support long-term functional cryopreservation and in-use stability of CellThRPE1, paving the way for the progression to a First-in-Human (FiH) Phase I clinical trial at St. Erik Eye Hospital, Sweden, targeting geographic

atrophy secondary to AMD.

CRISPR-CAS9-INDUCED DOUBLE-STRAND BREAKS DISRUPT MAINTENANCE OF EPIGENETIC INFORMATION

Li, Mo, *Bioscience, King Abdullah University of Science and Technology (KAUST), Saudi Arabia*
Bi, Chongwei, *Biological and Environmental Science and Engineering, KAUST, Saudi Arabia*
Wang, Mengge, *Biological and Environmental Science and Engineering, KAUST, Saudi Arabia*
Zhang, Yignzi, *Biological and Environmental Science and Engineering, KAUST, Saudi Arabia*

CRISPR-Cas9 genome editing enables precise genetic modifications by introducing targeted DNA double-strand breaks (DSBs). While prior studies by us and others have focused on the genetic consequences of CRISPR-Cas9 editing, there remains a lack of understanding of the epigenetic consequences arising from targeted DNA damage. Using Cas9-assisted targeted nanopore sequencing, we investigated how Cas9-induced DSBs affect DNA methylation patterns in human embryonic stem cells. We induced DSBs at differentially methylated regions (DMRs) of imprinted genomic loci and performed high-coverage, long-read native DNA sequencing to simultaneously obtain genetic variant and base-resolution methylation data in a haplotype-resolved manner. This deep-coverage, multidimensional data from multiple independent loci provided statistically robust evidence that DSBs consistently result in aberrant DNA methylation at the targeted sites. Our findings reveal that DSBs induce significant epigenetic disruptions through mechanisms such as homologous recombination, Cas9-induced large structural variations, and defective methylation maintenance during DNA repair. Notably, these epigenetic changes can occur either in conjunction with or independently of genetic alterations. Our results have significant implications for both basic research and clinical applications of genome editing technologies. In research settings, these findings underscore the need for comprehensive epigenetic assessments following genome editing to ensure that observed phenotypes are not attributable to unintended epigenetic alterations. This is critical for accurate interpretation of experimental outcomes. In clinical settings, given the increasing therapeutic use of CRISPR-Cas9, including its recent approval for treating sickle cell disease, our findings emphasize the urgent need to evaluate and mitigate unintended epigenetic consequences that could potentially lead to long-term side effects.

Funding Source: The research of the Li laboratory is supported by KAUST Office of Sponsored Research (OSR), under award number BAS/1/1080-01-01, and by funding from KAUST Center of Excellence for Smart Health (KCSH), under award number 5932.

ENGINEERED HUMAN TUBULES REVEAL PATHOPHYSIOLOGICAL MECHANISMS OF AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

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Locatelli, Laura, *Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Italy*
Trillini, Matias, *Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Italy*
Cerullo, Domenico, *Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Italy*
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Buttò, Sara, *Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Italy*
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Peracchi, Tobia, *Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Italy*
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Rubis, Nadia, *Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Italy*
Lavecchia, Angelo Michele, *Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Italy*
Remuzzi, Giuseppe, *Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Italy*
Xinaris, Christodoulos, *Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Italy*

Autosomal dominant polycystic kidney disease (ADPKD) is a rare monogenic disease, characterized by the formation of multiple cysts that cause gradual loss of renal function. To date there is no effective cure for this disease. Notably, our explorative clinical trial on ADPKD patients showed that the worsening of the renal functionality correlates with lower levels of freeT3, the active form of TH, and higher levels of reverseT3, a TH metabolite, suggesting that TH alterations could play a key role in the progression of the disease. With the aim of testing the therapeutic efficacy of THs and THs' analogues in human tissue, we established and used an in vitro human-derived polycystic kidney model. ADPKD patient-derived cells were used to generate 3D polycystic tubules that were treated with THs and their analogues. Both cysts' number and diameter in polycystic tubules were measured to evaluate the efficacy of each treatment under study. THs, and particularly T4, administration resulted in a significant reduction of cyst formation (cyst number) and growth (cyst diameter) in patient-derived engineered tubules. In addition, it was verified that this action was mediated by T4 binding to the αvβ3 integrin. The anti-cystogenic effect exerted in vitro by T4 was mediated by the activation of antioxidant pathways and the inhibition of proliferation. To verify furthermore the efficacy of this treatment, we tested THs administration in vivo on PCK rats. Consistent with the findings in human ADPKD, we observed that PCK rats have a lower TH serum level compared to the healthy control. Upon T4 administration, we revealed a significant reduction in macrocystic area in PCK rats treated with T4 compared to the vehicle-treated group. The anti-cystogenic effect observed was confirmed to be dependent on the activation of anti-proliferative and antioxidant processes. These data highlight the central role of THs signaling in ADPKD progression and the therapeutic potential of T4.

Funding Source: Fondazione Terzi-Albini, Fondazione Telethon ETS.

STEM CELLS IN DRUG DISCOVERY: FROM CONCEPT TO APPLICATION
8:30 AM – 10:00 AM
THEATER 1, LEVEL 1

CLINICAL TRIAL IN A DISH WITH INDUCED PLURIPOTENT STEM CELLS FOR DRUG DISCOVERY AND PRECISION MEDICINE

Pang, Paul, *Greenstone Biosciences, USA*

Drug discovery for rare diseases is often limited by the lack of relevant preclinical models that adequately capture the complexity and diversity of patients affected. Furthermore, clinical trials are burdened with challenges in patient recruitment and stratification to assess the safety and efficacy of a drug candidate. The ability to use in vitro cellular data for efficacy assessment will create drug development opportunities for rare diseases that were previously considered too challenging due to the small number of patients. To enable data from relevant cellular models

to refine clinical trials, establishing patient-derived induced pluripotent stem cells (iPSCs) will inform the development of general standards, quality control criteria, and best practices for human-based and genetically diverse in vitro models to assess drug candidate safety and efficacy. Greenstone Biosciences is building and growing an iPSC biobank, currently at 2,000+ iPSC lines, to model diseases with different genetic diversity for drug discovery and to further establish “clinical trial in a dish.” We differentiate iPSCs to multiple cell types from cardiomyocytes to endothelial cells to hepatocytes to perform multi-systemic evaluation of drug candidates. Furthermore, we synergize iPSC data with artificial intelligence (AI) methods to improve and de-risk the drug discovery process in pursuit of safer and more effective drugs for patients with unmet needs. Our services and resources are commercially available to help investigators in academia and industry accelerate their research and drug development pipelines.

SCALABLE GENERATION OF PATIENT-SPECIFIC 3D HUMAN NEUROMUSCULAR ORGANOIDS IDENTIFIES A NOVEL THERAPEUTIC CANDIDATE FOR AMYOTROPHIC LATERAL SCLEROSIS

Fu, Yuting, *Westlake University, China*
Wen, Shan, *Westlake University, China*
Gao, Chong, *Hangzhou City University, China*
Li, Zizhang, *Guangzhou National Laboratory, China*
Tian, Luyi, *Guangzhou National Laboratory, China*
Liu, Xiaodong, *Guangzhou National Laboratory, China*

Amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disease driven by genetic and microenvironmental complexity, lacks models that capture its multicellular pathology. Here, we developed a high-throughput platform to generate patient-specific neuromuscular organoids (NMOs) from induced pluripotent stem cells (iPSCs), preserving genetic diversity and neuromuscular tissue architecture. Single-cell analysis delineated developmental trajectories and identified ALS-specific disruptions in neurogenic, gliogenic, and myogenic networks. An optimized differentiation protocol accelerated organoid maturation while achieving high batch consistency. Using this platform, we discovered a therapeutic candidate, which restored neuromuscular junction integrity, reduced motor neuron degeneration, and mitigated ALS pathologies including apoptosis, proteostatic stress, and autophagic dysfunction. This scalable NMO system enables genotype-stratified drug screening and mechanistic dissection of neuromuscular interactions, offering a clinically translatable pipeline for ALS therapeutic development. Our work establishes patient-derived organoids as a transformative tool for accelerating targeted therapies in neurodegenerative disease.

COMBINING COMPUTATIONAL APPROACHES AND DISEASE-SPECIFIC IPSC MODELS TO ACHIEVE DRUG REPURPOSING FOR CHILDHOOD DEMENTIA

Oikari, Lotta, *Brain and Mental Health, QIMR Berghofer, Australia*
Gerring, Zachary, *Walter and Eliza Hall Institute of Medical Research, Australia*
Fernando, Amali, *QIMR Berghofer, Australia*
Chaves, Juliana, *QIMR Berghofer, Australia*
White, Anthony, *QIMR Berghofer, Australia*

Batten disease, also known as neuronal ceroid lipofuscinoses, is one of the most common causes of childhood dementia. It is a lysosomal storage disorder characterized by the accumulation of lipofuscin in lysosomes, which leads to loss of brain cell function. The onset of symptoms of Batten disease range from infantile to adolescence and include loss of vision and motor functions as well as seizures and dementia-like symptoms. There is no cure for Batten disease and treatment options are extremely limited with this disease often leading to loss of life before the age of 18. To tackle the lack of treatments for Batten disease, we investigated the ability to combine bioinformatic drug repurposing analysis with Batten disease-specific induced pluripotent stem cell (iPSC)-derived models to discover potential new therapeutic compounds. Brain cells (neurons and astrocytes) derived from iPSCs obtained from an individual with CLN3 type Batten disease were first characterized for a disease phenotype and were found to demonstrate abnormal inflammatory and lysosomal marker expression compared to isogenic control (CLN3 corrected) cells, supporting their use as a disease model. Drug repurposing analysis was then performed to identify candidate drugs for Batten disease. First, a comprehensive molecular network was constructed to identify highly interconnected genes in Batten disease. Then, the molecular network was used to identify existing compounds that target risk genes in Batten disease with the aim to normalize dysfunctional network activity. The analysis identified approximately 80 candidate compounds, of which 4 were selected for downstream testing in Batten disease iPSC-derived brain cells. Following drug treatment, effects on cell viability, inflammatory responses and lysosomal function were investigated. The tested compounds had minimal effects on cell viability, however, were able to decrease inflammatory cytokine secretion and increase lysosomal enzyme expression in Batten disease cells, suggesting therapeutic effects on disease phenotype. These results demonstrate that computational drug repurposing analysis along with disease-specific iPSC models is a potentially powerful approach to identify new therapeutics for rare but devastating disorders like childhood dementia.

Funding Source: Batten Disease Support and Research Association Australia.

MODELLING RESPIRATORY FIBROSIS USING INTEGRATED, MULTICELLULAR HIPSC-ALVEOLAR ORGANOIDS

Reed, Liam Allen, *University of Nottingham, UK*
Zuniga, Carlos Sainz *University of Nottingham, UK*
Azis, Rizal, *University of Nottingham, UK*
Serna-Valverde, Ana Lilia, *University of Nottingham, UK*
Cuevas-Ocaña, Sara, *University of Nottingham, UK*
Merry, Cathy, *University of Nottingham, UK*
Hannan, Nick, *University of Nottingham, UK*

Idiopathic pulmonary fibrosis (IPF) is a chronic disease resulting in irreversible scarring and thickening of normal lung tissue. IPF is driven by repeated damage to genetically susceptible alveolar epithelium, in particular type 2 alveolar epithelial cells (AT2s), and the complex interplay between tissue resident fibroblast and immune cell populations. To investigate the interactions that underly human IPF progression, we established hiPSCs from an IPF patient harbouring a mutation in pro-surfactant protein C (Y113C/WT) that causes protein misfolding and generated mutant Pro-SFTPC (Y113C/Y113C) and corrected Pro-SFTPC (WT/WT) hiPSC lines. We developed a universal, Xeno-free platform to differentiate hiPSCs to multiple

functional cell types including type 2 alveolar epithelial cells (AT2s), macrophages, endothelial cells, dendritic cells and fibroblasts and generated integrated, immune-competent alveolar organoids. We characterised corrected (WT/WT) and mutant (Y113C/Y113C) Pro-SFTPC multicellular lung organoids using single cell RNA-sequencing. After 16 days in culture, organoids contained all integrated cell types and several additional populations including distinct AT2 clusters and transitioning AT2-AT1 cells. Interestingly, we showed expansion of a neuroendocrine-like cell population and identified a transitioning KRT5-/KRT17+ aberrant basaloid-like cell cluster in Pro-SFTPC (Y113C/Y113C) organoids that were enriched for targets of NOTCH signalling that we did not observe in Pro-SFTPC (WT/WT) organoids. These transitioning KRT5-/KRT17+-like cells have recently been described in IPF patients. We identified significant interaction between KRT5-/KRT17+ like-cells and a late differentiating alveolar fibroblast-like cluster that had adopted lung tissue-specific markers. Further to this, immune cell populations in Pro-SFTPC (Y113C/Y113C) organoids were enriched for pro-inflammatory genes and we observed global expression of IL-4 and IL-13. Together, our multi-tissue organoid platform more faithfully resembles the alveolar niche in vivo and can recapitulate several clinical features of respiratory fibrosis, providing a robust model for improved understanding of IPF disease and for drug discovery.

Funding Source: NC3Rs.

STRATEGIC IPSC AND HESC APPROACH TO GENERATE DIFFERENTIATED PITUITARY CELLS FOR DISEASE MODELING AND DRUG SCREENING FOR NEW THERAPY

de Carvalho, Luciani Renata Silveira, *Endocrinology, University of São Paulo, Brazil*
Fattahi, Faranak, *University of California, San Francisco, USA*
Marques, Juliana Moreira, *University of São Paulo, Brazil*

Combined pituitary hormone deficiency (CPHD) is characterized by impaired production of multiple pituitary hormones, primarily due to mutations in the PROP1 gene. This deficiency leads to significant developmental and metabolic challenges owing to hormone insufficiency. Traditional methods to study CPHD are hindered by the inaccessibility of human pituitary tissue and the complex nature of pituitary development. The utilization of induced pluripotent stem cells (iPSCs) represents a pivotal advancement in disease modeling, enabling the in vitro generation of patient-specific pituitary cells. Our study focused on differentiating pituitary cells from individuals with and without PROP1 mutations to model CPHD and explore potential treatments. We successfully generated functional pituitary cells from iPSCs and human embryonic stem cells, accurately recapitulating the patient phenotype. This model highlighted the inability of CPHD cells to develop into hormone-producing cells, in contrast to the control group. Additionally, a high-throughput screening of drugs identified thirty compounds with potential therapeutic applications for pituitary disorders. These discoveries enhance our molecular understanding of CPHD and lay the groundwork for future therapeutic innovations

Funding Source: Sao Paulo Research Foundation Grants numbers: 2020/03299-0 (JMM), 2020/06792-0 (LRSC); PRONAS (National Program to Support Health Care for Persons with Disabilities) 25000.027842/2021-72 (LRSC).

ADVANCED HUMAN ORGANOID MODELS FOR DISSECTING TUMOR BIOLOGY
Artegiani, Benedetta and Hendriks, Delilah, *Princess Maxima Center, Netherlands*
Organoids have revealed to be invaluable tools for modelling and study organ physiology and their associated diseases. In this talk, we will present the latest advances from our group in generating human organoids from human brain and liver that better resemble specific in vivo features and capture multifaceted cellular interactions. We will further discuss how we are exploiting these novel models as a platform to unravel the complexity of specific human tumors and to discover mechanisms underlying cancer initiation. These advanced tumor initiation models are advancing our holistic understanding of cancer biology, and can be used to identify cancer vulnerabilities with therapeutic potential.

PLENARY II: DEVELOPMENT ACROSS SCALES: FROM FERTILIZATION TO TISSUES TO ANIMALS
10:30 AM – 12:00 PM
GRAND HALL, LEVEL 3

EX UTERO EMBRYOGENESIS OF NON-HUMAN PRIMATE EMBRYOS AND BEYOND
Tan, Tao, *Kunming University of Science and Technology, China*

Developmental failures and malformations that occur during gastrulation and early organogenesis can lead to early pregnancy loss and birth defects. Thus, investigating the molecular and cellular mechanisms involved during this critical "black box" period is essential for advancing our understanding of human embryogenesis and the underlying causes of early developmental disorders. However, the cellular and molecular dynamics that govern the transition from gastrulation to early organogenesis in primates remain largely unexplored due to limited access to research embryos. As humans' closest relatives, non-human primates serve as an excellent surrogate model for studying early human embryogenesis. Recent studies have examined significant molecular and cellular processes in cynomolgus monkeys. Despite these exciting advancements, the cellular and molecular dynamics involved in this transition remain elusive, underscoring the necessity for accessible models suitable for experimental manipulation. The establishment of in vitro culture systems for embryos provides a valuable opportunity to study development in a controlled setting. Additionally, modelling early human embryo development using pluripotent stem cells offers insights into developing stem cell systems for embryogenesis research.

IDENTIFYING AND UNDERSTANDING REGENERATION SUPRESSORS
Wang, Bo, *Stanford University, USA*

Although some animals can regenerate extensive body parts, the underlying molecular programs remain only partly understood, hindering our ability to induce regeneration in animals with limited tissue repair capabilities, including humans. While the focus in the field has long been on genes that promote regeneration, our work in highly regenerative planarian flatworms reveals an opposing regulatory program: regeneration suppressors. These genes must be downregulated for regeneration to begin – analogous to how tumor suppressors need to be

silenced for tumorigenesis – and they encode secreted proteins and extracellular matrix components broadly expressed in many differentiated cell types. We propose that their combined activities create a diffuse tissue microenvironment to restrict regeneration. This distributed repression can confer robustness: for the environment to become permissive, wound signal must propagate systemically and reduce suppressor levels throughout the body and across different cell types. In this talk, I will discuss our approach to identify these suppressors, the function of these genes in regulating regeneration, and the signaling circuitry that modulates their expression during the regeneration process. I will also address how variations in the regulation of regeneration suppressors may lead to the differences in regeneration competence across animals.

FROM LABORATORY TO SOCIETY: PUBLIC PERCEPTIONS ON HUMAN IN VITRO GAMETOGENESIS

Fujita, Misao, *Uehiro Research Division for iPS Cell Ethics, Center for iPS Cell Research and Application, Kyoto University, Japan*

In vitro gametogenesis (IVG) using human induced pluripotent stem cells (hiPSCs) is advancing rapidly, providing insights into human development, the causes of infertility, treatments for genetic disorders, and improvements in assisted reproductive technologies. However, as IVG research progresses, ethical, legal, and social challenges arise at various stages, from the creation of gametes to clinical applications. To explore public perceptions of this emerging technology, we conducted a survey with over 3,000 participants in Japan. The results showed that 78.6% of respondents accepted the creation and use of IVG-derived gametes for research, 51.7% accepted the creation of embryos with these gametes, and 25.9% accepted childbirth using such embryos. Those who have undergone infertility treatment showed a higher level of acceptance toward clinical applications of IVG compared to those who have not, and they were more likely to consider using IVG in their own treatment. A comparison of public perceptions in Japan with international perspectives revealed cultural differences in attitudes toward IVG use by same-sex couples and postmenopausal women. This presentation will discuss key ethical and social issues surrounding IVG research and provide new, unpublished data. Understanding how emerging research is perceived within society is essential to ensuring that such research develops in a trustworthy and socially responsible manner.

DECIPHERING THE MECHANISMS OF PRIMATE EARLY EMBRYOGENESIS AND PLACENTATION

Wang, Hongmei, *Institute of Zoology, Chinese Academy of Sciences, China*

Placental development and function are crucial for maintaining a healthy pregnancy. Abnormal placental development and function may lead to pregnancy complications and diseases. Our lab has focused on elucidating the mechanisms underlying placental development and function across multiple mammalian species (including human, macaque, and mouse) throughout gestation. These include mapping the cellular and molecular atlas of placentas by single-cell multi-omics studies, revealing the origin and differentiation trajectory of different placental cells, dissecting the mechanisms underlying trophoblast cell-cell fusion, epithelial-mesenchymal transition, the interaction between trophoblasts and the uterine endometrium, and hematopoiesis within the placental villous core. Based on the understanding of placenta, our

lab has also sought to develop various culture systems to support ex-utero embryogenesis and reveal the features of early mammalian embryonic development.

EVOLUTIONARY PERSPECTIVE OF DEVELOPMENT AND REGENERATION
2:00 PM – 3:30 PM
HALL C, LEVEL 1

KIN RECOGNITION AND CHIMERIC STEM CELL COMPETITION ENHANCE SURVIVAL IN A COLONIAL CHORDATE
Voskoboynik, Ayelete, *Biology, Stanford University, USA*

Chimerism, the presence of genetically distinct cells within an organism, is a widespread phenomenon in nature. In chimeric Botryllus schlosseri, stem cells from different individuals compete for dominance, potentially leading to the emergence of a single dominant genotype that takes over the gonads. To better understand the potential advantages of chimerism we investigated the likelihood of natural chimera formation in Botryllus colonies and assessed the impact of chimerism throughout a colony's lifespan. The colonial chordate Botryllus schlosseri consists of genetically identical zooids that reproduce both sexually and asexually. The sexually produced larvae swim, settle, metamorphose, and then bud asexually to form new zooids. Colonies can fuse with compatible neighbors, forming chimeras. We tracked larval settlement behavior and assessed the impact of chimerism on colony survival over a period of five and a half years. Larvae preferentially settled near kin, facilitating chimeric formation between 2-8 individuals. Early chimerism significantly enhanced survival during the initial 30-day die-off period and extended overall colony lifespan. Genomic analysis revealed that single genotype dominance emerged within 3 months post-fusion, encompassing both germline and somatic tissues, regardless of the initial number of contributing genotypes. These findings demonstrate a complex interplay between cooperation and competition within chimeric organisms. While chimerism provides significant survival benefits, it ultimately leads to a competition over continuing ones genetic line among individuals in the chimeras. However, in marine environments characterized by rapid environmental change, chimerism may offer an adaptive advantage by enabling the selection and expansion of stem cells best suited to prevailing conditions.

DIAPAUSING CAPACITY OF MOUSE AND HUMAN PLURIPOTENT CELLS IN PHYSIOLOGICAL CONDITIONS
Fan, Rui, *Max-Planck-Institute, Germany*

Embryonic diapause is a temporary arrest of development at the blastocyst stage, representing a fascinating reproductive strategy that enhances offspring survival in extreme environments. Embryonic diapause has been observed in over 130 mammalian species, including mice, while primates, including humans, are considered non-diapausing species. In this study, we asked whether the mouse and human pluripotent cells capture the species-specific properties for diapause under physiological conditions. Due to ethical and technical limitations in human embryo research, investigating whether human embryos can be “paused” in utero is not

feasible. Therefore, we incorporated naive human induced pluripotent stem cells (hiPSCs) or naive mouse pluripotent stem cells (mPSCs) into host mouse embryos to compare the diapausing capacity of these PSCs in the murine uterine environment. We found that both naive mPSCs and naive hiPSCs were efficiently incorporated into mouse blastocysts. However, in contrast to hiPSCs, only mPSCs showed an inherent capacity for embryo dormancy, capturing the diapausing differences between the species of origin. Next, we sought to decipher the molecular basis of the interspecies differences that govern the diapause competence of the PSCs. Molecular analysis showed that during diapause, the embryo experiences metabolic stress that is tolerated by mPSCs but not by hiPSCs. We therefore engineered hiPSCs to overcome the stress-induced barrier and integrated these cells into mouse embryos. Strikingly, we found that these hiPSCs can enter diapause in the context of mouse embryos. Furthermore, single-cell RNA-seq analysis revealed a number of common as well as species-specific transcriptional signatures in mouse and human PSC in response to the diapausing uterine conditions. Finally, our analysis also showed that the engineered hiPSC maintain their developmental potential during diapause and can subsequently be reactivated to a proliferative state, thus completing the cycle of entering, maintaining and exiting embryonic dormancy.

LINE-1 RETROTRANSPOSONS REGULATE THE EXIT OF HUMAN PLURIPOTENCY AND EARLY BRAIN DEVELOPMENT

Adami, Anita, *Lund Stem Cell Center, Lund University, Sweden*
Garza, Raquel, *Lund Stem Cell Center, Lund University, Sweden*
Gerdes, Patricia, *Lund Stem Cell Center, Lund University, Sweden*
Johansson, Pia, *Lund Stem Cell Center, Lund University, Sweden*
Sharma, Yogita, *Lund Stem Cell Center, Lund University, Sweden*
Douse, Christopher, *Lund Stem Cell Center, Lund University, Sweden*
Jakobsson, Johan, *Lund Stem Cell Center, Lund University, Sweden*

Retrotransposons are repetitive sequences that have colonised the human genome throughout evolution and now comprise around 50% of our DNA. Long-interspersed nuclear elements 1 (L1s) are the most abundant family of retrotransposons, occupying around 20% of the genome. If their promoter remains intact, L1s can drive their own transcription, have regulatory effects on nearby gene expression, and produce non-coding RNAs that may be involved in complex gene regulatory networks. Recently, L1s have been deemed essential for pluripotency maintenance, self-renewal, and embryo development in mouse, but their involvement is still contradictory, and their role in human early development remains largely unexplored. Using a multiomic approach that combines long- and short-read RNAseq, DNA methylation analysis, CUT&RUN epigenomic profiling and tailored bioinformatics, we found that thousands of L1s are expressed in human induced pluripotent stem cells (hiPSCs) and cerebral organoids. We show that L1 expression correlates with presence of the active promoter marker H3K4me3 and absence of DNA methylation by the L1 promoters, and describe the dynamic transcriptional and epigenetic status of L1s throughout differentiation. We then optimised and thoroughly characterised a CRISPR inhibition (CRISPRi) system in hiPSCs to silence the transcription of hundreds of evolutionarily young L1s that retain their promoter. Our results show that L1s have a cis-regulatory effect by acting as alternative promoters for nearly 100 protein-coding genes. Interestingly, L1 silencing does not affect pluripotency in hiPSCs or the ability of the cells to differentiate into hiPSC-derived cerebral organoids. However, L1s inhibition does affect the rate of differentiation of the cells: L1-CRISPRi cerebral organoids are consistently smaller by day 15 of differentiation compared to controls, and their transcriptional profile suggests an earlier

differentiation. In summary, our results provide an in-detail profiling of L1 transcriptional and epigenetic state in development-relevant human models and offer novel insights into the cis-regulatory role that L1s play in the fine-tuning of cell differentiation timing.

GENOME-WIDE SCREENING IN HUMAN EMBRYONIC STEM CELLS REVEALING DISTINCT CHARACTERISTICS OF LONG NON-CODING RNA GENES AND NOVEL REGULATORS OF THE PLURIPOTENT NETWORK

Sherman, Assa, *Genetics, The Hebrew University of Jerusalem, Israel*
Benvenisty, Nissim, *Hebrew University of Jerusalem, Israel*

The human genome encodes thousands of long non-coding RNAs (lncRNAs), transcripts of over 200 nucleotides in length that lack the potential for translation into functional proteins. LncRNAs have emerged as important players for diverse cellular processes, particular in tissue- and cell-type-specific contexts. In human pluripotent stem cells, lncRNAs play key roles in maintaining pluripotency, self-renewal, and cell fate determination, yet the functions of most lncRNAs remain poorly understood. Here, we performed a genome-wide CRISPR interference screen in haploid human embryonic stem cells (hESCs) and identified over 100 lncRNAs as essential and about 150 to be growth-restricting. These growth-modifying genes were found to be relatively specific to the pluripotent state, highly expressed in hESCs and more evolutionary conserved compared to lncRNAs who have not shown biological functionality. Notably, we discovered that essential lncRNAs are depleted from chromosome X whereas growth-restricting genes are enriched, suggesting a potential involvement in a dosage-sensitive regulation mechanisms. Furthermore, we observed a reduced expression of growth-restricting lncRNAs during teratoma formation, implicating their possible contribution to cancerous processes. Our investigation also identified two novel, primate-conserved essential lncRNAs that regulate their genomic neighboring pluripotent transcription factor (TF). Interestingly, mechanistic studies unveiled their distinct regulatory roles. One lncRNA, acts in cis as a positive regulator of its proximate TF, with knockdown triggering p53-mediated apoptosis. In contrast, the other functions as a negative regulator of its adjacent TF, with knockdown leading toward a mesendoderm fate, and overexpression driving conversion into a more formative state. Overall, our findings shed light on the roles of lncRNAs in the human-specific pluripotency network and provides new insights into the regulation of hESC growth and differentiation.

WNT/BETA-CATENIN SIGNALING AS A TEMPORAL REGULATOR OF PROGENITOR CELL FATE DECISIONS DURING HEMATOPOIESIS

Goins, Lauren, *Developmental Biology, Stanford University, USA*

Wnt/Beta-catenin signaling is a universal regulator of stem cell behavior, orchestrating self-renewal, mitosis, and differentiation across species. However, the mechanisms by which it achieves these functions remain unclear. Using Drosophila hematopoietic progenitors as a model, we have uncovered that Wnt/Beta-catenin controls stemness by regulating cell cycle progression and differentiation. Specifically, we recently demonstrated that active Beta-catenin is required for the G2-to-mitosis transition, while overactivation of Beta-catenin or Wnt6 signaling leads to G1 arrest and a failure to differentiate, thereby maintaining progenitors in an undifferentiated state. Building on this work, we reveal that early in development, Wnt/Beta-catenin signaling, mediated by the co-activator TCF/Lef, is essential for expanding the progenitor population. Loss of Wnt/Beta-catenin activity during early stages reduces

hematopoietic tissue size and depletes progenitors, underscoring its critical role in progenitor proliferation. Later in development, Wnt/Beta-catenin signaling functions to block differentiation, as overactivation of Beta-catenin (e.g., ArmS10 or Axin-RNAi) reduces markers of mature macrophage blood cells (e.g., Hml-DsRed and NimC1) and crystal cells (e.g. Hnt) while maintaining progenitors with low reactive oxygen species (ROS) levels. Loss of TCF also results in premature differentiation, though with less severity than dominant-negative TCF expression, suggesting temporally distinct roles for Wnt/Beta-catenin signaling. Our findings highlight a dual role for Wnt/Beta-catenin in the early expansion of progenitors and later inhibition of differentiation, demonstrating the importance of precise temporal control. These findings expand our understanding of Wnt signaling beyond its conventional association with G1 regulation, providing critical insights into stem cell-niche interactions and the integration of growth factor signaling with cell cycle progression. These results have broad implications for regenerative medicine, offering potential strategies to manipulate stem cell fate decisions for therapeutic applications.

RE-EMERGENCE OF STEM CELLS IN A MODEL FOR REGENERATIVE PLASTICITY
Raible, Florian, *Department for Neurosciences and Developmental Biology, University of Vienna, Austria*

Marine bristleworms exhibit excellent regeneration, including regeneration of the central nervous system. It has been known for more than 60 years that this regenerative capacity can be modulated by brain-derived factors. This makes bristleworms an well-suited model system to study regenerative plasticity and the modulation of stem cell systems involved in this process. In order to dissect the molecular principles underlying this modulation, we have characterized the cellular and molecular processes underlying successful regeneration in Platynereis dumerilii, a laboratory model system with a growing molecular and functional toolkit. We capitalized on a posterior regeneration paradigm in which the animals are challenged to re-establish a stem cell zone for posterior growth. By combining a time-resolved single-cell RNA sequencing approach with high-resolution imaging of markers in the emerging regenerate, we reveal that multipotent stem cell signatures emerge in differentiated cells, at the expense of differentiation markers. We further identified molecular markers for distinct stem cells produced in this process. Molecular comparisons draw parallels between the bristleworm system and dedifferentiation in vertebrate regeneration models. Our detailed characterization of this process now allows us to explore how this process is orchestrated by hormonal cues.

IN VIVO AND IN VITRO ORGAN GENERATION
2:00 PM – 3:30 PM
THEATER 2, LEVEL 1

HOST MACROPHAGE XENOPHAGOCYTOSIS CONSTITUTES A MAJOR BARRIER TO INTERSPECIES CHIMERISM
Nakauchi, Hiromitsu, *Genetics and Institute of Stem Cell Therapy and Regenerative Medicine, Stanford University, USA and Institute of Science, Japan*

The generation of interspecies chimeras using pluripotent stem cells (PSCs) is a promising approach for organ generation, yet xenogeneic donor cell contribution remains low due to

poorly understood barriers. While developmental incompatibilities have been proposed as a major obstacle, we identify an additional innate immune barrier-xenogeneic phagocytosis (xenophagocytosis)-mediated by host macrophages. Using a rat-to-mouse chimera model, we observed a sharp decline in donor chimerism between embryonic days 9.5 and 11.5, coinciding with the emergence of mouse primitive macrophages. Genetic depletion of host macrophages significantly increased rat donor chimerism up to 77% in the lung and 57% in the heart. Mechanistically, we show that host macrophages selectively phagocytose xenogeneic cells displaying elevated phosphatidylserine, an “eat-me” signal, through the phagocytic receptor Axl. Disruption of Axl in host embryos or overexpression of the “don’t eat-me” signal CD47 on donor cells markedly enhanced donor cell contribution. Importantly, blockade of xenophagocytosis also improved chimerism in human-to-mouse models, highlighting the broad applicability of this strategy. Our findings provide new mechanistic insights into xenogeneic barriers and suggest practical approaches to improve interspecies chimerism for organ generation.

Funding Source: Leducq Foundation, Dr. Ralph & Marian Falk Medical Research Trust.

IPSC-DERIVED THYMIC EPITHELIAL CELLS (ITECS) PROMOTE KEY FUNCTIONS OF THE HUMAN THYMUS IN A HUMANIZED ATHYMIC MOUSE MODEL

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The thymus instructs T cell immunity and central tolerance, yet the signals that drive thymic epithelial cell differentiation remain incompletely understood. Thus far, no clinically relevant strategies to regenerate thymic function exist, and its therapeutic potential has not yet been tapped into. To elucidate pathways instructing commitment and specialization of the human thymic epithelial stroma, we used complementary single-cell transcriptomic approaches. First, we identified gene regulatory networks that define fetal thymic epithelium in the context of other anterior foregut-derived organs; then, we characterized lineage trajectories within the thymic epithelial compartment across embryonic, fetal, and early postnatal stages. We have translated these findings into a morphogen-based approach to advance the differentiation of iPSCs into thymic epithelial cells (iTECs). iTECs derived from this protocol demonstrated high transcriptional fidelity to human fetal TECs and a diverse MHC I and MHC II-associated immunopeptidome. To test their functional characteristics, we transplanted iTECs into athymic NSG-nude mice (NSG-Foxn1Null) that were engrafted with human hematopoietic stem cells. Sixteen weeks after transplantation, thymocytes isolated from dissociated iTEC organoids showed physiologic TdT expression and maturation from double-negative to double-positive to single-positive stages. Flow cytometric analysis of the peripheral blood showed fully TCRab-

rearranged naïve and memory T cells with expected CD4 to CD8 ratio, as well as gd-T cells and FOXP3+ regulatory T cells. Experimental iTEC-transplanted animals developed a highly diverse human TCR repertoire, indistinguishable from control mice transplanted with primary fetal thymus. T cells showed strong responses to mitogens and proliferated in response to non-self MHC but not to self-MHC, suggesting iTECs can induce tolerance. These results provide proof of concept that transplantable human iPSC-derived TECs can perform key functions of the human thymus and have potential as a regenerative thymic cell therapy.

A SELF-ORGANIZED, MULTI-ORGAN BUD MODEL DERIVED FROM PLURIPOTENT STEM CELLS RECAPITULATES HUMAN HEPATO-BILIARY ORGANOGENESIS

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The biliary tree plays a key role in digestion by modifying and carrying bile from the liver to the intestine. During human development, the epithelial cells that line the biliary tree, known as cholangiocytes, are derived from different embryonic regions. While the extrahepatic cholangiocytes originate from the foregut endoderm, the intrahepatic ones differentiate from hepatoblasts in the liver bud. The impact of this divergent origin on adult tissue is unknown. Furthermore, the molecular mechanisms of cell fate choice during hepato-biliary organogenesis remain to be uncovered, especially in human, due to limited access to primary tissues and the lack of developmental models. To further understand the differences in the specification of extra and intrahepatic cholangiocytes, we established a self-organizing multi-organ bud model that mimics the hepato-biliary formation. First, we developed a new protocol using human pluripotent stem cells to generate a foregut system containing hepato-biliary progenitors and then established 3D culture conditions to grow the hepato-biliary bud in vitro. The resulting cells could give rise to self-renewing extrahepatic cholangiocyte and hepatoblast organoids (iECOs and iHBOs). Additionally, the iHBOs could be differentiated into self-renewing intrahepatic cholangiocyte organoids (iICOs). The generated iECOs and iICOs express cholangiocyte markers and show functional characteristics, resembling their primary counterparts. Moreover, exposure to Wnt signaling activates extrahepatic-specific markers in iECOs, but not in iICOs, uncovering key mechanisms that separate extra from intrahepatic biliary development. Thus, our platform not only recapitulates the organogenesis of the biliary tree but also reveals new mechanisms controlling its development. Finally, we show that iICOs and iECOs acquire epithelial plasticity when exposed to hepatic differentiation by transdifferentiating into hepatocyte-like cells, mimicking regenerative mechanisms occurring during chronic liver injury and suggesting that adult tissue repair could be related to developmental processes. Together, our results provide a new platform to dissect the mechanisms orchestrating hepato-biliary

lineage specification and epithelial cellular plasticity in health and disease.

GENERATION OF MULTI-LINEAGE KIDNEY ASSEMBLOIDS WITH INTEGRATION BETWEEN NEPHRONS AND A SINGLE EXITING COLLECTING DUCT

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A functional kidney requires a patent connection between all nephrons and the collecting duct network to ensure an exit path for the urinary filtrate. While it is now possible to separately direct the differentiation of pluripotent stem cells to nephron-forming or nephric duct-forming populations, the controlled integration of these two systems has not been demonstrated in a human context. We report the formation of an integrated kidney assembloid via the co-culture of distinct nephric duct progenitor and nephron-forming cultures in the absence of any requirement for cell enrichment. All nephrons within these kidney assembloids connect to a single exiting nephric duct with this connection forming with the early distal nephron prior to nephron segmentation. Using constitutional reporter lines, we show that the nephrons arise from the nephric progenitors and fuse with the nephric duct while the nephric duct progenitors form a single common duct without any branching. We further identify the stromal component of the nephric duct progenitor population contributing to the medullary stroma surrounding the nephrons. The cell aggregation and suspension culture method also provides a platform for large scale tissue generation. Such modular assembloids address the challenge of nephron unification in an engineered kidney tissue model, providing an advanced in vitro tissue system for developmental investigation, disease modelling, drug discovery and regenerative medicine studies.

Funding Source: MHL and SBW are funded by the Novo Nordisk Foundation Center for Stem Cell Medicine (NNF21CC0073729).

ESTABLISHING INTER-SPECIES CHIMERA WITH EMBRYONIC ORGANOID ENGRAFTMENT

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Human-mouse interspecies chimeras, which serve as humanized models for studying human cell development in vivo, are challenging to generate due to limited engraftment efficiency. Intravital embryonic imaging revealed that transplanted human stem or progenitor cells are outcompeted by endogenous murine cells due to a mismatched stem cell niche, leading to their disappearance postnatally. To address this, we explored a strategy of providing transplanted human cells with a predefined 3D stem cell niche to enhance chimeric engraftment. Here, we demonstrate the feasibility of generating human-mouse chimeras through the embryonic engraftment of human induced pluripotent stem cell (hiPSC)-derived organoids. Humanized brain, intestine, and liver tissues are successfully generated in vivo, detectable in adult mice. These chimeras exhibited tissue-specific tropism, with human cells detected only in organs corresponding to the transplanted organoid type. Notably, successful engraftment was observed with hiPSC-derived organoids but not with dissociated single cells and was less efficient with tissue-derived organoids, which lack the mesenchyme. These findings highlight the importance of an initial 3D human stem cell niche in facilitating interspecies embryonic engraftment. The result chimera models provide useful tools for study human tissue development, homeostasis, and disease conditions in an in vivo setting.

XENOGENEIC REGENERATION OF HUMANIZED MESONEPHROS/HEART IN PIGS
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The shortage of immunohistocompatible donors for organ transplantation is a universal problem with no direct solution. A promising alternative is to generate viable interspecies chimeras involving large mammals and human tissues through embryo complementation with pluripotent stem cells (PSCs). Pigs are a highly attractive target for growing xenogeneic human organs given their similarities with humans in body physiology and size, as well as in embryonic development. However, obtaining high degree of chimerism with species evolutionarily more distant than rodents is challenging. Although human endothelium and patches of skeletal muscle tissue have been generated inside pigs, no solid organs have been produced to date due to the overall poor contribution of human PSC-derived cells to chimeric porcine tissues. Here, to produce human cells with superior intra-niche competitiveness, we combined optimized naïve pluripotent stem cell culture conditions with the inducible over-expression of two pro-survival genes (MYCN and BCL2). The resulting cells had substantially enhanced viability in the xeno-environment of the interspecies chimeric blastocyst and successfully formed well-organized human heart and middle stage kidney until embryonic day 19 and 28 inside pig fetuses, respectively. Our findings demonstrate proof-of-principle of the possibility of generating human solid organs in organogenesis-disabled pigs, opening an exciting avenue for regenerative medicine and a unique artificial window for studying human development.

INSIGHTS INTO DISEASE MECHANISMS FROM STEM CELL RESEARCH
2:00 PM – 3:30 PM
HALL 3F, LEVEL 3

REGENERATION OF THE LIVER USING NUCLEOSIDE-MODIFIED MRNA ENCAPSULATED IN LIPID NANOPARTICLES (MRNA-LNP)

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The remarkable ability of the liver to regenerate by proliferation of hepatocytes constitutes the main repair mechanism named the hepatocyte-driven regeneration. Yet, during chronic liver injury or acute severe hepatocyte death, proliferation of mature cells becomes exhausted. In these cases, precursors of hepatocytes derived from cholangiocytes have been reported to generate healthy hepatocytes in a process defined as the cholangiocyte-driven regeneration. Despite these two mechanisms of liver repair, end stage liver disease remains the 12th most common cause of death in the United States, begging for therapeutic strategies to harness intrinsic mechanisms of liver repair. Alternatively, cell therapy using primary human hepatocytes or pluripotent stem cell-derived hepatocytes, if cell engraftment is harnessed, could also become a viable treatment for human liver diseases. We recently pioneered mRNA-LNP as a technology to deliver regenerative factors to the liver for liver regeneration application. We showed specific hepatotropism of mRNA-LNP via intravenous injection of luciferase encoding mRNA-LNP, with protein expression lasting about 3 days. In the liver, virtually all hepatocytes are transfected along with a subpopulation of endothelial and Kupffer cells. We demonstrate that delivery via mRNA-LNP to the liver of the key hepatocyte mitogen hepatocyte growth factor (HGF) and epidermal growth factor (EGF) enhances hepatocyte-driven repair by sharply reversing steatosis and accelerating restoration of liver function in a chronic liver injury model, while accelerating liver regeneration in an acute injury model. The combination of HGF and EGF mRNA-LNP injections also dramatically promotes primary human hepatocyte engraftment in a chronic model. To harness cholangiocyte-driven repair, we delivered vascular endothelial growth factor A (VEGFA) in acute and chronic injured mouse livers. We found that VEGFA mRNA-LNP induces robust cholangiocyte conversion to hepatocytes, and importantly reverts steatosis and fibrosis. Our study introduces mRNA-LNP as a potentially translatable safe therapeutic intervention to harness both hepatocyte- and cholangiocyte-driven liver regeneration as well as primary human hepatocyte cell therapy to alleviate human acute and chronic liver diseases.

CRISPR/Cas9-MEDIATED EXCISION OF EXPANDED “CTG” REPEATS AND CORRECTION OF SPLICEOPATHY IN MYOTONIC DYSTROPHY PATIENT-SPECIFIC IPSC-DERIVED MUSCLE AND CARDIAC CELLS IN VITRO AND DM1 MICE IN VIVO

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Myotonic dystrophy type 1 (DM1) is a genetic disorder, primarily affecting skeletal muscle and heart. It is caused by an CTG trinucleotide repeat expansion (CTGexp) in the 3’-untranslated region (UTR) of the dystrophia myotonica protein kinase (DMPK) gene, resulting in expanded CUGexp repeats in the corresponding DMPK transcripts. These CUGexp repeats cause

splicing abnormalities in genes that affect skeletal muscle and heart function. To reverse this splicing defect, we used CRISPR/Cas9-mediated gene editing based on a dual gRNAs that were designed to recognise a specific sequence upstream and downstream of the CTGexp repeats in the DMPK 3'-UTR. Efficient CTGexp was demonstrated in different "disease-in-a-dish" cellular platforms including DM1 patient-specific myoblasts, DM1 patient-specific induced pluripotent stem cells (DM1-iPSC) and DM1-iPSC-myogenic mesoangioblast-like cells This resulted in the disappearance of ribonuclear foci in the nuclei of corrected DM1 myoblasts, DM1-iPSC and their progeny upon myogenic and cardiomyogenic differentation. Consequently, the normal intracellular localization of the muscleblind-like splicing regulator 1 (MBNL1) was restored, resulting in the normalization of splicing pattern. Transcriptome-wide analysis of isogenic CRISPR-Cas9-corrected versus non-corrected DM1 iPSC-derived cardiomyocyte-like cells revealed a prominent difference in the splicing pattern for a number of candidate genes pertaining to genes that are associated with cardiac function and cellular signaling. Notably, normal splicing of MBNL1, MBNL2, INSR, ADD3, and CRTC2, was restored following CTGexp repeat excision. Subsequently, we administered the CRISPR/Cas9 components using AAV9 vectors in a newly developed inducible DM1 mouse model. This resulted in successful CTGexp excision with reversal of DM1-specific alternative splicing markers and decreased ribonuclear foci formation. In conclusion, this comprehensive in vitro and in vivo study supports the use of CRISPR/Cas9-mediated gene editing as a potential treatment for DM1.

Funding Source: VUB IOF-GEAR; Methusalem; SRP; FWO; EU Horizon 2020 No N°. 825825 (UPGRADE); Koning Boudewijn Stichting; VUB Duchenne Fund.

REGIMEN DEVELOPMENT ON DNAJA3 HAPLOINSUFFICIENCY MEDIATED SARCOPENIC OBESITY AND CANCER CACHEXIA WITH IMBALANCED MITOCHONDRIAL HOMEOSTASIS AND LIPID METABOLISM

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Muscle loss and impaired skeletal muscle regeneration are common ailments of aging and chronic disease including atrophy and sarcopenia. The molecular mechanisms of pathogenesis of age-related muscle wasting include mitochondria dysfunction, inflammation, intramuscular adipose tissue, and oxidative stress. Dnaja3, a mitochondrial co-chaperone, has been identified to regulate skeletal muscle physiology and act as a tumor suppressor on various cancer types. GMI, an immunomodulatory protein cloned from G. microsporum, is found to promote myogenesis. We generated transgenic mouse strains with Dnaja3 haploinsufficiency in skeletal muscle. We performed differential mitochondrial proteomes and analyzed mitochondrial function between young and old mice or mice with induced carcinogenesis. GMI acting as a Dnaja3 activator was tested to improve mitochondrial function both in vivo and in vitro using those transgenic mice strains. We observed impairment of physiological muscular function in young transgenic mice. The gene ontology analysis of the transgenic and wild-type mice indicated the association with mitochondria dysfunction, fatty acid metabolism, and oxidative stress response. Furthermore, GMI treatment could improve mitochondrial function ameliorate lipid accumulation in vivo, and accelerate myoblast regeneration in vitro. Overall, we characterize the mitochondria dysfunction associated molecular mechanisms, mediated by Dnaja3 haploinsufficiency, in the transition stage of obesity or cancer cachexia. Additionally,

GMI would be a potential therapeutic drug based on activating Dnaja3.

Funding Source: NSTC113-2811-B-A49A-015.

MODELING PARKINSON'S DISEASE INITIATION IN THE GUT: NOVEL INSIGHTS FROM IPSC-DERIVED ENTERIC NEURONAL LINEAGES

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More than 10 million people in the world suffer from Parkinson's disease (PD), a neurodegenerative disorder classically featuring motor manifestations due to the loss of dopaminergic neurons in the central nervous system. Yet, emerging evidence has demonstrated that gastrointestinal dysfunction precedes motor impairment by years, placing the enteric nervous system (ENS) at the center of disease initiation. While the PD hallmark protein alpha synuclein (α-syn) has been detected in the gut of PD patients, the mechanisms

underlying α-syn-driven toxicity in the human ENS remain poorly understood. The hypothesis of this study is that α-syn pathology in the ENS initiates PD progression, amplified by inflammatory signals. For the first time we established iPSC-derived enteric neuronal lineages (ENLs), consisting of enteric neurons and glial cells, from PD patients with a triplication in the SNCA gene locus (SNCA 3x) and their respective isogenic controls. Going beyond classical animal models, iPSC-ENLs offer a human-specific model to investigate enteric α-syn pathology and its interplay with inflammation. Using multiomics combined with functional assays, we uncovered disrupted neuronal differentiation, accelerated glial development, and impaired neuronal-glial communication in SNCA 3x ENLs. These cells were also more sensitive to TNF-α stimulation, which further enhanced α-syn-mitochondria interactions, induced a general hypometabolic state and promoted metabolic reprogramming towards glutamine oxidation. Additionally, TNF-α treatment strongly decreased neuronal activity in SNCA 3x ENLs, linking inflammation to impaired enteric neuronal function. Importantly, pharmacological intervention restored metabolic function of SNCA 3x ENLs, suggesting a potential therapy for early ENS dysfunction in PD. Overall, our study positions iPSC-ENLs as a robust platform for modeling ENS pathology in synucleinopathies. By revealing how intestinal inflammation drives PD progression via metabolic dysregulation, we highlight the ENS as a very promising therapeutic target. Our findings redefine the role of the ENS as merely a receptor of immune signals to an active initiator of PD, offering new insights into disease pathophysiology and drug screening for early PD.

Funding Source: This work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) - CRU5024 (A01).

APPLYING HUMAN ENDOMETRIAL ORGANOID AND IMPLANTATION MODELS TO DECIPHER UNDERLYING CAUSES OF ENDOMETRIOSIS-ASSOCIATED INFERTILITY

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One week after fertilization, the human embryo implants in the uterus, a process requiring highly coordinated interplay with the uterine lining (endometrium). To welcome and nest the nascent embryo (blastocyst), the endometrium must decidualize to reach a receptive state. Perturbed decidualization and receptivity are considered major causes of infertility. Here, we search for potential aberrations in these processes in a widespread burdening disease highly associated with infertility, i.e. endometriosis, to date only poorly understood. We apply endometrium biomimetics based on organoid models established from endometrial biopsies of healthy (fertile) and endometriosis (infertile) women, both recapitulating the original tissue characteristics. Bulk RNA-sequencing) and RT-qPCR analyses showed defective decidualization capacity of endometriosis' compared to healthy endometrium-derived organoids when exposed to hormones that regulate this process in vivo. In addition, we apply our in vitro

human embryo implantation model in which high-fidelity blastocyst models (blastoids) are combined with organoid-derived endometrium constructs, reliably capturing the first events of embryo-endometrium interaction. Blastoids successfully attach to the healthy endometrial layer, however only when these endometrial cells are hormonally primed (decidualized) to reach the receptive state. Interestingly, when blastoids are introduced to endometrial layers derived from endometriosis patients, their attachment is significantly reduced. We subsequently performed single-cell RNA-seq analysis comparing the healthy and endometriosis implantation model, which identified molecular factors potentially contributing to the decreased blastoid adhesion rate on endometriosis' endometrial layer. Further exploration and validation of these markers will offer insight into the disruptions occurring at the embryo-woman interface in infertility associated with endometriosis. Taken together, our stem cell-derived organoid(-based) models offer great value to decipher (patho)physiological processes of endometrial behavior and embryo-endometrium interactions.

ENGINEERED HEART TISSUE FOR DISEASE MODELING AND EFFICACY TESTING
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Human induced pluripotent stem cell (hiPSC)-cardiomyocytes (CM) are increasingly used for modeling cardiac diseases, but exhibit developmental immaturity, lack of cell diversity and limited functional readout. We have pioneered the 3D engineered heart tissue (EHT) technology to overcome some of these limitations. EHTs induce better organized sarcomere structure, high degree of mitochondrial, oxidative metabolism and mature electrophysiological characteristics of hiPSC-CM. We used hiPSC-EHT to model neonatal cardiomyopathy caused by homozygous or compound heterozygous truncating mutations in myosin binding protein C gene (MYBPC3). While force of contraction was only reduced under high afterload compared to isogenic controls, time of contraction was always shortened and terminal relaxation was relatively prolonged. This phenotype was used as an efficacy assay for the commercial development of a gene therapy of MYBPC3-related cardiomyopathy (Dinaqor/Biomarin). In a second project, we evaluated the hypothesis that multicellular hiPSC-derived EHT provide a better in vitro model of cardiac hypertrophy than standard EHT from CM only. To this end, we generated cardiac fibroblasts, smooth muscle cells, macrophages, endothelial cells in addition to CM from the same hiPSC line. Mixing them at a ratio of 5 : 5 : 10 : 20 : 60 resulted in EHT that developed much faster than EHTs from CM only, exhibited a better sarcomeric and mitochondrial ultrastructure and showed typical distribution of the different cell types throughout EHTs. Importantly, multicellular EHTs showed a clear hypertrophic (increase in CM size) and fibrotic response (gene expression) to the pro-hypertrophic agonists phenylephrine and endothelin-1 that is lacking in EHTs from CM only. Taken together, we have optimized the EHT system to a point that EHT-data have been part of an FDA IND procedure. And we have developed the first EHT model with 5 cardiac cell types that can serve as a new human hypertrophy model.

METABOLISM, STEM CELLS, AND CANCER
2:00 PM – 3:30 PM
HALL B, LEVEL 1

METABOLIC REPROGRAMMING IN HEPATOCELLULAR CARCINOMA TO DRIVE CANCER STEMNESS
Ma, Stephanie, *School of Biomedical Sciences, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong*

Hepatocellular Carcinoma (HCC), in particular those driven by hepatitis B virus and metabolic dysfunction, is one of the most prevalent and aggressive malignancies the world. Despite definite improvements in the outcome of patients with this disease, the overall prognosis is still unsatisfactory because of late presentation, drug resistance and frequent tumor recurrence. Unlocking phenotypic plasticity has become known as a new emerging hallmark of cancer, with the feature endowing cancer cells with the capacity to shift dynamically between a differentiated state, with limited tumorigenic potential, and an undifferentiated or cancer stem-like cell (CSC) state, which is responsible for long-term tumor growth. There is now ample evidence to show a more stemness or less differentiated state represents an important root of HCC recurrence and therapy resistance. Recent discoveries in the lab have identified a number of metabolic-associated molecular targets and mechanisms driving cancer stemness, providing novel insights into new therapeutic interventions. This talk will cover the latest findings in our lab in this area.

ONCOGENIC KRAS INITIATES SKIN TUMOR IN A NICHE DEPENDENT MANNER
Xin, Tianchi, *Yale School of Medicine, USA*
Regot, Sergi, *Johns Hopkins School of Medicine, USA*
Greco, Valentina, *Yale University School of Medicine, USA*

Tissue regeneration and maintenance rely on the coordinated behaviors of stem cells. However, this orchestration can be disrupted by oncogenic mutations, resulting in the breakdown of the healthy tissue architecture and, ultimately, tumor formation. Despite its significance, how oncogenes disrupt stem cell functions to initiate tumorigenesis remain poorly understood. Through intravital imaging of mouse skin together with dynamic ERK signal sensor, our recent work uncovered that KrasG12D mutation, a driver of squamous cell carcinoma, induces early tissue abnormality by converting pulsatile into sustained ERK activation in hair follicle stem cells. To further interrogate the mechanism of tumor initiation, we longitudinally tracked the same KrasG12D mutant hair follicles over several months in the mouse ear. Surprisingly, as tumors emerged in other body parts, no tumor formation was observed in the ear despite the dramatic architectural changes in individual hair follicles, suggesting a tumor resistant microenvironment provided by the ear skin. We then introduced a wound following mutation induction to stimulate tumorigenesis, as wounding has been shown to promote tumor formation originating from hair follicles. Interestingly, our preliminary data indicate that tumor initiation only occur in a hair follicle stage-specific manner, suggesting that remodeling hair follicles provide a tumor permissive microenvironment. To uncover the mechanism of this niche-dependent tumorigenesis, we are analyzing the cellular and molecular dynamics during tumor initiation. Tracking ERK signal dynamics in vivo shows that KrasG12D induces sustained ERK activation exclusively at the tumor permissive hair follicle stage. Molecular profiling suggests that Kras mutant stem cells adopt a new cellular state, which may synergize with

wound-induced signal to enable tumor formation. Collectively, our findings support a niche-dependent mechanism of oncogenesis, demonstrating that oncogenic mutations must cooperate with specific microenvironments to drive tumor initiation.

IDENTIFICATION OF LINEAGE-SPECIFIC CANCER-RELATED MUTATIONS IN HUMAN PLURIPOTENT AND ADULT STEM CELLS

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Lezmi, Elyad, *The Hebrew University of Jerusalem, Israel*
Benvenisty, Nissim, *The Hebrew University of Jerusalem, Israel*

Human pluripotent stem cells PSCs accumulate genomic defects, impacting their growth and tumorigenic potential. In a recent large-scale analysis of over 140 PSC lines, we revealed cancer-related pathogenic point mutations, as identified in human tumors, in about 20% of PSC samples and their differentiated derivatives. Furthermore, we suggested effects of such mutations on the cellular phenotype during differentiation. To compare the rate and type of mutations in PSCs versus adult stem cells (ASCs), we analyzed cancer-related mutations, in over 700 samples of human ASCs, finding mutations in approximately 18% of mesenchymal stem cell samples and 41% in neural stem cell samples. The analysis identified involvement of 25 tumor-suppressor genes and 9 oncogenes with interconnection between them. Surprisingly, ASCs exhibited dramatically different profiles of genes compared to those found in PSCs. These lineage-specific profiles of cancer-related genes are suggested to be correlated with their gene expression levels and with tumor-specific mutations in patients. In addition, we demonstrated in all three lineages cancer mutations that are acquired in vitro. Our findings emphasize the importance of genetic screening in all stem cell lines, both for accurate research interpretation and for ensuring safety in clinical applications. The initial part of this work was recently published in Nature Biotechnology (2024).

METABOLIC REWIRING DRIVES TOLERANCE TO ONCOGENIC MUTATIONS IN THE SKIN STEM CELLS

Hemalatha, Anupama, *Multicellular Systems, Friedrich Miescher Institute for Biomedical Research, Switzerland*
Perry, Rachel, *Yale University, USA*
Greco, Valentina, *Yale University, USA*

Phenotypically normal human and mouse epithelial tissues accumulate oncogenic mutations with age. To understand why some of these mutations lead to disease while others are tolerated, it is essential to define the temporal trajectory of tissue as it transitions from a pre-cancerous to cancerous state. Cellular energy metabolism is a key driver of both regulated and aberrant growth. How is metabolism altered to support oncogenic tolerance versus cancer emergence? To track the metabolic changes within the skin stem cells of live mice over time as they encounter oncogenic mutations, I adapted optical redox imaging into a live-imaging platform so as to capture the endogenous fluorescence of metabolites NAD(P)H and FAD in parallel with cell morphology and behaviors in the mouse skin. Different oncogenes invoke different modes of epithelial tolerance: while wild-type (WT) cells outcompete β-catenin-Gain-of-Function (βcatGOF) mutant cells, HrasG12V mutant cells outcompete WT cells, yet are

integrated into functional tissue. By tracking the redox ratio (NAD(P)H/FAD) with single cell resolution in the same mouse over time, we discovered that both βcatGOF and HrasG12V mutations lead to a rapid drop in redox ratios (NAD(P)H/FAD), before any other tissue aberrancy, making it a first line of response to the mutations. Glucose flux studies reveal that both βcatGOF and HrasG12V mutant epidermis upregulate flux and fractional contribution of glucose through TCA cycle, and thereby glucose oxidation, in line with the observed drop in redox ratio- but contrary to the expectations for cancer cells. Using pharmacological inhibition of mitochondrial oxidation, we find that upregulation of glucose oxidation is essential for the downstream phenotype of both oncogenic mutations. Strikingly, their cell competition outcome is reversed upon inhibition of metabolic changes - the βcatGOF cells are no longer effectively eliminated and the HrasG12V cells no longer expand. Is the deviation from the expected metabolic rewiring a reason for oncogenic tolerance? To answer this, future studies will combine HrasG12V mutants with p53 loss-of-function; conditions under which cancer will develop. My lab will investigate the earliest redox and metabolic changes, tracking them over time as they diverge between tolerance and disease phenotypes.

TRANSCRIPTION FACTORS TFEC AND MAFB SPECIFY HUMAN IPSC TO BLOOD STEM CELL-SUPPORTIVE SINUSOIDAL VASCULAR ENDOTHELIAL CELLS

Zon, Lenonard, *Stem Cell Program, Boston Children's Hospital, USA*Han, Tianxiao, *Boston Children's Hospital, USA*
Gunagem Rajesh, *Boston Children's Hospital, USA*
Jing, Ran, *Boston Children's Hospital, USA*
Khosraviani, Negin, *Boston Children's Hospital, USA*
Stockard, Oliva, *Boston Children's Hospital, USA*
Cordin, Alexandra, *Boston Children's Hospital, USA*
Xu, Anthony, *Boston Children's Hospital, USA*
Hensch, Luca, *Boston Children's Hospital, USA*
Konovov, Martin, *Boston Children's Hospital, USA*
Tang, Yang, *Boston Children's Hospital, USA*
Chen, Kenny, *Boston Children's Hospital, USA*
Yang, Song, *Boston Children's Hospital, USA*
Zhou, Yi, *Boston Children's Hospital, USA*
Daley, George, *Boston Children's Hospital, USA*
Schlaeger, Thorsten, *Boston Children's Hospital, USA*

Sinusoidal endothelial cells (ECs) and other perivascular supportive cells constitute a specialized niche microenvironment for the hematopoietic stem and progenitor cells (HSPCs). HSPC niches are found in the fetal liver and the adult bone marrow. The transcriptional programs that orchestrate the HSPC niche functions remain unclear. We elucidated the transcription factor code that specifies the venous sinusoids of the marrow. Using zebrafish, we performed single-cell RNA-sequencing analyses between HSPC niche sinusoidal ECs in the adult marrow and the non-HSPC niche sinusoidal ECs in the adult liver. These results were compared to murine marrow sinusoids. By overexpressing the differentially expressed transcription factors (TFs) tfec and mafbb in the adult zebrafish liver sinusoids, we reprogrammed liver sinusoids to upregulate key genes known for HSPC niche-supportive functions, including mrc1a (log2FC=4.9, $p< 0.005$), lyve1b (log2FC=3.6, $p< 0.005$) and dab2 (log2FC=5.0, $p< 0.005$). These reprogrammed sinusoidal ECs attracted primary runx1+ spi1b+ HSPCs. Transplantation of whole liver cells in the reprogrammed liver achieved functional

multi-lineage engraftment in immunocompromised recipients by transplant assay (7/26 in the reprogram group vs. 0/20 in the control not programmed group, p=0.0296). Using this information, we reprogrammed human iPSC into HSPC niche-like sinusoidal ECs by DOX-inducible overexpression of human TFs ETV2, TFEC, and MAFB. These HSPC niche-like sinusoidal ECs significantly upregulated putative HSPC niche-supportive genes, including MRC1 (log2FC=3.6, p< 0.005), STAB2 (log2FC=10.3, p< 0.005), JAG1 (log2FC=1.7 p< 0.005), and CXCL12 (log2FC=5.1 p< 0.005). Co-culture with primary human cord blood-derived CD34+ HSPCs revealed that these HSPC niche-like sinusoidal ECs could better preserve the co-cultured HSPC stem cell properties, shown by higher RNA expression of stem cell marker genes such as CD34 and MECOM, and by a greater number of multipotent colonies in the colony-forming unit (CFU) assay. More importantly, these HSPC niche-like sinusoidal ECs helped the co-cultured HSPCs improved HSPC engraftment compared to generic ECs (p=0.0164). Our findings unveiled the transcription factor code TFEC and MAFB in specifying sinusoidal ECs to have HSPC niche function.

METABOLISM IN THE TISSUE MICROENVIRONMENT REGULATES KIDNEY (RE)GENERATION
Rabelink, Ton, *Internal Medicine, Leiden University Medical Center, Netherlands*

Chronic kidney disease (CKD) is a global health concern, and the limited treatment options for end-stage kidney disease have remained unchanged for decades. Concurrent insults to the kidney can exacerbate disease progression, but the underlying potential to have kidney repair after such injury remains poorly understood. The tricarboxylic acid (TCA) cycle, a fundamental metabolic pathway, plays a vital role in energy production and biomolecule synthesis. Recent research by my group has revealed that failed repair and subsequent fibrosis in kidney epithelial cells are linked to anaplerotic failure in the TCA cycle, resulting in the inability to maintain sufficient levels of TCA intermediates. These metabolites regulate chromatin remodeling and cell signaling pathways, influencing cell fate decisions. Using a spatial multi-omics approach we identify regenerative and profibrotic niches in the kidney that are characterised by unique metabolic profiles and cellular metabolite communication. These metabolic features recapitulate the metabolite communication during kidney development. I will discuss how these insights can subsequently be applied to improve procurement and outcome of kidney transplantation.

MODULATING THE IMMUNE SYSTEM TO FIGHT DISEASE
2:00 PM – 3:30 PM
THEATER 1, LEVEL 1

BUILDING AN ENHANCED IMMUNE SYSTEM TO FIGHT DISEASE
Levings, Megan, *School of Biomedical Engineering, University of British Columbia, Canada*

Regulatory T cells (Tregs) regulate immune homeostasis through a variety of mechanisms and are being explored as a cell-based therapy to dampen inflammation and induce tolerance in the settings of transplantation and autoimmunity. Evidence shows that Treg therapy is safe and well-tolerated, but efficacy remains undefined and could be limited by poor persistence in vivo and lack of antigen specificity. With the advent of new genetic engineering tools, it is now

possible to create bespoke “designer” Tregs that not only overcome possible limitations of polyclonal Tregs, but also introduce new features. I will discuss our recent work to engineer Tregs with chimeric antigen receptors as a strategy to increase potency. I will also discuss how different types of progenitor cells can be leveraged to make “on demand” human T cells for application as allogeneic cell therapies in a wide variety of immune-mediated diseases.

IMMUNE RESPONSE IN IPSC-BASED ALLOGENEIC CELL THERAPY FOR PARKINSON'S DISEASE

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Yamasaki, Emi, *Kyoto University, Japan*
Shindo, Takero, *Kyoto University Graduate School of Medicine, Japan*
Anazawa, Takayuki, *Kyoto University Graduate School of Medicine, Japan*
Sawamoto, Nobukatsu, *Kyoto University Graduate School of Medicine, Japan*
Yamakado, Hodaka, *Kyoto University Graduate School of Medicine, Japan*
Nakanishi, Etsuro, *Kyoto University Graduate School of Medicine, Japan*
Sawamura, Masanori, *Kyoto University Graduate School of Medicine, Japan*
Taruno, Yosuke, *Kyoto University Graduate School of Medicine, Japan*
Doi, Daisuke, *Kyoto University, Japan*
Kikuchi, Tetsuhiro, *Kyoto University, Japan*
Kawasaki, Yuri, *Kyoto University, Japan*
Saito, Megumu, *Kyoto University, Japan*
Kikuchi, Takayuki, *Kyoto University Graduate School of Medicine, Japan*
Arakawa, Yoshiki, *Kyoto University Graduate School of Medicine, Japan*
Miyamoto, Susumu, *Kyoto University Graduate School of Medicine, Japan*
Nakamoto, Yuji, *Kyoto University Graduate School of Medicine, Japan*
Takahashi, Ryosuke, *Kyoto University Graduate School of Medicine, Japan*
Takahashi, Jun, *Kyoto University, Japan*

Because the central nervous system (CNS) is an immune-privileged organ, it requires different immunosuppression strategies for cell therapies using induced pluripotent stem cells (iPSCs) compared with the ones for organ transplantations. We conducted the first in-human clinical trial of a cell therapy for Parkinson's disease using allogeneic iPSCs (jRCT2090220384) to seven subjects. All patients were transplanted with dopaminergic neural progenitors differentiated from iPSCs (iPSC-DANs), which was derived from a healthy volunteer with homozygous human leukocyte antigen (HLA) haplotypes, through immunosuppression with tacrolimus (trough value 5-10 ng/mL) alone for about one year. No significant immune response was detected by positron emission tomography and serological study regardless of HLA compatibility. A mixed lymphocyte reaction with a stimulator of iPSC-derived dendritic cells, but not iPSC-DANs, showed the activation of lymphocytes from HLA mismatch-grafted recipients. The low expression of HLA in iPSC-DANs might contribute to the successful engraftment in the immune-privileged CNS. Although the number of cases is small, this study indicated the possibility that immunosuppression with tacrolimus alone could control the postoperative immune response in allogeneic iPSC-based cell therapy for Parkinson's disease. Appropriate immune suppression strategies using cost-effective immune monitoring methods should be further developed for stem cell therapies in CNS.

Funding Source: This study was supported by a grant from the Research Project for Practical Application of Regenerative Medicine of the Japan Agency for Medical Research and

Development (AMED) [23bk0104126h0003].

Clinical Trial ID: jRCT2090220384.

SYNNOTCH/CAR-BASED COMBINATORIAL IMMUNOTHERAPY AGAINST COLORECTAL CANCER WITH HER2 AMPLIFICATION IN NK AND IPSC-DERIVED NK CELLS

Cortese, Marco, *Oncology, Candiolo Cancer Institute, Italy*
D'Andrea, Alice, *Università di Torino, Italy*
Landra, Indira, *Candiolo Cancer Institute, Italy*
Taebi, Sahar, *Università di Torino, Italy*
Petti, Consalvo, *Candiolo Cancer Institute, Italy*
Franco, Letizia, *Candiolo Cancer Institute, Italy*
Medico, Enzo, *Università di Torino, Italy*

HER2 amplification occurs in about 5% of colorectal cancer (CRC) cases and is associated only partially with clinical response to combined human epidermal growth factor receptor 2 (HER2)/epidermal growth factor receptor (EGFR)- targeted treatment. An alternative approach based on adoptive cell therapy (ACT) using T-cells engineered with anti-HER2 chimeric antigen receptor (CAR) proved to be toxic due to "on-target off-tumor" activity. Here we describe a combinatorial strategy to safely target HER2 amplification and CEA expression in CRC using a synNotch-CAR based artificial regulatory network. The natural killer cell line NK-92 was engineered with an anti-HER2 synNotch receptor driving the expression of a CAR against CEA only when engaged. After being transduced and sorted for HER2-driven CAR expression, cells were cloned. The clone (5F) with optimal performances in terms of specificity and amplitude of CAR induction demonstrated significant activity in vitro and in vivo specifically against HER2amp/CEA+ CRC models, with no effects on cells with physiological HER2 levels. To further improve survival, tumor penetration and in vivo efficacy of the NK-92.5F clone, we adopted two approaches. First, we tried to increase cytotoxicity building a more complex and potent system, in which HER2 synNotch engagement drives expression of both the CEA-CAR and different cytokines (5Fck). In fact, 5Fck cells displayed further increased cytotoxicity in vitro, and drastically increased the survival of mice carrying HER2amp CRC xenografts. The second approach was to employ the combinatorial system in a different effector cell type, i.e. iPSC-derived NK cells, characterized by longer survival (no irradiation required) and higher penetration and cytotoxicity. In conclusion the HER2-synNotch/CEA-CAR-NK system provides an innovative, scalable and safe off-the shelf cell therapy approach with potential against HER2amp CRC resistant or partially responsive to HER2/EGFR blockade.

GENO-WRITING™ PLATFORM: TRANSFORMATIVE GENOME ENGINEERING FOR HUMAN IPSCS

Aizawa, Yasunori, *Logomix, Japan*

Human iPSCs are revolutionizing research and therapy, but existing genome engineering tools fall short in enabling the large-scale, precise modifications essential for advanced cellular models and therapeutic prototypes. Geno-Writing™ bridges this gap, offering a groundbreaking genome engineering platform that enables (1) bi-allelic, 100-kb-scale deletion of multiple endogenous gene loci, and (2) integration and stable expression of 8–12 transgenes in human iPSCs in under two months. This unprecedented capability to perform large-scale genome

modifications and complex cell functional enhancements represents a paradigm shift in iPSC engineering. A defining innovation of Geno-Writing™ is its automation and high-throughput capability. The current automated transgene screening system enables the parallel generation of up to 5,000 isogenic iPSC lines, each carrying unique transgene combinations. This high-throughput workflow transforms the conventionally lengthy process of transgene optimization into a rapid, systematic endeavor. The resulting isogenic iPSC library provides a powerful resource for dissecting the effects of distinct transgene combinations on cellular phenotypes. By analyzing these phenotype datasets, the platform identifies optimal transgene configurations with precision, accelerating both basic research and translational applications. Internally, we are leveraging Geno-Writing™ to develop next-generation hypoimmune iPSCs tailored for regenerative medicine and autoimmune disease therapies. Simultaneously, the platform is advancing the creation of iPSC-derived immune cells, addressing the unmet therapeutic needs in refractory tumors. By combining large-scale transgene integration with streamlined screening workflows, Geno-Writing™ significantly reduces timeline from conceptualization to therapeutic application, enhancing rapid development of innovative therapies. This presentation will showcase the latest data on Geno-Writing™ , highlighting its transformative potential to bridge the gap between complex genome engineering and real-world applications. By setting new standards for precision, scalability, and innovation, Geno-Writing™ is reshaping the future of regenerative medicine and therapeutic development.

GENERATION OF ALLOGENEIC CAR-NKT CELLS FROM HEMATOPOIETIC STEM AND PROGENITOR CELLS USING A CLINICALLY GUIDED CULTURE METHOD

Yang, Lili, *University of California, Los Angeles (UCLA), USA*

Cancer immunotherapy with autologous chimeric antigen receptor (CAR) T cells faces challenges in manufacturing and patient selection that could be avoided by using ‘off-the-shelf’ products, such as allogeneic CAR natural killer T (AlloCAR-NKT) cells. Previously, we reported a system for differentiating human hematopoietic stem and progenitor cells into AlloCAR-NKT cells, but the use of three-dimensional culture and xenogeneic feeders precluded its clinical application. Here we describe a clinically guided method to differentiate and expand IL-15-enhanced AlloCAR-NKT cells with high yield and purity. We generated AlloCAR-NKT cells targeting seven cancers and, in a multiple myeloma model, demonstrated their antitumor efficacy, expansion and persistence. The cells also selectively depleted immunosuppressive cells in the tumor microenviroment and antagonized tumor immune evasion via triple targeting of CAR, TCR and NK receptors. They exhibited a stable hypoimmunogenic phenotype associated with epigenetic and signaling regulation and did not induce detectable graft versus host disease or cytokine release syndrome. These properties of AlloCAR-NKT cells support their potential for clinical translation.

Funding Source: This work was supported by funding from the California Institute for Regenerative Medicine (DISC2-11157 and TRAN1-12250), Department of Defense (CA200456), and UCLA (BSCRC Innovation Award and Ablon Scholars Award).

Friday, June 13, 2025

AGING AND REGENERATION
8:30 AM – 10:00 AM
THEATER 2, LEVEL 1

STEM CELL FATE DECISION FOR PIGMENTED HAIR REGENERATION, AGING AND CANCER DEVELOPMENT

Nishimura, **Emi K.**, *The Institute of Medical Science, The University of Tokyo, Japan*

The accumulation of an individual's life-long environmental exposure, known as the "exposome," significantly impacts health. Somatic tissues undergo functional decline with age, exhibiting characteristic ageing phenotypes such as hair graying and cancer. However, specific genotoxins and signals driving each phenotype and their underlying cellular mechanisms remain largely unknown. Our previous studies identified melanocyte stem cells (McSCs) in mammalian hair follicles as somatic stem cells that generate pigment-producing melanocytes. We also demonstrated that these cells are depleted with aging and various types of genotoxic stress, contributing to hair-graying phenotypes. However, the precise fate of DNA-damaged stem cells remains largely unknown in most tissues. In this study, we found that depending on the type of genotoxic damage that occurs, melanocyte stem cells (McSCs) and their niche coordinately determine individual stem cell fate through antagonistic stress-responsive pathways at a single stem cell level. Chronological stem cell fate-tracking in mice revealed that McSCs undergo cellular senescence-associated differentiation (seno-differentiation) in response to DNA double-strand breaks (DSBs) and downstream pathways, resulting in their selective elimination and resultant hair graying at the cost of cancer defense. Conversely, carcinogens can effectively rescue the seno-differentiation of McSCs, even those with DSBs, through the upstream niche-derived KITL, a master niche factor for McSC self-renewal. Collectively, our data demonstrate that the fate of individual stem cell clones - expansion versus exhaustion - cumulatively and antagonistically governs a degenerative ageing phenotype and/or cancer development through the stem cell niche, depending on the exposome.

DECODING THE IMPACT OF PREGNANCY ON AGING OF STEM/PROGENITOR CELLS IN THE MAMMARY GLAND

Olander, Andrew, *University of California, Santa Cruz, USA*
Ramirez, Cynthia*, Department of Applied Mathematics, University of California, Santa Cruz, USA*
Medina, Paloma*, Department of Biomolecular Engineering and Bioinformatics, University of California, Santa Cruz, USA*
Haro-Acosta, Veronica*, Department of MCD Biology, University of California, Santa Cruz, USA*
Dijkgraaf, Marijs*, Department of MCD Biology, University of California, Santa Cruz, USA*
Kaushik, Sara*, Department of MCD Biology, University of California, Santa Cruz, USA*
Jonsson, Vanessa*, Department of Biomolecular Engineering and Bioinformatics, University of California, Santa Cruz, USA,*
Sikandar, Shaheen*, Department of MCD Biology, University of California, Santa Cruz, USA*

A woman's age and reproductive history have significant influence over her risk of developing breast cancer through their impact on stem/progenitor cells. Stem/progenitor cells are critical in the context of tumor initiation and cellular plasticity, as they possess the ability to both self-renew and differentiate, properties that are co-opted during oncogenesis to drive tumor progression and heterogeneity. While several studies have explored the effect of aging or pregnancy on stem/progenitor cells in silo, the combined effects remain poorly understood. This knowledge gap is particularly relevant given that more than 60% of women over the age of 44 have experienced pregnancy globally, underscoring the need to study biological aging in a context that reflects the broader human population. Here, we establish a map of the long-term impact of pregnancy on the aging of stem/progenitor cells in the mammary gland using flow cytometry, functional assays, and single-cell transcriptomics. Our findings reveal that pregnancy counteracts age-induced imbalances in stem/progenitor cells, promoting a more normalized cellular landscape and inducing a differentiated cell state. Notably, we identify a rare population of Il33-expressing hybrid cells with high cellular plasticity that accumulate in aged nulliparous mice but are significantly diminished in aged parous mice. Functionally, IL33 treatment of young mammary glands recapitulates aging phenotypes in vitro and in vivo. Collectively, our study demonstrates that pregnancy blocks the age-induced loss of lineage integrity through a decrease in Il33+ hybrid cells, potentially contributing to pregnancy-induced breast cancer protection. Future studies will determine the cell-intrinsic role of IL33 in cellular plasticity of stem/progenitor cells and test whether blocking Il33 expression may serve as a strategy for breast cancer prevention in women who have not undergone pregnancy. Thus, our study for the first time comprehensively characterizes the aged nulliparous and parous mammary glands to determine the long-term impacts of pregnancy on the aging of stem/progenitor cells.

Funding Source: Hellman Fellows Award, NIH T32 Training Grant.

UNVEILING THE ROLE OF THE HYPOXIA RESPONSE MACHINERY IN STEM CELL REGULATION AND TISSUE RENEWAL AT PHYSIOXIA

Salvo, Paula Martos, *Lund University, Sweden*
Islam, Mazharul, *Lund University, Sweden*
Moraitis, Ilias, *P-CMR[C] - Program for Clinical Translation of Regenerative Medicine in Catalonia; IDIBELL - Bellvitge Biomedical Research Institute, Spain*
Arozamena, Borja, *P-CMR[C] - Program for Clinical Translation of Regenerative Medicine in Catalonia; IDIBELL - Bellvitge Biomedical Research Institute, Spain*
Mazariegos, Marina, *Lund University, Sweden*
Wienskowska, Olga, *P-CMR[C] - Program for Clinical Translation of Regenerative Medicine in Catalonia; IDIBELL - Bellvitge Biomedical Research Institute, Spain*
Mohlin, Sofie, *Lund University, Sweden*
Guiu, Jordi, *P-CMR[C] - Program for Clinical Translation of Regenerative Medicine in Catalonia; IDIBELL - Bellvitge Biomedical Research Institute, Spain*
Hammarlund, Emma, *Lund University, Sweden*

The Hypoxia-Inducible Factor (HIF) pathway plays a critical role in tissue renewal and stem cell maintenance across species. We investigate how the overall hypoxia response machinery is involved in that pathway since the difference in the HIF pathway between invertebrates and

vertebrates may constrain tissue renewal under oxygen-rich conditions. Here, we explore how these differences influences stem cell activity and tissue renewal under varying oxygen conditions in vertebrates, alongside complementary insights from invertebrate models. We begin by examining hypoxia-responsive mechanisms in invertebrates, focusing on Drosophila melanogaster. Our experiments indicate that flies exposed to hyperoxia (40% oxygen) display reduced stemness and shortened lifespans. We interpret this as if there are potential limits of tissue renewal in high-oxygen environments. Next, we explore the role of hypxoia respone machineries for tissue renewal in oxygenated vertebrate tissues, using mouse intestinal organoids cultured under physiologically oxic concentrations (3% oxygen). Organoids show upregulation of hypoxia-responsive genes and a shift toward developmental, stem cell-like and regenerative states, as revealed by enriched fetal intestinal and spheroid-like gene expression signatures. We interpret this as if the hypoxia response machinery may facilitate stemness control at also physiologically oxic settings. Finally, we investigate the in vivo role of the hypoxia response machinery in mice, with a focus on intestinal stem cell maintenance and regeneration. In an knockout model, we assess tissue renewal and wound healing following radiation-induced damage, uncovering compensatory mechanisms that may operate in the absence of a functional hypoxia response machinery. By integrating invertebrate and vertebrate models, our findings provide new insights into how oxygen availability and the overall HIF pathway regulate stem cell dynamics, tissue renewal, and organismal lifespan. This work may advance our insights regarding, and implications for, regenerative medicine and developmental biology.

GDF11 SECRETING CELL TRANSPLANT EFFICIENTLY AMELIORATES AGE-RELATED PULMONARY FIBROSIS

Guo, Lily, *Lunenfeld-Tanenbaum Research Institute, Canada*
Duchesneau, Pascal, *Lunenfeld-Tanenbaum Research Institute, Canada*
Jong, Eric, *Oxford University, UK*
Li, Chengjin, *Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Canada*
Sawula, Evan, *Lunenfeld-Tanenbaum Research Institute, Canada*
Waddell, Tom, *Lunenfeld-Tanenbaum Research Institute, Canada*
Nagy, Andras, *Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Canada*

Idiopathic pulmonary fibrosis (IPF) is an age-related, devastating lung disease without a cure. The age-related lung degeneration significantly contributes to the pathogenies and development of IPF, leading to irreparable tissue damage, further impaired function, and high mortality rates. Therefore, developing interventions to counteract age-related deterioration of this organ and promote effective fibrosis resolution is crucial. Here, we present a combination of cell and gene therapy that leverages the lung-regenerative properties of GDF11. We integrated a GDF11 transgene, regulated by a doxycycline-inducible promoter, into mouse embryonic stem cells (mESCs) that were equipped with a genetically encoded suicide-switch system, the FailSafe™ system, enabling the elimination of unwanted proliferating cells. Furthermore, the cells allowed efficient differentiation of lung progenitors. When these progenitor cells were transplanted into lung-injured aged mice, besides acting as reparative cells to restore the damaged alveolar epithelium, they served as an “in situ factory," enabling the production of GDF11 in response to the inducer drug. This approach attenuated age-associated senescence and led to the successful resolution of fibrosis. Our study presents a promising method for treating pulmonary fibrosis. Moreover, this approach offers a versatile platform that can be expanded to incorporate other regenerative and anti-aging factors. By transitioning this approach to hypoimmunogenic off-the-shelf available therapeutic cells, we can overcome

limitations associated with traditional protein-based therapies, such as high production costs and the need for frequent administration.

Funding Source: This study was supported by the Canadian Institutes of Health Research Foundation Grant [143231], Canada Research Chair [950-230422] to AN, and Grosman-Lubin Fellowship Award to LG.

TITLE AND ABSTRACT NOT AVAILABLE AT THE TIME OF PUBLISHING
Rando, Thomas, *Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, University of California Los Angeles, USA*

AI AND CANCER EVOLUTION
8:30 AM – 10:00 AM
HALL B, LEVEL 1

MOLECULAR DIAGNOSIS AND TARGETED THERAPY IN SPINAL CORD DIFFUSE GLIOMAS
Wang, Jiguang, *Hong Kong University of Science and Technology, Hong Kong*

H3 K27-mutated diffuse spinal cord glioma (H3-DSG) represents poorly characterized tumors with limited therapeutic options. Here we generated and analyzed multi-omics and spatially resolved single-cell data from H3-DSG and stratified the disease into two distinct subtypes: pons-like and thalamus-like. Whereas the two subtypes genetically and clinically mirrored two classes of H3 K27-mutated gliomas in the brain, both originated from dorsal PAX3+ oligodendrocyte progenitor cells in the spinal cord. Thalamus-like H3-DSG was characterized by NF1 mutations, active immune niches housing abundant proinflammatory myeloid cells and CD8+ cytotoxic T cells, the glycolytic/plurimetabolic tumor cell state and accumulation of chemoattractant cytokines. The inflammatory microenvironment sensitized thalamus-like H3-DSG to anti-PD1 therapy. Conversely, pons-like H3-DSG exhibited an enriched frequency of TP53 mutations, high proliferative niches, along with elevated DNA replication stress and genomic instability. From newly established patient-derived cellular and animal models, we found that targeting master kinases involved in DNA repair enhanced the efficacy of radiotherapy in pons-like, but not thalamus-like H3-DSGs. Thus, we uncovered two distinct H3-DSG subtypes, provided molecular biomarkers for accurate subtype identification and delivered experimentally validated precision therapeutics opportunities for patients with spinal cord glioma.

ENGINEERED UNIVERSAL IPSC-DERIVED DENDRITIC CELLS DRESSED WITH TUMOR-MHC-PEPTIDE COMPLEXES AS A CANCER VACCINE
Xu, Huaigeng, *University of California, San Francisco (UCSF), USA*
Kamei, Chisato, *University of California, San Francisco (UCSF), USA*
Goekbuget, Deniz, *University of California, San Francisco (UCSF), USA*
Wang, Li, *University of California, San Francisco (UCSF), USA*
Soriano, Jason, *University of California, San Francisco (UCSF), USA*

Blelloch, Robert, *University of California, San Francisco (UCSF), USA*

Dendritic cells (DCs) play a critical role in initiating anti-tumor immune responses by presenting tumor-associated antigens (TAAs) to T cells. Based on this role, DCs have been developed as cell-based vaccines for cancer therapy; however, their efficacy has been constrained by the limited availability of patient-derived DCs and the narrow spectrum of TAAs that can be presented using current methods. In this study, to address these challenges, we introduce a novel strategy to create personalized DC vaccines by using the scalability of human induced pluripotent stem cells (hiPSCs) and the broad antigen presentation enabled by tumor-derived MHC-antigen complexes. The B2M and CIITA loci were mutated in the hiPSCs, removing the presentation of endogenous MHCI and MHCII. We then optimized a method to produce mature immune activating DCs. Flow cytometry and scRNA-seq analysis revealed a migratory DC phenotype for the resulting DCs, with high expression of the migratory receptor CCR7 and costimulatory molecules CD80 and CD86. The resulting “universal” hiPSC-DCs were then dressed with membrane vesicles derived from tumor cells such that the DCs presented the entire repertoire of the tumor antigens on their surface. Resulting DCs dressed with tumor cells expressing the NY-ESO antigen in the context of HLA-A2 strongly stimulated CD8 T cells that were transduced with the cognate T cell receptor. The dressed DC cells were also resistant to the NK cell directed killing that was seen with the parental MHC deficient DCs. Furthermore, the dressed DC stimulated naïve allogenic T cells confirming their priming function. The immune stimulating properties of the DCs were further enhanced by deletion of the immune checkpoint proteins PD-L1 and PD-L2. Our approach shows great promise for the use of off-shelf engineered iPSC-derived DCS dressed with tumor membrane as a new tumor vaccine approach, which allows for the presentation of a complex mix of tumor antigens in the context of a highly stimulatory DC. Such an approach could have broad applications across tumors that normally evade the anti-tumor immune response. We are currently testing the approach on matching tumor and immune cells from acute myeloid leukemia patients, a tumor notoriously resistant to immune checkpoint therapies.

GLOBAL IN VIVO DETECTION OF DNA REPAIR OUTCOMES INDUCED BY CRISPR–CAS9 IN ENDOGENOUS MUSCLE STEM CELLS

Fu, Yang, *Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Hong Kong*
He, Liangqiang, *The Chinese University of Hong Kong, Hong Kong*
Wang, Ziliu, *The Chinese University of Hong Kong, Hong Kong*
Sun, Hao, *The Chinese University of Hong Kong, Hong Kong*
Wang, Huating, *The Chinese University of Hong Kong, Hong Kong*

CRISPR–Cas9 has revolutionized genome editing by enabling precise, programmable, and targeted modifications, showcasing significant potential for therapeutic applications in human diseases. However, despite substantial insights gained from in vitro studies, comprehensive in vivo assessments of editing patterns and safety remain limited, hindering the rational and effective clinical translation of this technology. To address this gap, we conducted in vivo AAV/CRISPR/Cas9 gene editing and evaluated global DNA repair outcomes at 98 genomic loci targeted by single or paired guide RNAs in mouse muscle stem cells using amplicon deep sequencing. Our results reveal that the outcomes of in vivo editing are site-specific and nonrandom, primarily introducing small insertions and deletions. Importantly, we observe widespread occurrence of deleterious DNA repair byproducts, including large deletions and

insertions. Interestingly, the pattern of large insertions is random, and their formation is affected by local three-dimensional chromatin interactions. As a notable form of large insertions, AAV integration occurs at all targeted loci and accounts for 31.3% of the large insertions on average. Increasing the AAV dose significantly enhances the overall editing efficiency but also leads to elevated AAV integration at the target sites. In summary, this study represents the first large-scale in vivo application of CRISPR/Cas9 genome editing in mice, providing valuable insights into the fundamental principles of CRISPR/Cas9-induced endogenous DNA repair and highlighting the need for more cautious application of this technology in therapeutic genome editing.

ARE WE MAKING THE RIGHT KIND OF HEART MUSCLE? USING A HUMAN FETAL HEART ATLAS TO ASSESS MATURITY, PURITY, AND SUBTYPES IN STEM-CELL DERIVED CARDIOMYOCYTE MODELS

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Bayraktar, Semih, *Cambridge Stem Cell Institute, University of Cambridge, UK*
Kanemaru, Kazumasa, *Cambridge Stem Cell Institute, University of Cambridge, UK*
Cranley, James, *Cambridge Stem Cell Institute, University of Cambridge, UK*
Teichmann, Sarah, *Cambridge Stem Cell Institute, University of Cambridge, UK*
Sinha, Sanjay, *Cambridge Stem Cell Institute, University of Cambridge, UK*

Cardiomyocytes (CMs) derived from human pluripotent stem cells (hPSC-CMs) offer a solution to the global clinical burden of cardiovascular disease and cardiac-related drug reactions. However, the human heart contains many CM subtypes depending on specification, developmental stage, or anatomical region. This diversity is pertinent for hPSC-CMs in regeneration, precision medicine, and drug discovery as evidence suggests a lack of functional integration of current hPSC-CM grafts, while in vitro assays may not be relevant for modelling disease nor cover the breadth of CMs implicated in cardiotoxicity. With a multitude of hPSC-CM models now available, it is unclear on how to select the most relevant model for application, or whether the hPSC-CMs available are a good reproduction of human CMs in vivo. To address this, we leveraged single-cell and spatial transcriptomics to build a high-resolution human fetal heart atlas, and applied machine learning and statistical modelling to generate a reference model, encompassing 63 cardiac cell types. We then benchmarked published 2D and 3D hPSC-CM models to determine subtypes and maturities of the CMs in the context of the developing heart. Initially, our results highlighted successes in the field; protocols aimed at improving cardiomyocyte maturation were predicted largely as compact CMs, validating our model. Most protocols were predicted to contain high purities of CMs. However, heterogeneity was seen in CM subtypes, revealing broader trends in hPSC-CM models. Interestingly, we found a right vs left bias in ventricular hPSC-CMs and a tendency for hPSC-CMs to be conduction-system-like at early stages of differentiation, perhaps explaining arrhythmogenicity in clinical application. Our model and findings have broad implications for regenerative medicine and drug discovery, allowing researchers to evaluate the hPSC-CMs and optimise stem-cell protocols, for intended clinical, pharmaceutical or research applications.

Funding Source: BBSRC, BHF, Wellcome Trust, Kusuma Trust.

CHARACTERIZATION OF TRANSCRIPTIONAL REGULATIONS OF TELOMERASE IN CANCER AND STEM CELLS

Tse, **Lap Hang**, *School of Biomedical Sciences, The University of Hong Kong, Hong Kong*
Shih, David J. H., *School of Biomedical Sciences, The University of Hong Kong, Hong Kong*

Telomerase reverse transcriptase (TERT) play crucial roles in maintaining chromosomal stability through telomere elongation, conferring cells the capacity for unlimited replication. While TERT expression is predominantly restricted to stem cells and absent in differentiated cells due to transcriptional repression, its re-activation is a prevalent feature in approximately 90% of cancers, representing a hallmark of oncogenic transformation. Elevated expression of TERT in cancer is frequently associated with promoter mutations that introduce or disrupt binding motifs. Hypermethylation occurring upstream of the TERT transcription start site has also been identified as a key oncogenic feature across many cancers. However, the transcriptional regulation of TERT is still incompletely understood. By integrating genomic data from stem and cancer cell lines, including DNA sequencing, DNA methylation profiling, histone modification profiling, and RNA sequencing, we dissect how TERT promoter sequence variants, epigenetic marks, and transcriptional factors modulate TERT expression in stem cells and cancer cells. We develop statistical models to characterise the transcriptional regulations of TERT across different cell types. By identifying the differences in TERT transcriptional regulation between cancer and stem cells, we hope to provide insights for potential therapeutic strategies targeting TERT dysregulation in cancer cells while preserving its physiological function in normal stem cells.

UNSUPERVISED APPROACHES TO DECODE THE FUNCTIONAL IMPACT OF GENETIC VARIATION

Palpant, Nathan, *Institute for Molecular Bioscience, The University of Queensland, Australia*

The increasing availability of large-scale genomic data has transformed our ability to study genetic regulation of cell states. However, understanding how genetic variation governs cellular function and complex diseases remains a challenge, requiring new analytical frameworks capable of integrating diverse genomic datasets to infer functional relationships. This seminar will present new unsupervised computational approaches for dissecting genetic regulation of cellular phenotypes. Our analysis of evolutionary and epigenetic conservation across human cell types has revealed domains under cellular constraint that encode functional determinants of cell identity. By calculating genome-wide, single base resolution cellular constraint scores, I will demonstrate their utility in fine-mapping causal variants from genome-wide association studies, improving polygenic risk models, and predicting clinical outcomes in machine learning-based cancer survival models. These findings form the basis for development of multi-omic genome-wide unsupervised machine learning frameworks and variant-to-trait models that provide powerful approaches for functional annotation of non-coding variants and partitioning disease-associated genetic variants governing complex trait and disease sub-phenotypes. I will illustrate the versatility of these methods in studying multi-lineage differentiation from pluripotent stem cells and highlight ongoing efforts to study population-scale data to parse the genetic basis of complex diseases. These studies illustrate new strategies to bridge the gap between genomic variation and cellular function for guiding scalable and interpretable solutions to advance our understanding of human development, disease, and therapeutic discovery.

CLINICAL TRANSLATION OF BIOENGINEERED TISSUE-BASED THERAPEUTICS
8:30 AM – 10:00 AM
HALL 3F, LEVEL 3

CLINICAL APPLICATION OF PATIENT-DERIVED ORGANOIDS IN THE TREATMENT OF INFLAMMATORY BOWEL DISEASE
Okamoto, Ryuichi, *Department of Gastroenterology and Hepatology, Institute of Science Tokyo, Japan*

Inflammatory Bowel Disease (IBD), which includes ulcerative colitis (UC) and Crohn's disease (CD), is a chronic inflammatory disorder of the gastrointestinal tract. The incidence of IBD is rising in Japan, with an estimated 300,000 individuals currently affected. The pathophysiology of IBD is complex, involving immune system dysregulation, alterations in the gut microbiome, and various environmental factors. The introduction of treat-to-target (T2T) strategies has transformed IBD management, setting new therapeutic objectives that include the resolution of clinical symptoms and mucosal healing, the latter serving as a key marker for long-term remission. Despite the success of biologic therapies, particularly anti-TNF-α agents, a subset of patients continues to experience refractory disease with persistent mucosal injury, underscoring the need for innovative therapeutic approaches to promote mucosal repair. Recent advancements in intestinal stem cell (ISC) research have enabled the generation of organoids from both murine ISCs and human patient biopsies, providing a promising model for regenerative therapies. These organoids have shown potential as a novel treatment for refractory IBD ulcers, supported by promising findings from animal models. This presentation will review a clinical study exploring the application of organoid-based regenerative therapy for UC and discuss the emerging role of regenerative medicine in IBD treatment, which may offer new possibilities for achieving long-term remission in patients with difficult-to-treat disease.

GENE THERAPY CLINICAL TRIAL FOR HEART FAILURE WITH PRESERVED EJECTION FRACTION (MUSIC-HFPEF) INFORMED BY IN VITRO SCREENING WITH STEM CELL-BASED BIOENGINEERED MINI-HEARTS
Costa, **Kevin D.**, *Novoheart, Medera Biopharm, USA*
Fudim, Marat, *Duke University Medical Center, USA*
Rudy, Jeffrey, *Sardocor, Medera Biopharm, USA*
Levine, Benjamin, *UT Southwestern Medical Center, USA*
Guerrero, Janet, *Sardocor, Medera Biopharm, USA*
Chan, Camie, *Medera Biopharm, USA*
Li, Ronald, *Sardocor, Medera Biopharm, USA*

Heart failure with preserved ejection fraction (HFpEF) is a major health problem with increasing prevalence. HFpEF has few effective treatments but is associated with high mortality and morbidity. A critical characteristic of HFpEF is abnormal intracellular Ca2+ relaxation, which is due to a decrease in expression and function of the cardiac sarcoplasmic reticulum calcium ATPase pump (SERCA2a). Direct SERCA2a activation through gene therapy holds promise for more potent improvements in myocardial relaxation, potentially leading to clinical benefits in HFpEF. Delivery of an adeno-associated type 1 vector carrying SERCA2a (AAV1.SERCA2a) improved lusitropy in a dose-dependent manner in bioengineered human mini-heart tissue

models of HFpEF created using human pluripotent stem cell-derived ventricular cardiomyocytes. Our ongoing phase 1/2a clinical trial for the treatment of HFpEF (MUSIC-HFpEF) delivers two doses of AAV1.SERCA2a, 3E13 viral genome(vg)/patient and 4.5 E13 vg/patient, through intracoronary infusion in HFpEF patients who have a Pulmonary Capillary Wedge Pressure of 25 mmHg or higher at rest. To date five patients have received a dose of 3E13 vg/patient of AAV1.SERCA2a and the follow-up period has been 4 to 13 months. There have been no gene therapy or procedure–related serious adverse events. Four out of the five patients in the 3E13vg/patient group have shown improvements in NYHA heart failure classification at 6 months. Clinically meaningful improvements in 6-minute walk test (6MWT), and decrease/stabilization in NT-Pro-BNP and in high sensitivity troponin, two clinical biomarkers for heart failure, have also been observed in some of the patients. The enrollment of patients at a higher dose of 4.5E13 vg/patient is ongoing. These encouraging results of AAV1.SERCA2a in patients with HFpEF may offer alternative treatment strategies to patients with HFpEF where a large unmet need remains. We note that the FDA accepted our application for Fast Track Designation of the MUSIC-HFpEF trial in part due to efficacy data from the stem cell-derived human mini-heart models, demonstrating our team's success in developing alternatives to animal testing in alignment with the FDA Modernization Act 2.0.

Clinical Trial ID: US FDA, NCT06061549.

AUTOLOGOUS TRANSPLANTATION OF AIRWAY BASAL STEM CELLS IN COPD PATIENTS: A RANDOMIZED, SINGLE-BLIND, PLACEBO-CONTROLLED PHASE 2 TRIAL

Zuo, Wei, *School of Medicine, Tongji University, China*
Li, Shiyue, *Guangzhou Medical University, China*
Niu, Lingyun, *Tongji University, China*
Zhang, Ting, *Regend Therapeutics, China*

Chronic obstructive pulmonary disease (COPD) is the third leading cause of death worldwide characterized by irreversible lung tissue damage and progressive decline of pulmonary function, which prompts the exploration of regenerative therapies. Airway basal progenitor cells (BCs) have demonstrated great lung repair capacity in preclinical models as well as in pilot clinical trials. The current study aims to investigate the safety and efficacy of BC transplantation in COPD patients with gas transfer capacity deficiency, in a multicenter, randomized, single-blind, placebo-controlled, phase 2 clinical trial in China. Autologous BCs were isolated from healthy lung tissue via bronchoscopic brushing, manufactured into cell product in GMP facilities, and then transplanted into the patient lung via bronchoscopy at the dose of 2.5×10^6 cells per kilogram of body weight. 50 patients (n = 25 in placebo control, n=25 in intervention group) were evaluable within 24 weeks post cell transplantation. After cell transplantation, the occurrence of adverse events showed no substantial difference between groups. Participants in the control group experienced averagely −0.159 mmol/min/kPa decline of diffusing capacity of the lung for carbon monoxide (DLCO) from baseline, whereas the intervention group displayed averagely +0.117 mmol/min/kPa improvement of DLCO (AUC p-value=0.0356), which indicated significant improvement of lung gas transfer capacity after cell therapy. Additionally, participants in the control group exhibited averagely +0.84 points increase in the COPD Assessment Test (CAT) score, while the intervention group exhibited averagely −2.33 points reduction in CAT score (AUC p-value=0.0397), indicating significant improvement of COPD symptoms after cell therapy. The six-minute walking distance test (6MWD) also indicated significantly improved

exercise capability (+46 meters) in the intervention group patients. In conclusion, autologous transplantation of cultured BCs is safe and may represent a promising therapeutic strategy for COPD patients with gas transfer capacity deficiency. This study was approved by China National Medical Product Administration and registered at ClinicalTrials.gov (NCT05638776).

Funding Source: This research was supported by Jiangxi Xianhe Medical Technology Co., Ltd.

Clinical Trial ID: NCT05638776.

PRECLINICAL STUDY OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED ENDOTHELIAL CELLS FOR PERIPHERAL ARTERY DISEASE

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Oh, Jee Eun, *Karisbio, South Korea*
Kim, Yonghak, *Yonsei University, South Korea*
Sohn, Dongchan, *Karisbio, South Korea*
Jung, Cholomi, *Yonsei University, South Korea*
Bae, Jung Yoon, *Yonsei University, South Korea*
Kim, Sangsung, *Karisbio, South Korea*
Kim, Hyun Ok, *Yonsei University, South Korea*
Choi, Donghoom, *Yonsei University, South Korea*
Yoon, Young-sup, *Emory University, USA*

Peripheral artery disease (PAD), affecting over 230 million people globally, can progress to chronic limb-threatening ischemia (CLTI), a condition often resulting in limb amputation. Human induced pluripotent stem cell-derived endothelial cells (hiPSC-ECs) have emerged as a promising regenerative therapy for PAD; however, their clinical use remains limited due to the absence of regulatory criteria and preclinical validation. To address this, we conducted a comprehensive study evaluating the feasibility, efficacy, and safety of clinical-grade hiPSC-ECs generated from PAD patients in the context of developing future autologous therapies. We reprogrammed peripheral blood mononuclear cells from three PAD patients into hiPSC lines using episomal plasmids. These lines exhibited stable karyotypes, expressed pluripotency markers, and formed teratomas. Directed differentiation yielded hiPSC-ECs that displayed cobblestone morphology, expressed endothelial markers (CDH5: 98.4 ± 0.2%, VWF: 94.4 ± 1.3%), and maintained genomic stability confirmed by CGH array. Functional assays revealed robust nitric oxide production and tube formation. In a murine hindlimb ischemia model, PAD-hiPSC-ECs significantly improved perfusion (~3.3-fold), reduced limb loss (~8.8 ± 0.6%), and enhanced vascular density (~2.7-fold) versus controls. Long-term toxicity, biodistribution, and tumorigenicity assessments in nude mice showed no adverse findings over 12 months. Based on these results, our cell therapy received regulatory approval for clinical trials in Korea targeting moderate-to-severe PAD and patient recruitment has begun. This first-in-field preclinical validation of autologous hiPSC-EC therapy demonstrates its therapeutic potential and safety, and provides critical translational and regulatory groundwork for clinical applications.

Funding Source: RS-2024-00509295, RS-2013-00214899, RS-2024-00333839.

DEVELOPING A PSC-DERIVED THERAPY FOR HEARING LOSS: PAVING THE WAY FOR A FIRST-IN-HUMAN CLINICAL TRIAL

Rivolta, Marcelo N., *University of Sheffield and Rinri Therapeutics, England*

Disabling hearing loss is a growing global concern having a significant social, personal, and economic impact. While sensory hair cells are essential for hearing, mounting evidence suggests that the loss of neural connections between hair cells and the brainstem often precedes and exceeds hair cell damage, as seen for instance in presbycusis. Auditory neuropathy is another example of the importance of cochlear neural health, characterized by neuronal loss despite preserved hair cell function. Currently, there are no disease-modifying therapies; hearing aids and cochlear implants are the only interventions available. Cochlear implants can partially compensate for hair cell loss, but no regenerative treatment to target cochlear neuron degeneration exists to date. To address this, we are using human PSCs to target the auditory nerve. We have shown that we can restore hearing in a deafened gerbil model of auditory neuropathy, by transplanting hESC-derived otic neuroprogenitors (hONPs) into the cochlear nerve. Adaptations to the manufacturing process for hONPs have not impacted efficacy, as different batches display a similar functional profile. To model cochlear implantation, we used a fully-implantable rodent stimulator in which the electrode is activated by a magnetic field – with this, we demonstrated functional integration between the transplanted cells and the implant. To monitor for safety, we have carried out long-term studies exploring the distribution of transplanted cells. Animals were followed for up to a year, with whole-body MRI scans performed at termination to identify any potential lesions. Additionally, biodistribution studies for human-specific DNA sequences were performed by QPCR. No tumours attributed to the test article were found and there was no detection of cells spreading systemically. Similar safety studies were undertaken using the Rag2/Il2rg (SRG) double knock-out rat. Rinri is now preparing to initiate a first-in-human trial using Rincell-1, a preparation of ONPs derived from human embryonic stem cells. A novel surgical approach for injecting Rincell-1 into the cochlear neural spaces via the round window has also been developed. The initial clinical trial will focus on assessing the safety of Rincell-1 as an adjunct to a cochlear implant, in patients with neural hearing loss.

Funding Source: The clinical trial will be supported and sponsored by Rinri Therapeutics.

ENDODERM STEM-CELL DERIVED ISLET TISSUE FOR THERAPEUTICS AND DISEASE MODELING

Cheng, Xin, *Shanghai Institute of Biochemistry and Cell Biology, China*

Stem cell-derived islet transplantation has emerged as a promising therapy to restore normoglycemic islet function in both T1D and T2D patients, but questions remain regarding the optimal strategy of immunosuppression in the setting of T1D. We initiated a pilot study to evaluate the safety and effectiveness of the autologous or allogeneic Endodermal Stem Cell-derived islet (E-islet) transplantation in both T2D and T1D patients with partial or complete loss of β cell function. The hepatic portal venous infusion of the autologous E-islets led to the restoration of islet function and euglycemia in the first patient with T2D and a prior functioning renal transplant for diabetes-induced end-stage renal failure after 25 years of diabetes. Chronic

immunosuppression was given to maintain the kidney transplant, but likely was not needed for autologous E-islets. The second patient who has a 20-year history of T1D complicated by severe hypoglycemia received autologous E-islets under ultra-low-dose calcineurin-inhibitor-free immunosuppression for the initial transplant and full-dose immunosuppression for a second transplant one year later. "While the first transplant failed to achieve graft function, by week 23 post 2nd transplant, time-in-range (TIR) improved from 48% to 97%. And pre-/postprandial C-peptide levels at 0·33/0·43 nmol/L, respectively. The third and fourth patients who had brittle T1D and received healthy donor-derived allogeneic E-islets were free from sever hypoglycemic events by week 6, and achieved robust graft function and complete insulin independence with TIR reaching 100% by weeks 30 and 36, respectively. The E-islet transplants in the four patients were tolerated well, and there was no tumor formation observed throughout the period of follow up for at least one year. These observations indicate that both autologous and allogeneic E-islet therapies have demonstrated potential to restore glycemic control in T1D under sufficient immunosuppression. Reduced-dose immunosuppression warrants exploration in autologous stem cell-derived islet transplant, though full-dose chronic maintenance immunosuppression is currently necessary to prevent autoimmune recurrence and sustain graft function. Future progress in hypoimmune engineering is key to removing immunosuppression need.

FATE CHOICES IN DEVELOPMENT AND REGENERATION
8:30 AM – 10:00 AM
THEATER 1, LEVEL 1

SYNTHETIC EX UTERO EMBRYOGENESIS: FROM NAIVE PLURIPOTENT CELLS TO BONA FIDE EMBRYO MODELS
Hanna, Jacob, *Department of Molecular Genetics, Weizmann Institute of Science, Israel*

The identity of somatic and pluripotent cells can be epigenetically reprogrammed and forced to adapt a new functional cell state by different methods and distinct combinations of exogenous factors. The aspiration to utilize such in vitro reprogrammed pluripotent and somatic cells for therapeutic purposes necessitates understanding of the mechanisms of reprogramming and differentiation and elucidating the extent of equivalence of the in vitro derived cells to their in vivo counterparts. In my presentation, I will present my group’s recent advances toward understanding these fundamental questions and further detail our ongoing efforts to generate developmentally unrestricted human naive pluripotent cells with embryonic and extra-embryonic developmental potential. I will expand on new avenues for utilizing custom made electronically controlled ex utero platforms and optimized conditions for growing natural mammalian embryos ex utero for extended periods capturing development from pre-gastrulation until advanced organogenesis, for better studying of stem cell transitions during embryogenesis and organogenesis. I will detail how the latter platforms offered an exclusive technical platform to demonstrate and unleash the self-organizing capacity of mouse naïve PSCs to generate post-gastrulation synthetic Bona Fide synthetic whole developmental models with both embryonic and extraembryonic compartment ex utero, as well as our ability to extend these findings with naïve human PSCs and generate complete structured day 14 human developmental models and beyond. Collectively, I will be highlighting prospects for new platforms for advancing human disease and embryogenesis developmental modelling.

A NOVEL PROTEIN CYTB-187AA ENCODED BY THE MITOCHONDRIAL GENE CYTB MODULATES MAMMALIAN EARLY DEVELOPMENT

Liu, Xingguo, *Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, China*
Hu, Zhijuan, *Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, China*
Yang, Liang, *Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, China*
Zhang, Maolei, *Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, China*

The mitochondrial genome transcribes 13 mRNAs coding for well-known proteins essential for oxidative phosphorylation. We demonstrate here that cytochrome b (CYTB), the only mitochondrial-DNA-encoded transcript among complex III, also encodes an unrecognized 187 amino-acid-long protein, CYTB-187AA, using the standard genetic code of cytosolic ribosomes rather than the mitochondrial genetic code. After validating its existence using mass spectrometry and antibodies, we show that CYTB-187AA is mainly localized in the mitochondrial matrix and promotes the pluripotent state in primed-to-naïve transition by interacting with SLC25A3 to modulate ATP production. We further generated a transgenic knock-in mouse model of CYTB-187AA silencing and found that reduction of CYTB-187AA impairs females' fertility by decreasing the number of ovarian follicles. For the first time we uncovered a dual translation pattern of a mitochondrial mRNA and demonstrated the physiological function of this 14th protein encoded by mtDNA.

CO-DEVELOPMENT BETWEEN SOMITES, NEURAL TUBE, AND NOTOCHORD ESTABLISHES DORSOVENTRAL PATTERNING IN HUMAN TRUNK-LIKE ORGANOIDS

Wu, Tianming, *School of Biomedical Science, The Chinese University of Hong Kong (CUHK), Hong Kong*
Yu, Hao, *School of Biomedical Science, The Chinese University of Hong Kong (CUHK), Hong Kong*
Wong, Brian, *School of Biomedical Science, The Chinese University of Hong Kong (CUHK), Hong Kong*
Teng, Kexin, *School of Biomedical Science, The Chinese University of Hong Kong (CUHK), Hong Kong*
Xiang, Weiman, *School of Biomedical Science, The Chinese University of Hong Kong (CUHK), Hong Kong*
Xu, Ling, *School of Biomedical Sciences, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China*
Zhang, Jianan, *School of Biomedical Sciences, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China*
Ng, Ethel, *School of Biomedical Science, The Chinese University of Hong Kong (CUHK), Hong Kong*
Kam, Angel, *School of Biomedical Science, The Chinese University of Hong Kong (CUHK), Hong Kong*
Vong, Joaquim, *School of Biomedical Science, The Chinese University of Hong Kong (CUHK), Hong Kong*

Zhang, Jiannan, *Key Laboratory of Bio-resources and Eco-environment of Ministry of Education, College of Life Sciences, Sichuan University, China*
Gao, Bo, *School of Biomedical Science, The Chinese University of Hong Kong (CUHK), Hong Kong*
Tsui, Stephen, *School of Biomedical Science, The Chinese University of Hong Kong (CUHK), Hong Kong*
Dalton, Stephen, *School of Biomedical Science, The Chinese University of Hong Kong (CUHK), Hong Kong*

Recent advancements in human stem cell-based embryo models have enabled in vitro studies of trunk formation during early embryogenesis that were previously difficult to address. These models recapitulate some aspects of anteroposterior (A-P) asymmetry breaking and elongation. However, they fail to achieve coordinated dorsoventral (D-V) patterning and consequently, generate embryonic tissues with either dorsal-biased or ventral-biased cell identities. Here we present the co-development of neuromesodermal and notochordal progenitors to form a progressively A-P breaking-patterned organoid including a neural tube, a notochord and paired somites. We show that this trunk-like organoid has morphometric features and transcriptome profiles resembling post-implantation human organogenesis from Carnegie Stages 8 to 10. Modulation of signaling activities by addition of exogenous WNT, FGF, BMP, SHH, and Retinoic Acid resulted in the formation of a D-V axis in both the neural tube and somites, as confirmed by time-course single-cell RNA sequencing (scRNA-seq) and end-point spatial scRNA-seq. In addition, loss of function mutations in VANGL2 leads to neural tube truncation and compaction of segmented somites, consistent with observations in mouse embryos and supporting the important role of VANGL2 in early human miscarriages. Our findings provide a valuable model to assess signaling modulation at early human embryo stages. This lays the foundation for future studies of the mechanisms underlying human A-P and D-V body axis establishment.

Funding Source: The Hong Kong Jockey Club Charities Trust.

NEUROGENESIS IS SPARED DURING FETAL GROWTH RESTRICTION VIA INDUCTION OF ATF4-DDR2 SIGNALING IN RADIAL GLIA

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Broncel, Goska, *The Francis Crick Institute, UK*
Gould, Alex, *The Francis Crick Institute, UK*
Ryohei, Iwata, *VIB Center for Brain and Disease Research, Belgium*
Serpente, Patricia, *The Francis Crick Institute, UK*
Vanderhaeghen, Pierre, *VIB Center for Brain and Disease Research, Belgium*

Allometric scaling has fascinated developmental biologists for decades. A striking example of this is brain sparing during fetal growth restriction. This important survival strategy, conserved from insects to mammals, is often triggered by developmental malnutrition. In humans, brain sparing occurs in 1:7 births worldwide and, although it was documented well over 60 years ago, the underlying mechanisms remain unclear. Here we identify a cellular and molecular mechanism for brain sparing in the context of a maternal low protein (LP) mouse model of fetal growth restriction. The LP model restricts fetal growth of the body by ~30% and the liver by ~50%, yet the neocortex remains relatively unperturbed. Proliferation of neural progenitors (radial glia) in the neocortex is fully spared and, at late gestational ages, moderately higher than

in controls. Furthermore, layer-specific subtypes of cortical projection neurons are generated largely as normal. This impressive brain sparing requires mTORC1 and LP-dependent induction of ATF4 in radial glia. Conditional knockout of ATF4 in cortical progenitors from the mid-gestational stage indicates that its brain sparing function is not mediated via a typical integrated stress response. Instead, we find that a key transcriptional target of ATF4 is Discoidin Domain Receptor Tyrosine Kinase 2 (Ddr2), a receptor tyrosine kinase we identified in a brain sparing proteomics screen. ATF4 is both necessary and sufficient for Ddr2 upregulation in radial glia. Importantly, conditional knockout of Ddr2 indicates that it is required for radial glial proliferation during brain sparing but not normal brain development. Together, these findings identify a specific mechanism for brain sparing that is activated in radial glia to protect them from the potentially harmful effects of maternal malnutrition.

Funding Source: Sir Henry Wellcome Fellowship, The Francis Crick Institute core funding.

A STEPWISE MODE OF TGFB-SMAD SIGNALING AND DNA METHYLATION REGULATES NAÏVE-TO PRIMED PLURIPOTENCY AND DIFFERENTIATION

Wang, Qiong, *Department of Histoembryology, Genetics and Developmental Biology, Shanghai Jiao Tong University School of Medicine, China*

The formation of transcription regulatory complexes by the association of Smad4 with Smad2 and Smad3 (Smad2/3) is crucial in the canonical TGFβ pathway. Although the central requirement of Smad4 as a common mediator is emphasized in regulating TGFβ signaling, it is not obligatory for all responses. The role of Smad2/3 independently of Smad4 remains understudied. Here, we introduce a stepwise paradigm in which Smad2/3 regulate the lineage priming and differentiation of mouse embryonic stem cells (mESCs) by collaboration with different effectors. During the naïve-to-primed transition, Smad2/3 upregulate DNA methyltransferase 3b (Dnmt3b), which establishes the proper DNA methylation patterns and, in turn, enables Smad2/3 binding to the hypomethylated centers of promoters and enhancers of epiblast marker genes. Consequently, in the absence of Smad2/3, Smad4 alone cannot initiate epiblast-specific gene transcription. When primed epiblast cells begin to differentiate, Dnmt3b becomes less actively engaged in global genome methylation, and Smad4 takes over the baton in this relay race, forming a complex with Smad2/3 to support mesendoderm induction. Thus, mESCs lacking Smad4 can undergo the priming process but struggle with the downstream differentiation. Our two-step model is also consistent with the in vivo data. Conditional knockout of Smad4 in the epiblast using the Cre-LoxP system does not prevent the development from the epiblast to the gastrulation initiation, but shows focal defects in the primitive streak. As gastrulation proceeds, Smad4-deficient embryos fail to form derivatives of the anterior primitive streak, including definitive endoderm, sharing many phenotypic similarities with the downregulation of the Nodal/Smad2/3/Foxh1 pathway. Thus, Smad4 has a limited role during the priming process but is required for primitive streak/mesendoderm differentiation. These findings highlight the distinct roles of Smad2/3 and Smad4 in the process of lineage priming and differentiation, providing a fresh viewpoint on the complex mechanisms underlying TGFβ signaling. In addition, Dnmt3b is identified as a newly discovered partner of receptor-phosporylated Smad2/3, thereby shedding light on their collaborative regulatory mechanism underlying the priming process.

UNRAVELING LSD1'S ROLE IN REGULATING HUMAN BRAIN-ENRICHED ECM/CELL ADHESION GENES AND PROGENITOR DYNAMICS
Muralidharan, Bhavana, *Modelling Neurodevelopment and Disease, Institute for Stem Cell Science and Regenerative Medicine, India*

The study of the mouse brain has significantly advanced our understanding of mammalian neurogenesis, including regional patterning. Yet, our grasp of human-specific developmental attributes of brain development is still evolving. Recent research from our lab has revealed these differences, particularly focusing on LSD1, a highly conserved histone demethylase and subunit of the NuRD complex. LSD1 plays a pivotal role in neuronal differentiation, serving as a regulatory switch between cell proliferation and differentiation by negatively influencing the Notch signaling pathway, which is associated with cell proliferation. Additionally, LSD1 represses cell adhesion and extracellular matrix-associated genes enriched in human neural stem cells. Our comparative analysis of histone methylation marks at LSD1 target gene promoters and gene regulatory elements revealed increased methylation at these enhancer sites in the human genome, which is absent or minimal in mouse loci. By overexpressing human brain-enriched ECM genes in the mouse brain, we have been able to introduce human-specific features into the mouse neocortex, such as the generation of minor gyri and sulci (in an otherwise lissencephalic, or smooth, mouse brain). This process is regulated by ECM genes through the generation of increased numbers of progenitors. This study highlights the unique functions of LSD1 in humans, despite its evolutionary conservation, by targeting human-gained enhancers and genes prevalent in human neural stem cells, thereby enriching our understanding of LSD1's role in human neurogenesis and highlighting aspects unique to the human brain.

MANUFACTURING WITH COMMERCIALIZATION IN MIND
8:30 AM – 10:00 AM
HALL 3G, LEVEL 3

TITLE AND ABSTRACT NOT AVAILABLE AT THE TIME OF PUBLISHING
Baptista, Ricardo, *SmartCella, Sweden*

QC AND CQAs: QUALITY ASSESSMENT FOR PSC CELL BANKS TODAY AND A VISION FOR THE FUTURE
Ludwig, Tenneille, *WiCell Stem Cell Bank, WiCell Research Institute, USA*
As more groups seek to move discoveries to clinically relevant treatments, it is clear that successful translation is dependent on more than just innovative science and technical excellence. Early, careful selection of starting materials can ease the path to clinical and commercial translation and prevent good science from being overcome by insurmountable hurdles in regulatory compliance. This talk will focus specifically on critical factors for consideration in the selection and generation of seed material for cGMP PSC Master Cell Bank production. Significant efforts in diligence are necessary to enable regulatory compliance and promote clinical and commercial success. Such efforts include but are not limited to ensuring complete cell line documentation which includes ethical and technical provenance, appropriate cell line procurement which assures freedom to operate, and confirming critical quality attributes of the material.

SCALING MOUNT COMMERCIAL: NAVIGATING THE PEAKS OF MANUFACTURING COMPARABILITY

Stefan, Irion, *BlueRock Therapeutics, USA*

Scaling manufacturing processes from the small-scale environment of process development to commercial large-scale production is a critical, yet often challenging, step on the path to bringing a product to market. While the fundamental process may appear similar, the practicalities of larger volumes, different equipment, and altered process dynamics can introduce significant variability. A key hurdle is the demonstration of comparability – ensuring that the product manufactured at scale is analytically and functionally equivalent to that produced at smaller scales used for clinical trials. This requires a robust strategy that goes beyond simply increasing batch size. This session will delve into the multifaceted challenges inherent in demonstrating comparability during manufacturing scale-up with a commercialization mindset. We will explore the complexities of establishing meaningful comparability protocols when transitioning between different process scales. A significant focus will be placed on the challenges associated with sampling strategies and the analytical assays used to assess product quality. Obtaining representative samples from large volumes can be difficult, and assay variability, suitability, and the need for method validation at different scales must be carefully considered. Insights from our recent work on comparing complex biological datasets, as detailed in our paper on scCompare, offer valuable perspectives on tackling the inherent heterogeneity and analytical challenges relevant to demonstrating comparability across scales. Finally, we will address the critical role of functional assays in demonstrating that the biological activity and efficacy of the product remain consistent despite scale-up, highlighting the challenges in developing and validating assays that are sensitive and predictive across different manufacturing scales. Successfully navigating these challenges through proactive planning, rigorous testing, and a deep understanding of the process is paramount to ensuring product quality, regulatory approval, and ultimately, commercial success.

CELL IDENTITY CONVERSION: LIVER REGENERATION AND CELL THERAPY

Hui, Lijian, *Shanghai Institute of Biochemistry and Cell Biology, Center for Excellence in Molecular Cell Science, CAS, China*

Our team studies molecular pathology of liver diseases, with the focus on cell identity conversion, i.e., dedifferentiation and transdifferentiation, in liver regeneration and tumorigenesis. We demonstrated the conversion from fibroblasts into hepatocytes in vitro, thereby providing one of the first evidence of transdifferentiation in mammalian cells. We were also among the first to report cell identity conversion in liver injury and repair. Based on these fundamental findings, we generated functional hepatocytes, hiHep and ProliHH, for clinical application. Innovative therapies based on these cells, namely bioartificial liver devices and encapsulated liver organoids, are now being tested in clinics.

INCREASING TRANSLATION BY INBUILT REGULATORY CONSIDERATIONS

Hidalgo-Simon, Ana, *Internal Medicine, Leiden University Medical Center, Netherlands*

The translation of cell-based therapies from bench to bedside remains a complex and arduous path, and success rates for clinical translation remaining low. A significant challenge is the

knowledge gap between academic research and established regulatory pathways, where academic-originated ideas often struggle to align with the rigorous standards required for approval and commercialization. Traditional engagement of academic developments with regulatory considerations tends to be limited or delayed, hindering the timely progression of therapies to market. Incorporating regulatory thinking early in the research and development process is crucial for improving the likelihood of successful translation. By integrating regulatory insights from the outset, researchers can better align their manufacturing strategies with the standards set by regulatory bodies, optimizing the chances for approval and reducing delays. Regulatory thinking can direct developers choices from the selection of starting materials, to the assessment of final product comparability and analytical assays. This proactive approach not only enhances the development process but also provides an invaluable opportunity to address regulatory hurdles before they become insurmountable challenges. In addition, considerations regarding the final step of accessing patients, not only the market, should also be a strategically element of early research.

Funding Source: The Novo Nordisk Foundation Center for Stem Cell Medicine is supported by Novo Nordisk Foundation grants (NNF21CC0073729).

STEM CELLS AND INFECTIOUS DISEASE
8:30 AM – 10:00 AM
HALL A, LEVEL 1

INFECTION, INFLAMMATION AND CANCER IN THE GUT - ORGANOIDS AS MODEL
Bartfeld, Sina, *Medical Biotechnology, Technische Universität Berlin, Germany*

Infections are still a major health threat worldwide. We need new models for infectious diseases to reach a better understanding and develop new therapies. In the gastrointestinal tract, the epithelial lining acts as physical and immunological barrier between the microorganisms of the gut and the body. The epithelial cells can sense microorganisms, which activates innate immune signaling pathways and leads to an inflammatory response. Chronic inflammations, for example caused by infection with the gastric pathogen Helicobacter pylori, can cause many diseases, including cancer. We analyse the molecular basis of innate immune signaling in the epithelium of the gastrointestinal tract and the host cell response to infections such as with H. pylori, but also Enteropathogenic E. coli, or SARS-CoV-2. To these ends, we use adult stem cell-derived organoids. We generated a biobank of human and murine organoids covering 6 sites from stomach to colon. RNA-sequencing showed that the tissue identity is conserved in the adult stem cells. Moreover, components of the epithelial innate immune sensing, such as toll like receptors, are part of the tissue identity and highly organized along the gastrointestinal tract. Infection of organoids or organoid-derived monolayers identified target cells and the molecular mechanisms of tropisms of different infections.

SPATIAL EPITRANSCRIPTOMICS UNVEILS A-TO-I RNA EDITING DYNAMICS AND HOST-VIRUS INTERACTIONS IN ZIKA VIRUS-INFECTED BRAIN ORGANOIDS
Lee, **Amos Chungwon**, *Business, Meteor Biotech, Korea*

Kang, Kyung-Sun, *Seoul National University, Korea*
Kim, Da-Hyun, *Sungshin Women's University, Korea*
Kim, Gyeongjun, *Seoul National University, Korea*
Kwon, Sunghoon, *Seoul National University, Korea*
Lee, Sumin, *Meteor Biotech, Korea*

Zika virus (ZIKV) poses a significant neurodevelopmental threat, particularly during early brain development, where its infection of neural progenitor cells (NPCs) leads to devastating outcomes such as microcephaly. Current studies employing iPSC-derived brain organoids have shed light on the phenotypic impacts of ZIKV infection but lack the spatial and molecular resolution necessary to elucidate the underlying mechanisms driving host-virus interactions. To address these gaps, we integrated Spatially-resolved Laser Activated Cell Sorting (SLACS) with Select-seq to achieve spatially resolved, base-resolution mapping of host transcriptomes, viral genomes, and RNA editing landscapes within ZIKV-infected brain organoids. This approach revealed distinct spatial patterns of Adenosine-to-Inosine (A-to-I) RNA editing, catalyzed by ADAR enzymes, which modulate ZIKV replication, host immune responses, and mitochondrial dysfunction. SOX2+ NPC-enriched regions exhibited heightened A-to-I editing associated with viral replication and immune evasion, while TUJ1+ differentiated neuron regions displayed distinct transcriptional profiles linked to synaptic function. Additionally, ZIKV envelope protein-expressing cells demonstrated unique editing and gene expression patterns, suggesting potential viral adaptation mechanisms. Pathway analysis highlighted the disruption of critical neurodevelopmental pathways, such as Notch signaling, and mitochondrial processes in ZIKV-infected regions, providing novel insights into the virus's neuropathogenicity. This study represents the first spatially resolved epitranscriptomic investigation of ZIKV pathogenesis in brain organoids, offering a transformative framework for understanding the molecular dynamics of host-virus interactions. By identifying spatially defined biomarkers and therapeutic targets, our findings establish a foundation for precision medicine in combating ZIKV and other neurotropic viral infections, while advancing the application of spatial epitranscriptomics in developmental biology.

Funding Source: This research was funded by the Ministry of Science and ICT (RS-2023-00266110, RS-2024-00454407, RS-2024-00468338) and Ministry of Trade, Industry and Energy of Republic of Korea (20024391, RS-2024-00451981, RS-2024-00508416).

HUMAN RESPIRATORY ORGANOIDS SUSTAINED PROPAGATION OF PREVIOUSLY UNCULTIVABLE HUMAN RHINOVIRUSES AND ELUCIDATION OF VIRUS-HOST INTERACTION

Zhou, Jie, *The University of Hong Kong, Hong Kong*
Li, Cun, *The University of Hong Kong, Hong Kong*
Wan, Zhixin, *The University of Hong Kong, Hong Kong*
Yu, Yifei, *The University of Hong Kong, Hong Kong*

We have established the first organoid culture system of the human respiratory epithelium directly from primary lung tissues and nasal epithelial cells. We induce maturation in long-term expandable organoids and generate nasal, airway, and alveolar organoids that contain all major epithelial populations in the human respiratory tract. Thus, the respiratory organoid culture system allows us to rebuild and propagate the entire human respiratory epithelium in culture

plates with excellent efficiency and stability. The lack of a robust system to reproducibly propagate HRV-C, a family of viruses refractory to cultivation in standard cell lines, has substantially hindered our understanding of this common respiratory pathogen. We sought to develop an organoid-based system to reproducibly propagate HRV-C, and characterize virus-host interaction using respiratory organoids. We demonstrate that airway organoids sustain serial virus passage with CYT387-mediated immunosuppression, whereas nasal organoids that more closely simulate the upper airway achieve this without any intervention. Nasal organoids are more susceptible to HRV-C than airway organoids. Intriguingly, upon HRV-C infection, we observe an innate immune response that is stronger in airway organoids than in nasal organoids, which is reproduced in a Poly(I:C) stimulation assay. Treatment with α-CDHR3 and antivirals significantly reduces HRV-C viral growth in nasal organoids. Collectively, we develop an organoid-based system to reproducibly propagate the poorly cultivable HRV-C, followed by an in-depth characterization of HRV-C infection and innate immunity in physiologically active respiratory organoids. The organoid-based HRV-C infection model can be extended for developing antiviral strategies. More importantly, our study has opened an avenue for propagating and studying other uncultivable human and animal viruses.

LINKING PRENATAL INFLAMMATION TO SYNAPTIC DYSFUNCTION IN PSYCHIATRIC DISORDERS

Cota Coronado, **Jose Agustin**, *Monash University, Australia*
Hill, Rachel, *Monash University, Australia*
Kim, Dong-Hyun, *Monash University, Australia*
Suwakulsiri, Wittaya, *University of Queensland, Australia*
Gibbons, Andrew, *Monash University, Australia*
Law, Kevin, *University of Sydney, Australia*
Thompson, Lachlan, *School of Medical Sciences, University of Sydney, Australia*
Lee, Joohyung, *Monash University, Australia*
Sundram, Suresh, *Monash University, Australia*

Maternal exposure to viral infections during pregnancy is a robustly verified risk factor for the future development of neurodevelopmental disorders, including Schizophrenia (SCZ) and Autism Spectrum Disorder (ASD). Our recent study showed severe maternal SARS-CoV-2 infection resulted in post-infection maternal elevation of serum cytokines, IL-6 and IL-17, and was associated with significant DNA methylation changes in synaptic genes in their infants at birth and delayed neurodevelopment at 12 months. This suggested a link between maternal immune activation (MIA), synaptic dysfunction and altered neurodevelopment. Using single-nuclei RNA sequencing, we also assessed, post-mortem prefrontal cortical tissue from adults diagnosed with SCZ (n=17), Major Depressive Disorder MDD (n=19) and healthy controls (n=18). Pathway analysis revealed postsynaptic and cell-projection alterations, particularly within the glutamatergic synapse. Investigating causal relationships, we tested the direct effect of pro-inflammatory cytokines (IL-6, IL-17, IL-1b, TNF, cytokine storm (all cytokines) on human pluripotent stem cell hPSC-derived neuronal cultures at the neural progenitor stage and a mature neuronal stage (DIV 50), with targeted molecular profiling of synaptic and neurodevelopmental markers. We show dynamic transcriptional changes in developmental genes and cell proliferation when exposed to cytokines at the progenitor stage. Subsequently, synaptic genes, glutamatergic and GABAergic receptors were disrupted in the mature cytokine exposed cultures. Notably, we observed a remarkable convergence between the SARS-CoV-2 exposed infant data, the cytokine-treated cultures and post-mortem adult patient data. This

pioneering study, incorporating data sets from the cradle to the grave, evidenced a critical link between prenatal inflammation and the synaptopathy observed in certain psychiatric disorders and revealed novel molecular targets amenable to precision-medicine interventions.

Funding Source: We gratefully acknowledge the funding support for this work from the HOMER HACK Foundation, the NHMRC Ideas Grant #2001907, and the One in Five Foundation.

DYSPLASTIC EPITHELIAL REPAIR DRIVES THE TISSUE RESIDENCE OF LYMPHOCYTES TO IMPAIR ALVEOLAR REGENERATION

Xi, Ying, *ShanghaiTech University, School of Life Science and Technology, China*
Liu, Li, *ShanghaiTech University, China*
Lu, Tiantian, *ShanghaiTech University, China*
Wu, Pei, *ShanghaiTech University, China*

Severe respiratory viral infections lead to extensive damage to the alveolar epithelium and also induce a robust immune response. How immune microenvironment interacts with lung stem/progenitor cells and impact alveolar regeneration is poorly understood. Here, we found that dysplastic epithelial repair, which emerge after severe viral infections, preferably recruit and sequester both effector T cells in the lung after viral clearance. Persistent effector T cells impair alveolar regeneration mediated by airway secretory cells through secretion of IFNγ, thereby inhibiting lung functional repair. Importantly, anti-IFNγ treatment improves alveolar regeneration and lung function in vivo. Overall, our study reveals the pathogenetic role of dysplastic epithelial cells in alveolar regeneration, serving as a niche for tissue-resident lymphocytes that specifically inhibit airway secretory cell-mediated alveolar regeneration.

RESEARCH FOR VIRAL RESPIRATORY INFECTIONS USING ORGANOIDS AND ORGAN-ON-A-CHIP

Takayama, Kazuo, *Center for iPS Cell Research and Application (CiRA), Kyoto University, Japan*

Our experience with COVID-19 has underscored the importance of viral infection research and drug discovery efforts. Addressing a pandemic caused by a viral infection requires a thorough understanding of how the virus impacts the human body and the rapid development of therapeutic drugs to mitigate symptoms. Organoids and organ-on-a-chip technologies, which enable the recapitulation of structure and function of human organs in vitro, hold significant promise for accelerating research for viral infections. We previously developed respiratory organoids and airway/alveoli-on-a-chip, demonstrating their utility in infection studies with various respiratory viruses, including SARS-CoV-2, SARS-CoV, MERS-CoV, HCoV-229E, HCoV-OC43, and RSV. Our respiratory organoids and airway/alveoli-on-a-chip enable the identification of infected cells, as well as the analysis of innate immune responses, inflammatory responses, and fibrosis. Furthermore, our model has the potential to facilitate the development of antiviral drugs. In addition to the respiratory models, we have recently developed intestinal and liver models and applied them to the study of viral infections. In this presentation, we will share the latest findings from our research on viral infections using organoids and organ-on-a-chip technologies.

PLENARY III: ADULT HOMEOSTASIS: STEM CELLS IN PHYSIOLOGY AND DISEASE
10:30 AM – 12:00 PM
GRAND HALL, LEVEL 3

CARDIOPEDIA: COMPREHENSIVE MODEL OF LIGAND-FUNCTION-OMICS FOR PREDICTION OF THERAPEUTIC TARGETS

Hudson, James, *Infection and Inflammation, QIMR Berghofer, Australia*

Cardiac ligands mediate cell-cell communication and control cardiac biology and function. Adrenergic drivers of disease have been extensively studied and targeting these pathways remains the front line of cardiac therapeutics. However, the full repertoire of how ligands control heart function remains unknown and can be difficult to dissect in vivo when many ligands may impact other cardiovascular parameters such as vessel dilation or fluid retention. To address this, we have catalogued the contractile function and transcriptional signatures for cardiac ligands across ~500 human cardiac organoids (hCO) to create an encyclopedia of these responses - Cardiopedia. A library of 80 endogenous ligands and 10 small molecule agonists was curated to target >100 plasma membrane receptors expressed in both the human heart and hCO. The library included catecholamines, peptide hormones, cytokines, lipid mediators, and growth factors. hCO contractility parameters were assessed 4 and 24 hours after ligand stimulation (n = 5 hCO per ligand). A semi-automated single hCO RNA-seq pipeline was developed to assess the transcriptional responses to each ligand which we could directly correlate with hCO contractile parameters (i.e. rate, force). Dimensionality reduction of the transcriptomic data revealed distinct clusters, including inotropes, endothelins, fibrotic factors, and multiple inflammatory clusters separating interleukins and interferons. By overlaying contractile data, we identified cluster-specific functional differences, for example opposite activation and relaxation parameters for inotropes vs endothelins despite both increasing force and rate. Using differential expression analysis, unique ligand-induced transcriptional signatures have been identified which we have started exploring in human heart failure transcriptomes to predict aberrant cellular signalling. Together, this study provides a novel resource for a systems-level approach to understanding ligand control of cardiac function with the goal of understanding disease drivers and improving patient stratification.

WAKING UP “SLEEPING” NEURAL STEM CELLS

Wang, Hongyan, *Duke-NUS Medical School, Singapore*

The ability of stem cells to switch between quiescence and proliferation is crucial for tissue homeostasis and regeneration. Most neural stem cells (NSCs) in mammalian adult brains are in a mitotically dormant, quiescent state but can exit quiescence and become reactivated, in response to physiological stimuli such as the presence of nutrition. Drosophila NSCs, also known as neuroblasts, have emerged as a powerful model to study the mechanisms underlying NSC quiescence and reactivation in vivo. Quiescent NSCs are morphologically characterized by a primary cellular protrusion extending from their cell body. Recently, we demonstrated the enrichment of microtubules and F-actin in this protrusion. We showed that in quiescent NSCs, microtubules are predominantly acentrosomal and oriented plus-end-out toward the tip of the primary protrusion. We also found that the Golgi apparatus functions as a microtubule organizing centre in quiescent NSCs and that Golgi-resident GTPase Arf1 plays a new role in

microtubule assembly. We have further identified Mini Spindles (Msps)/XMAP215 as a new effector of Arf1 and a key microtubule regulator that governs NSC reactivation by regulating acentrosomal microtubule growth. We will discuss further molecular mechanisms that promote the reactivation of quiescent NSCs.

2025 ISSCR MOMENTUM AWARD LECTURE: PASSAGE OF TIME IN BRAIN ORGANOIDS: THE JOURNEY TO UNDERSTAND HUMAN BRAIN DEVELOPMENT AND MATURATION
Arlotta, Paola, *Stem Cell and Regenerative Biology, Harvard University, USA*

Much remains unknown regarding the mechanisms governing human brain maturation and aging. Human brain organoids offer a unique platform for these studies. Here, we investigated human cortical organoids cultured for periods ranging from 6 months to over 5 years in vitro. Module scores of maturations trained on the endogenous tissue show that organoids continue to develop and mature while in culture for these extended time frames. In agreement, methylation profiling revealed a strong correlation between predicted age of the organoids and time in culture. Using extracellular single-unit recordings with multielectrode arrays (MEA), we detected network bursts and action potentials with features that changed over developmental and maturation trajectories in culture. Notably, we find that human brain organoids are capable of “recording and recalling” developmental time as demonstrated by the ability of “old” progenitors to rapidly produce late progeny when exposed to inductive developmental signals. The work indicates that the human brain can develop, mature and age outside the context of the embryo.

HIGH MITOCHONDRIAL MASS CHARACTERIZES ENHANCED SELF-RENEWAL IN AGED HEMATOPOIETIC STEM CELLS
Suda, Toshio, *Institute of Hematology and Blood Disease Hospital, China*

Hematopoietic stem cells (HSCs) are a source of blood cells throughout the lifespan, but their properties and functions change with age. Healthy HSCs balance self-renewal and differentiation, with mitochondria playing a crucial role in their fate. Aging causes mitochondrial dysfunction and decreased autophagy, leading to damaged mitochondria accumulation. Yet, the role of mitochondria in aged HSCs, particularly how their mass affects HSCs characteristics, is not fully understood. We used mito-Dendra2 mice, expressing the mitochondria-specific fluorescent protein Dendra2, to show that aged HSCs with high mitochondrial mass are not exhausted but have high self-renewal potential and prefer residing in aged bone marrow. Conversely, mitochondrial mass did not directly affect peripheral output. To elucidate the genetic signature of aged HSCs, we sorted young and aged HSCs into two groups based on mitochondrial mass (low and high) and performed single-cell RNA-seq and single-cell ATAC-seq. We found that aged mitochondrial-high (mito-high) HSCs were enriched in IFNα signaling, oxidative phosphorylation (OXPHOS), and the p53 pathway, and Mitocarta3.0 showed enhanced iron homeostasis alongside OXPHOS. Furthermore, we identified 17 common genes across three cohorts, including genes specific to aged mito-high HSCs by single-cell multiomics, genes specific to long-term HSCs by bulk RNA-seq, and genes repeatedly associated with aging HSCs in a meta-analysis. Among these was Gpr183, a receptor for 7α,25-dihydroxycholesterol. GPR183-positive aged HSCs maintained higher

mitochondrial mass, increased autophagy activity, and greater self-renewal capacity compared to GPR183-negative aged HSCs. These findings suggest that GPR183 could be a useful marker for identifying functionally preserved aged HSCs, aiding in understanding and assessing HSCs during aging.

ENGINEERING REGENERATION
2:00 PM – 3:30 PM
HALL A, LEVEL 1

GUT ORGANOID-BASED MICROPHYSIOLOGICAL SYSTEMS TO STUDY MUCOSAL REGENERATION AND DEFENSE MECHANISMS
Park, Tae-Eun, *Biomedical Engineering, UNIST, Korea*

Disruption of gastric epithelium homeostasis by various foreign agents leads to numerous gastric diseases. Helicobacter pylori (H. pylori) infection, which affects over half of the global population, dysregulates the gastric mucosal barrier and can cause severe peptic ulcers or stomach cancer. The pathogenic mechanisms of H. pylori remain unclear due to the lack of human-relevant responses from current gastric barrier models, impeding the study of causal factors and treatment strategies. Recently, adult stem cell-derived 3D gastric organoids have emerged as a promising in vitro model, offering better physiological relevance compared to traditional stomach cell lines. However, these organoid systems have significant limitations, such as the absence of multiple cell interactions and physiological mechanical stimuli, which restrict the functional maturity of the model. To address these limitations, we have developed a functionally advanced human stomach micro-physiological system (MPS) that mimics the biochemical and physical interactions between cells and their microenvironment. Human gastric antral organoids were cultured on a porous membrane within a microchannel, interfaced with antrum-specific mesenchymal stem cells under controlled flow conditions. This stomach MPS exhibited physiologically relevant internal barrier functions, including in vivo levels of epithelial integrity and external barrier functions, highlighted by increased mucin production and molecules that mediate protection against H. pylori. Furthermore, we demonstrated the impact of dynamic flow conditions on epithelial morphology and stem cell renewal within the microfluidic device. Ultimately, the H. pylori pathogenic process was successfully replicated in the stomach MPS co-cultured with peripheral blood mononuclear cells, which emulated the relevant immune responses and breakdown of the gastric mucosal barrier.

COMPLETE CARDIAC REGENERATION BY INDUCING ENDOGENOUS CARDIOMYOGENESIS AND ANGIOGENESIS
Liu, Yuan-Hung, *Section of Cardiology, Cardiovascular Center, Far Eastern Memorial Hospital, Taiwan*
Hsieh, Hsiao-Ting, *Far Eastern Memorial Hospital, Taiwan*
Lin, Jiunn-Lee, *Taipei Medical University-Shuang Ho Hospital, Taiwan*

The adult mammalian heart has limited regenerative capabilities after myocardial injury. Current approaches in cardiac regeneration mostly aim to regenerate cardiomyocytes, but of limited efficacy. We recently found that Nkx2.5+ cardiomyoblasts reactivate after myocardial infarction.

They reside mostly in the subepicardium. We also documented that Nkx2.5+ cardiomyoblasts originate from the embryonic epicardial cells. Thymosin ß4 is a G-actin monomer binding protein, involved in cell proliferation, migration, and differentiation. We hypothesized that Thymosin ß4 would promote Nkx2.5+ cardiomyoblasts, an embryonic epicardial-derived cells, mobilization and cardiomyogenesis, after myocardial injury. Various microRNAs (miR) have been reported to control cardiac development and promote cardiac regeneration. We have transfected neonatal Nkx2.5 cardiomyoblasts with various microRNAs and found miR-590 markedly promotes cardiomyogenesis capacity of cardomyoblasts. To prove that miR-590 plus Thymosin ß4 may enhance cardiomyogenesis and functional repair of heart function, we created myocardial infarction in B6 mice. In the experiment group, miR-590 was injected intramyocardially into mice heart immediately after myocardial infarction. Thymosin ß4 was injected peritoneally post-MI. Our data showed that miR-590 (intracardiac injection) plus Thymosin ß4 (intraperitoneal injection) markedly improved murine cardiac function after myocardial infarction. In the treatment group with miR-590 plus Thymosin ß4, LVEF improved from 45% (Day 0) to 77% (Day 7), to 83% (Day 28), to 85% (Day 56) with no LV regional wall motion abnormality. In comparison, the control group with miR-mimic plus PBS, persistent hypokinesia at apex and anterior wall with impaired LV contractility. Enhanced cardiomyocytes proliferation was documented at miR-590 plus Thymosin ß4 –treated 1-week post-MI hearts. Enhanced angiogenesis was documented at treated 8-week post-MI hearts. Masson Trichrome stain demonstrated minimal fibrosis in the treated heart sections at 8 weeks post-MI. In conclusion, administration of miR-590 plus Thymosin ß4 synergistically enhances cardiac repair/ regeneration almost completely by inducing endogenous cardiomyogenesis and angiogenesis.

Funding Source: Far Eastern Memorial Hospital.

SPATIOTEMPORAL TUNABLE MICROENVIRONMENT FOR NEXT-GENERATION ORGANOIDS: RECAPITULATING PRIMITIVE STREAK FORMATION TO NOTOGENESIS

Wang, Zhe, *Institute for Life and Medical Science, Kyoto University, Japan*
Hao, Ruolin, *Kyoto University, Japan*
Eiraku, Mototsugu, *Kyoto University, Japan*

Organoids and embryoids have transformed regenerative medicine and developmental biology, providing unprecedented insights into human development and disease modeling. Our lab has been at the forefront of this revolution, developing a variety of organoids, including cortex, eyecup, retina, and more recently, limb bud, branchial arch, and olfactory epithelium. Despite these advances, organoids still face major challenges such as limited reproducibility and stochastic self-organization, primarily due to the lack of spatiotemporal control in current culture environments. These limitations hinder the modeling of more complex biological phenomena, such as early developmental events. Traditional organoid generation methods, based on cell aggregation followed by differentiation cues, have seen little innovation. The simplicity of in vitro environments, compared to the complexity of in vivo conditions, remains a significant limiting factor. My work addresses these challenges by engineering a spatiotemporal tunable artificial microenvironment that offers precise control over the culture conditions. This allows the generation of organoids with enhanced complexity and functionality. We have developed a photocurable hydrogel platform for cell attachment culture that enables 2D patterning and 3D printing with exceptional flexibility. This platform offers control over: 1. Spatial stiffness, 2. Spatial cell attachment. 3. Geometry and 3D structure, 4. Degradability, 5. Cell differentiation,

6. Spatial cell deposition, enabling localized signaling, 7. Protein binding, achieving up to a 10-fold improvement over glass dishes, 8. Transparency for live imaging. We have also pioneered methods for local application, allowing spatiotemporal control of signaling around organoids. Using this system, we have successfully recapitulated primitive streak formation and notogenesis, key early developmental processes, in a controlled and reproducible manner. In this presentation, I will discuss how we used our system to model primitive streak morphogenesis and eventually derived notochord, advancing our understanding of these critical events in embryogenesis.

MULTI-LAYERED CELL SPHEROID SHEETS USING FIBRINOGEN-BASED SIMPLE GEL FORMATION FOR HINDLIMB ISCHEMIA

Chung, Youngdoo, *Dankook University, Korea*
Lim, Juhan, *Dankook University, Korea*
Lee, Min Suk, *Dankook University, Korea*
Jeon, Jin, *Dankook University, Korea*
Yang, Hee Seok, *Dankook University, Korea*

Stem cell therapy has been studied as an effective tissue regeneration of damaged or degenerative tissue by transplantation of single cells or spheroids with additional scaffold platforms. However, stem cell therapy has shown some disadvantages of poor cell viability when applied in the form of a single cell, a decrease in overall cellular activity and vascularization when transplanted with an incomplete extracellular matrix (ECM). Cell sheet engineering is one of the effective options that include ECM of cultured cells without any scaffolding system in a desire to mimic the microenvironment in natural tissues and to promote vascularization after transplantation. Our previous study had a novel strategy for fabricating cell sheets that were prepared simply by dispensing the fibrinogen stock solution to the cell growth medium without using any synthetic polymers or chemical agents. The goal of this study was to investigate the effect of transplantation of cell spheroid sheets on the regeneration of the hindlimb ischemic region. The fibrinogen-based cell spheroid sheet was developed to be used as an experimental group to compare differences with the single cell sheet group for further application as a treatment of ischemic disease. The fibrinogen-based cell spheroid sheet showed much higher in vitro tubular formation and release of angiogenic relative factors compared to fibrinogen-based single cell sheet. The fibrinogen-based cell spheroid sheet showed significantly higher physiological status and blood perfusion rate due to their angiogenic potential compared to the other group. These results demonstrate that the fibrinogen-based cell spheroid sheet is an effective treatment for ischemic disease. The application of fibrinogen-based 3D cell spheroid sheets would be a promising therapeutic candidate for ischemic diseases as tissue engineering platforms.

HARNESSING SELF-ASSEMBLING DENTAL STEM CELLS ENCAPSULATED IN RAPIDLY DEGRADABLE POLYSACCHARIDE MICROGELS FOR RAPID ORGANOGENESIS

Liang, **Chao Christina**, *Faculty of Dentistry, The University of Hong Kong, Hong Kong*
Lee, Sangjin, *The University of Hong Kong, Hong Kong*
Wu, Shuxuan, *The University of Hong Kong, Hong Kong*
Wu, Zhaoming, *The University of Hong Kong, Hong Kong*

Regenerating complex tissues and organs requires biomaterial systems that support highly organized cell assembly and integration while degrading rapidly to promote cell-driven morphogenesis. However, many conventional biomaterials degrade too slowly and leave residual scaffolds that interfere with efficient cellular communication, thereby limiting tissue maturation and function. Here, we present an innovative approach using oxidized alginate microgels encapsulating high-density human dental stem cells (hDSCs) to achieve rapid and scalable 3D tissue fabrication. Sodium periodate oxidation was optimized to produce oxidized alginate microgels with tunable degradability, enabling spontaneous cell condensation and robust tissue formation both in vitro and in vivo. Upon transplantation, the high-density hDSC-laden oxidized alginate microgels promoted immediate integration with host tissues in a mouse model, demonstrating the system's potential for broad tissue engineering applications. Furthermore, by facilitating rapid cell release and self-assembly, this oxidized alginate microgel platform can recapitulate epithelial-mesenchymal interactions which is a key developmental process across various tissue types. Thus, the high-density hDSC-laden oxidized alginate microgel system offers a versatile and cost-effective strategy for organ-level reconstruction, promising improved clinical outcomes in regenerative medicine.

ENGINEERING 3D HUMAN TISSUE FOR DISEASE AND REPAIR
Zhou, Linna, *Nuffield Department of Medicine and Department of Chemistry, University of Oxford, UK*

Human tissue and organs contain defined and often complex 3D cellular architectures that are crucial for their function. The disruption of their structure, due to genetic mutations or injuries, likely leads to pathological conditions. Currently, there are limited tools to engineer realistic 3D tissue models. We are developing novel technologies, including 3D droplet printing and microfluidics combined with stem cell technologies, to build defined and functional 3D human tissue models. The 3D tissues can be used to model developmental processes and diseases, as well as implants for repair.

EXPANDING THERAPEUTICS THROUGH STEM CELLS AND REPROGRAMMING
2:00 PM – 3:30 PM
HALL C, LEVEL 1

STRATEGIES AND ADVANCES IN REGENERATING AND SUSTAINING NEURAL TISSUE
Cosma, **Maria Pia**, *Centre for Genomic Regulation, Spain*

Our group is dedicated to unraveling the intricate mechanisms that govern somatic cell reprogramming and tissue regeneration in mammals. We investigate diverse biological systems, ranging from the molecular level to entire organs. Our research has highlighted the crucial role of the Wnt/beta-catenin signaling pathway in orchestrating cell fusion-mediated regeneration of retinal cells, dopaminergic neurons, and hepatocytes in mice. Specifically, we discovered that Wnt-activated bone marrow-derived cells can fuse with Müller glia cells in degenerated retinas, leading to temporary reprogramming and subsequent differentiation into neuronal lineages. We have also observed this cell fusion-mediated regeneration in human retinal organoids and retinal explants. In a recent breakthrough, we identified chemokines

released from damaged human and mouse retinas and elucidated the chemokine-receptor interactions that enable the migration and integration of transplanted cells into the mouse retina. Our current focus is on identifying key Wnt-dependent master regulators of reprogramming and regeneration. Additionally, we are developing methods to sustain the viability of the human retina in cadaveric eyes for extended periods, enabling us to explore therapeutic approaches ex vivo.

ENGINEERING IPSC-DERIVED MICROGLIA FOR IMMUNE CELL-BASED THERAPIES FOR NEUROLOGICAL DISEASES

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Studies have sought to circumvent the protective qualities of the blood-brain barrier to deliver therapeutic proteins for the treatment of neurodegenerative diseases. Yet, brain-specific uptake, peripheral toxicity, off-target effects, and repeated dosing present considerable challenges. In this study, we sought to determine whether human iPSC-derived microglia (iMG) could be genetically engineered ex vivo to enable pathology-responsive delivery of therapeutic proteins to the brain. We first examined the transcriptional responses of human iMG in chimeric models of breast cancer brain metastasis, multiple sclerosis-associated demyelination, and Alzheimer's Disease (AD), demonstrating iMG adopt diverse transcriptional responses in response to differing brain pathologies. Using a novel xenotolerant model of AD lacking the Csf1r-FIRE enhancer (5x-hFIRE) we further found a strong correlation between microglial CD9

expression and beta-amyloid deposition throughout the brain. As proof of principle, we then CRISPR-edited human iPSCs to express the beta-amyloid degrading enzyme neprilysin (NEP) or secreted neprilysin(sNEP) downstream of the endogenous CD9 promoter. iPSC-microglial progenitors were then differentiated and transplanted into 2-month-old WT-MITRG and 5x-MITRG mice for 4.5 months before brains were harvested for immunohistochemistry and biochemical analysis. Compared to WT cells, CRISPR-engineered NEP and sNEP xenotransplanted microglia (xMG) reduced AB-plaques and oligomers, prevented synaptic degeneration, and significantly reduced levels of astrogliosis. To further determine whether increased engraftment of therapeutic microglia could provide additional disease-modifying efficacy, sNEP-iMG were further modified to enable CNS-wide engraftment. Surprisingly, sNEP delivery by microglia adjacent to the injection site alone was sufficient to achieve almost all disease-modifying outcomes including reducing inflammatory markers and plasma NfL levels as effectively as CNS-wide sNEP-microglia engraftment. Together, these results demonstrate iPSC-derived microglia can be engineered as a promising immune cell therapeutic platform to provide widespread and pathology-responsive delivery of biological therapeutics for treating neurodegenerative disease.

Funding Source: NIA T32 AG073088 (JP.C.); NIA T32 AG00096 (JP.C. and J.H.); NIH AG061895 (H.D.); CIRM EDUCA4-12822 (S.K.S.); Larry L. Hillbolm Foundation #2024-A-023-FEL (G.E.-S.); NIH AG048099, AG055524, AG056303, U19 AG06970101 (M.B.J.).

LYOPHILIZED EXTRACELLULAR VESICLES PROMOTE CARDIOMOCYTE SURVIVAL AND PROLIFERATION VIA STC2- MEDIATED GLUCOSE METABOLISM IN ISCHEMIC HEART INJURY

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Ischemic heart disease, especially myocardial infarction (MI) with resultant heart failure, remains a major cause of death and a substantial socioeconomic burden worldwide. Severe MI causes an extensive and irreversible loss of cardiomyocytes, eventually leading to the progression of heart failure. Based on various preclinical studies, stem cell-derived extracellular vesicles (EVs) exert great therapeutic effects for the repair of damaged myocardium. To promote the successful implementation of EV-based therapeutics in the clinical setting, the creation of a safe, ready-to-use, off-the-shelf product and storage conditions need to be optimized. Here, we optimized lyophilization methods to process EVs from human pluripotent stem cell-derived mesenchymal stem cells (hPSC-MSC), and systematically compared their cardiac repair efficacy with that of freshly isolated hPSC-MSC-EVs in a mouse MI model. We found that lyophilized EVs, even following long-term storage (up to four months) at room temperature, showed similar therapeutic efficacy with that of freshly isolated EVs after infarction, which was associated with improved myocardial function, increased neovascularization, enhanced cardiomyocyte survival and proliferation, as well as reversed cardiac fibrosis. Using EV proteomics analysis, we found that stanniocalcin 2 (STC2) was one of the most abundant proteins in lyophilized EVs that were highly enriched in glucose metabolism. STC2 knockdown in EVs significantly impaired cardiomyocyte proliferation and cardiac functional recovery, while elevated expression of STC2 in EVs exhibited augmented cardiac regeneration and function outcomes. Mechanistically, lyophilized EVs could transfer

STC2 into cardiomyocytes, phosphorylated STAT3, and promoted the transcription of hexokinase2 (HK2), ulteriorly enhancing cardiomyocyte glucose metabolism. These findings emphasized the clinical translational potential of lyophilized EVs in cardiac repair and highlighted a novel mechanism of STC2-pSTAT3-HK2-mediated glucose metabolism for enhancing cardiac survival and proliferation after MI.

Funding Source: This work was supported by National Key Research and Development Program of China (No. 2023YFA1800700, No. 2023YFA1800702), and National Natural Science Foundation of China (No. 81770484 and No. 31101052).

PHASE 1/2A CLINICAL TRIAL OF HUMAN ESC-DERIVED DOPAMINE NEURONS FOR PARKINSON'S DISEASE

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Parkinson's disease (PD) is caused by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta. Conventional treatments, such as L-dopa and deep brain stimulation, primarily manage symptoms but do not modify disease progression. To overcome these limitations, we developed a cell therapy using dopaminergic neurons derived from human embryonic stem cells (hESCs). Our approach refines signaling pathways with small molecules to optimize neural induction and regional patterning. Specifically, we used inhibitors of Activin/nodal and BMP signaling to prime hESCs for neuroectodermal specification, and activators of Wnt and Shh signaling to induce midbrain ventralization. This protocol yielded a high percentage of ventral midbrain dopaminergic (mDA) progenitors (99.57% of LMX1A/B+ FOXA2+ cells). These cells were used in both non-clinical and clinical trials. The therapeutic potential and safety of hESC-derived mDA progenitors (TED-A9) were evaluated in rodent and primate models. Transplantation into PD rats significantly improved dopamine function, while PD monkeys demonstrated behavioral improvements for up to 28 weeks with increased dopamine activity on PET-CT following transplantation. Comprehensive safety assessments conducted in GLP-compliant facilities showed no toxicity or tumorigenicity concerns. TED-A9 received IND approval from the Korean MFDS. The phase 1/2a clinical trial (NCT05887466) was conducted on 12 participants who have been diagnosed with Parkinson's disease for more than 5 years and exhibited motor complications such as wearing-off, freezing of gait or dyskinesia. Participants were aged 50 to 75 years. TED-A9 was administered to 6 participants in the low-dose group (3.15 million cells) and to another 6 participants in the high-dose group (6.30 million cells). One-year follow-up data revealed no significant adverse events, confirming the safety of TED-A9. Efficacy was demonstrated by improvement in motor symptoms. FP-CIT PET confirmed successful neuron engraftment, which correlated with clinical recovery. In this talk, I will present a detailed analysis of the one-year safety and efficacy data following transplantation.

Funding Source: This work was supported by National Research Foundation of Korea (NRF) grants funded by the Korean government (MSIT) (2022R1A2C2091165 and 2022R1A2C1091800) and the Korean Fund for Regenerative Medicine (KFRM) (RS-2024-00332790).

Clinical Trial ID: NCT05887466.

PRECURSOR OF CHEMICALLY EXPANDED HEPATOCYTES (PRE-CHEP) WITH 1 MILLION-FOLD EXPANSION POTENTIAL AND LIVER REPOPULATION CAPACITIY

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Hepatocytes constitute approximately 80% of liver mass and are essential for various physiological functions, including detoxification, drug metabolism, and the regulation of blood levels of fats, glucose, and amino acids. Commercially available cryopreserved primary hepatocytes (PHH) have been critical materials for laboratory assays such as toxicology testing, drug screening, and disease modelling in the pharmaceutical industry. However, the limited batch sizes and significant batch-to-batch variability of donated hepatocytes pose challenges to standardisation. Their limited availability also hampers the development of hepatocyte-mediated therapies. Pluripotent stem cell-derived hepatocyte-like cells, induced hepatocytes generated by reprogramming fibroblasts, and immortalised hepatocyte cell lines all exhibit limited hepatocyte functionality. Recently, reprogramming mature hepatocytes into a proliferative state has emerged as an alternative strategy to expand fully functional hepatocytes. However, these reprogrammed cells lose their re-differentiation capacity over successive passages, which presents a significant limitation. Here, we report an improved culture condition that enables the reprogramming of mature human hepatocytes into precursor cells. Our precursors of chemically expanded hepatocytes (pre-cHep) can proliferate for over 30 days, achieving an expansion potential of up to 10^12 cells from 1 million hepatocytes (a 1-million-fold expansion). During this period, they maintain liver repopulation capacity equivalent to cryopreserved hepatocytes in immunocompromised mice—the gold-standard test for hepatocyte functionality. Furthermore, pre-cHep can differentiate into chemically expanded hepatocytes (cHep) in 3D culture conditions within 3-6 days. These cHep display global gene expression profiles similar to those of 3D-cultured adult PHH, one of the most effective strategies for maintaining hepatocyte function in vitro. We successfully derived pre-cHep lines from 5 out of 7 donor hepatocytes, demonstrating the robustness of our culture system. Thus, pre-cHep/cHep represent a bona fide alternative to primary hepatocytes for various in vitro assays and hold promise as a potential cell source for future therapeutic applications.

Funding Source: Medical Research Council, Novo Nordisk.

HUMAN EXPANDED POTENTIAL STEM CELLS FOR FUNDAMENTAL RESEARCH AND TRANSLATIONAL APPLICATIONS

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We have developed the expanded potential stem cell (EPSC) technology that enables establishment of stem cells from preimplantation embryos of multiple mammalian species. This is achieved by inhibiting signal pathways implicated in the early preimplantation embryo development. Mouse EPSCs are derived from individual 4-cell and 8-cell blastomeres. A single mouse EPSC can contribute to both the embryo proper and the TE lineages in chimera assay. Molecular analyses have revealed that EPSCs had some enriched features of cleavage stage

embryos. EPSCs of these species share similar molecular features and developmental potentials in that they are genetically and epigenetically stable, can be maintained as homogenous long-term cultures, and permit efficient precision genome editing. EPSCs thus provide a new tool for studying human development in vitro and open up new avenues for translational research in biotechnology, agriculture, and regenerative medicine. For example, knocking out a cancer gene impairs extraembryonic development which is relevant to its functions in fertility, development and cancer. Furthermore, early syncytiotrophoblasts generated from human TSCs are highly susceptible to coronavirus infection and are sensitive to antiviral treatment which facilitates antiviral drug discovery and stem cell-based vaccine development. We have also established a rapid and robust platform for producing high-quality patient EPSC lines that permit disease cellular phenotyping and drug candidate evaluation and screens for precision medicine. Finally I will discuss using the EPSC-based technology for anti-aging discovery.

REGULATORY FRAGMENTATION AND UNPROVEN STEM CELL THERAPIES: BEYOND HARMONIZATION
2:00 PM – 3:30 PM
HALL 3G, LEVEL 3

TITLE AND ABSTRACT NOT AVAILABLE AT THE TIME OF PUBLISHING
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JUDICIAL VARIABILITY IN THE REGULATION OF THE STEM CELL INDUSTRY IN CHINA
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With the rapid advancement of stem cell research, unproven stem cell therapies have proliferated worldwide. In China, this trend has led to an increasing number of court cases involving such interventions and their commercialization. These legal disputes are shaping the trajectory of clinical translation, both directly and indirectly. This talk critically examines recent judicial decisions in China related to unproven stem cell therapies and associated trade practices. We analyze the legal foundations and judicial reasoning behind these rulings, and assess their broader implications for industry stakeholders, academic researchers, and public perception. Based on these findings, we propose targeted strategies to improve judicial practice, curb the spread of unproven therapies, and support the sustainable development of the stem cell sector.

TITLE AND ABSTRACT NOT AVAILABLE AT THE TIME OF PUBLISHING
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REGULATORY FRAGMENTATION AND UNPROVEN STEM CELL THERAPIES: BEYOND HARMONIZATION - EUROPEAN PERSPECTIVE

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The aim of the paper is to define the requirements and limits of the applicability and responsibility for the use of Advanced Therapy Medicinal Products (ATMPs) under the European Hospital Exemption (ATMP-HE) in experimental therapies in the light of the current legal framework and the future reform of EU law in this area. The paper examines the role of the European ATMP-HE concept in the innovation and development of advanced therapeutic treatments, including unlicensed (unproven) cellular interventions known as "stem cell therapies", and the general principles governing the use of ATMPs under EU law. The market for unlicensed experimental human therapies continues to grow due to the lack of scientific positions defining the limits of acceptable use of innovative therapeutic methods. The paper analyzes the main aspects of the ATMP-HE concept, including the scope of EU legislation, the conditions for the authorized production of ATMPs as HE and the principles of its application. It is also important to distinguish the ATMP-HE concept from other specific EU "procedures" for the use of medicinal products outside the registration process, such as off-label use and compassionate use. The answers to the research questions on EU law will serve as a basis for considerations and research on the scope and method of transposition of EU law into the laws of the Member States law, including Polish law. The current provisions of the ATMP-HE Regulation in EU law are also unclear and give rise numerous interpretation doubts. The Hospital Exemption (HE), as well as the specific requirements allowing its manufacture and use, are further regulated at the Member State level. This has led to differences in the implementation of the HE across the European Union (EU). As a result, the use of the Hospital Exemption (HE) varies considerably between Member States, depending on the national legal implementation and the interpretation of the HE by policy makers. The amendment of the ATMP-HE Regulation is part of the reform of EU pharmaceutical legislation proposed by the European Commission, and it will be presented to the audience.

STEM CELL MODELS OF TISSUE INJURY AND REPAIR
2:00 PM – 3:30 PM
THEATER 2, LEVEL 1

UNDERSTANDING AND HARNESSING THE REGENERATIVE POTENTIAL OF THE BRAIN

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There is an unmet need for repair following injury in humans, particularly in the brain where endogenous stem cell activity is minimal. An understanding of neural progenitor diversity and flexibility in their fate choices is crucial for understanding how complex organs like the brain are generated or undergo repair. The neonatal mouse cerebellum is a powerful model system to uncover regenerative responses due to its high regenerative potential. We previously showed that the cerebellum can recover from the loss of at least two types of neurons via distinct regenerative mechanisms. However, the regenerative potential of the cerebellum decreases once development ends, despite the presence of stem-like cells in the adult cerebellum. We hypothesize that the lack of regeneration is due to a lack of pro-regenerative developmental

signals in the adult brain in addition to epigenetic silencing of stem cell differentiation programs and inhibitory cellular mechanisms as development is completed. The signalling pathways and the gene regulatory networks that drive lineage decisions and plasticity in the postnatal cerebellum remain unknown. Understanding these mechanisms is the crucial first step to building a road map for successful regenerative therapies. Using a combination of single cell genomics and in vivo and in vitro assay, we are interested in answering two overarching questions: i) What are the cellular and molecular mechanisms that enable regeneration in the developing brain and inhibit it in the adult? ii) Can we facilitate regeneration in the brain by stimulating stem/stem-like cells?

DECODING THE REGENERATIVE LANDSCAPE: INSIGHTS FROM SPONGE SINGLE-CELL TRANSCRIPTOMICS

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Caglar, Cuneyt, *Australian National University, Australia*
Adamski, Marcin, *Australian National University, Australia*
Adamska, Maja, *Australian National University, Australia*

Rapid and precise activation of wound healing and regeneration in response to mechanical injury is crucial for animal survival and health. While the majority of animals can heal wounds, morphologically complex organisms, such as vertebrates and insects, often lack the capacity to regenerate tissues and organs. In contrast, sponges, with their simple body plans, possess remarkable regenerative abilities, exemplified by rapid tissue repair and the capability to reconstruct their bodies from dissociated cells. Building on our previous work that characterised the microscopic cellular dynamics and bulk gene expression changes in sponges during the early injury response, we sought to identify specific genetic regulators of these processes. However, the genes controlling key cellular behaviours, such as migration and transdifferentiation critical for wound coverage, remain largely unknown. To delve into these mechanisms, I generated single-cell transcriptomic data for Sycon capricorn, an Australian calcareous sponge. The deconstructed 10,747 cells were identified as distinct eleven cell types and states through computational cell clustering and in situ hybridisation with identified marker genes. By sequencing six sponges that were sampled twice each, I accounted for individual variance, enabling effective demultiplexing and the identification of twelve distinct regeneration time points. This comprehensive dataset, ranging from immediately after dissociation to 90 minutes post-injury, allowed me to trace injury response at single-cell resolution. Visualising the gene expression patterns across cell types revealed both widespread expression across cell families and restricted expression within specific ones. My findings suggest that genes with potential roles in regulating cell migration and differentiation exhibit particular expression patterns, dynamically contributing to injury response and wound repair. Supported by bulk sequencing data, my single-cell analysis offers a more detailed perspective on understanding regenerative mechanisms. As the first known single-cell transcriptomic atlas of calcareous sponges, my study reinforces the utility of Sycon capricorn as a powerful model for developmental biology. This provides new insights into distinct aspects, including germ layer evolution and regeneration, and bridging the cognitive gap between simple-bodied organisms and more complex life forms.

IN VITRO MODELLING OF RESPIRATORY INFECTIONS IN IDIOPATHIC PULMONARY FIBROSIS USING IMMUNE-COMPETENT MULTI-TISSUE HIPSC-DERIVED LUNG ORGANOIDS

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Reed, Liam, *University of Nottingham, UK*
Sainz-Zuñiga, Carlos, *University of Nottingham, UK*
Azis, Rizal, *University of Nottingham, UK*
Cuevas-Ocaña, Sara, *University of Nottingham, UK*
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Idiopathic Pulmonary Fibrosis (IPF) is a chronic interstitial lung disease characterised by progressive scarring of the lungs with a median survival of 2–3 years. Central to its pathogenesis is the dysfunction of alveolar epithelial type 2 (AT2) cells, which produce pulmonary surfactant and regenerate the alveolar epithelium after injury. Genetic mutations, such as those in surfactant protein genes, and environmental stressors like infections can impair AT2 cell function, driving aberrant fibrotic responses. Despite the pivotal role of AT2 cells in IPF, progress in understanding these genotype-phenotype interactions has been hindered by the lack of physiologically relevant human models. To address this, we developed a novel xeno-free differentiation protocol to generate homogeneous AT2 cells from human induced pluripotent stem cells (hiPSCs). The protocol combines fluorescent-activated cell sorting to enrich NKX2.1-expressing lung progenitors with 3D culture using human recombinant laminin-111 and peptide hydrogels to mature cells into surfactant protein C (SFTPC)-expressing AT2 cells. Using IPF patient-derived hiPSCs carrying a SFTPC mutation and CRISPR/Cas9-corrected isogenic controls, we established immune-competent, multi-tissue lung organoids incorporating fibroblasts, macrophages, dendritic cells, and endothelial cells, all derived from the same hiPSCs. To validate the platform, we exposed the organoids to H1N1 influenza A virus. Transcriptomic profiling revealed infection-induced stress responses, including inflammation, immune activation, and oxidative stress. Mutant IPF organoids showed heightened fibrotic responses, increased secretion of SFTPC, and disrupted AT2-specific functions compared to healthy controls. Healthy organoids mounted a stronger acute-phase inflammatory response, revealing differences in immune competence and repair mechanisms. This immune-competent, multi-tissue organoid platform provides a robust in vitro model for studying the interplay between genetic predisposition and environmental insults in IPF. By recapitulating key disease features, it offers valuable insights into genotype-phenotype interactions and could support the development of targeted therapies to limit fibrosis progression.

INVESTIGATING THE CROSSTALK BETWEEN MICROGLIA AND RELEVANT CELLULAR NICHES USING HUMAN PLURIPOTENT STEM CELL DERIVED CO-CULTURES AND ORGANOIDS

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Battacharya, Afrin, *The Hospital for Sick Children, Canada*
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The development of the human cerebral cortex hinges on the precise regulation of neural precursor (NP) proliferation and differentiation into neurons and glia, governed by intrinsic and extrinsic signals. Disruptions in these processes are linked to numerous disorders. Recent studies highlight microglia, the brain's immune cells, as pivotal in brain development and function. Yet, their specific roles within the human NP niche remain largely unexplored. To bridge this knowledge gap, we utilized human pluripotent stem cell-derived cultures to model interactions between isogenic microglia-like cells (pMGLs) and NPs in 2D co-cultures. We refined a protocol to integrate fluorescently tagged pMGLs into 3D cortical organoids, achieving a density and microglia-to-NP ratio akin to the human fetal cortex. Both models revealed a density-dependent effect of pMGLs in enhancing the proliferation and abundance of SOX2+ NPs. This effect was replicated by pMGL-conditioned medium alone, indicating a mechanism mediated by secreted growth factors. To uncover the molecular underpinnings, we performed receptor-ligand interaction analyses on pMGL and NP transcriptomic datasets. Insulin-like Growth Factor-1 (IGF1) emerged as a key proliferative factor predominantly expressed by microglia during cortical development. Disrupting the IGF1-IGF1-receptor (IGF1R) pathway partially mitigated the trophic effect of pMGLs on NPs. In mature organoids, single-cell RNA sequencing revealed astrocytes as the primary cell type interacting with microglia, identifying key receptor-ligand pairs in this interaction. Assembloids with adjustable proportions of neurons and astrocytes demonstrated the necessity and sufficiency of astrocytes for microglial residency and maturation. In organoids matured to an oligodendrogenic stage, microglia were shown to expedite myelin formation. Our models also facilitated the study of microglia in human demyelination events and their participation in subsequent regenerative responses. These studies aim for a better understanding of the role of human microglia during critical periods of development and brain maturation, in an experimentally tractable system amenable to genetic and tissue engineering. Elucidation of these interactions paves the way for microglial modulation therapies.

Funding Source: CFREF/Medicine by Design; CIHR Canada Research Chair Program; NARSAD/BBR; Brain Canada; Stem Cell Network.

HUMAN SPINAL INTERNEURONS REPAIR THE INJURED SPINAL CORD THROUGH SYNAPTIC INTEGRATION

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Pelonero, Angelo, *Gladstone Institutes, USA*
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Lane, Michael, *Drexel University, USA*
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Advances in cell therapy offer promise for some of the most devastating neural injuries, including spinal cord injury (SCI). Endogenous VSX2-expressing V2a spinal interneurons have

been implicated as a key component in plasticity and therapeutically driven recovery post-SCI. However, the integration of these cells with injured host networks after transplantation has been unexplored. Here, we show that transplanted human stem cell-derived spinalized V2a-enriched neurons (SpINs) synaptically and functionally integrate with host injured motor networks in a traumatic model of SCI. Neuroanatomical tracing and immunohistochemistry demonstrated transplant integration and synaptic connectivity with injured host tissue in a sub-acute model of cervical contusion. Optogenetic activation of transplanted human V2a-enriched SpINs revealed functional synaptic connectivity to injured host circuits, culminating in improved diaphragm activity assessed by electromyography. Furthermore, optogenetic activation of host supraspinal pathways revealed functional innervation of transplanted cells by host neurons, which also led to enhanced diaphragm contraction indicative of a functional neuronal relay. Single-cell transcriptomics analyses pre- and post-transplantation suggested the in vivo environment resulted in maturation of transplanted SpINs that mediate the formation of neuronal relays, as well as differentiation of glial progenitors involved in repair of the damaged spinal cord. This study rigorously demonstrates feasibility of generating human cervical spinal V2a-enriched interneurons that develop functional host-transplant and transplant-host connectivity resulting in improved muscle activity post-SCI.

Funding Source: F32 NS119348, DISC2-14180, R01 NS104291, F31 NS125975, 1S10OD028511-01, P40 OD010996, The Lisa Dean Moseley Foundation, UCSF HIVE initiative, STAR Scholars Program, Roddenberry Foundation.

HUMAN IPSCs IN ACTION: REVOLUTIONIZING DRUG SAFETY AND HEART DISEASE TREATMENT

Hsieh, **Patrick C. H.**, *Cardiovascular and Metabolic Diseases, Institute of Biomedical Sciences, Academia Sinica, Taiwan*

In recent years, human induced pluripotent stem cells (hiPSCs) have emerged as a groundbreaking tool in biomedical research, offering unprecedented opportunities to revolutionize drug safety and heart disease treatment. This presentation delves into two pivotal studies that highlight the transformative potential of hiPSCs in these domains. Firstly, we explore a pioneering study that establishes a population-based hiPSC drug screening platform. By recruiting 1,000 healthy donors and identifying 13 HLA-homozygous "super donors," we have created a representative model that encompasses the genetic diversity of nearly half a billion people worldwide. Differentiated into cardiomyocytes and neurons, these hiPSCs facilitate high-throughput toxicity screening, enabling the identification and validation of drug-induced cytotoxicity. This innovative approach not only enhances our understanding of interpopulation differences in drug responses but also promises to improve the safety and efficacy of new therapeutics. Secondly, we examine a landmark study on the regenerative potential of hiPSCs in heart disease treatment. We demonstrated that the combined transplantation of hiPSC-derived cardiomyocytes and endothelial cells can effectively regenerate infarcted heart tissue in both mice and non-human primates. This dual-cell approach addresses critical challenges such as poor cell survival and insufficient vascularization, promoting the maturation and integration of transplanted cells. The results reveal significant improvements in cardiac function and tissue repair, paving the way for clinical translation of hiPSC-based therapies for myocardial infarction. Together, these studies

exemplify the dynamic capabilities of hiPSCs in addressing some of the most pressing challenges in medicine.

STEM CELLS AND CELL METASTASIS
2:00 PM – 3:30 PM
HALL B, LEVEL 1

PLASTICITY, THERAPY RESISTANCE AND IMMUNE EVASION IN METASTATIC COLORECTAL CANCER
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Only a small proportion of the observed transcriptomic variation between different subclones in a given colorectal cancer (CRC) can be attributed to genetic or epigenetic changes. Instead, intratumor heterogeneity primarily arises from phenotypic plasticity—the ability of cancer cells to adopt different transcriptional states without underlying heritable (epi)genetic alterations. A significant portion of the phenotypic and functional cellular heterogeneity observed in CRC can be traced to the footprints of homeostatic stem cell renewal and differentiation programs expressed by tumor cells. Plasticity is both pervasive and essential for disease progression. Here, I will share our latest discoveries on how tumor cells co-opt different cell states during metastatic dissemination, relapse, outgrowth, and therapy resistance. I will also discuss the impact of tumor cell heterogeneity on immune evasion throughout metastatic evolution.

RHO-MEDIATED CELLULAR EXTRUSION DRIVES DIFFERENTIAL OUTCOMES OF KRAS AND HRAS MUTANT CELLS IN THE INTESTINE
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Intestinal stem cells are essential for tissue homeostasis and serve as the cell-of-origin for tumor initiation, yet how specific mutations in these cells influence their behavior and tumorigenic potential remains poorly understood. Mutations in RAS family genes, such as KRAS and HRAS, activate pathways that promote cell survival and proliferation. While both mutations are associated with cancer, KRAS mutations are significantly more prevalent in colorectal cancer (CRC) than HRAS mutations. To uncover the factors contributing to this disparity, we investigated how KRAS and HRAS mutations affect the fate of intestinal stem and progenitor cells in vivo. Using lineage tracing and intravital microscopy, we longitudinally tracked KRAS and HRAS mutant cells over several months in living mice. We found that, while both mutations conferred a short-term proliferative advantage, their long-term outcomes differed significantly. KRAS-mutant clones expanded and persisted in the tissue, while HRAS-mutant clones were eliminated via apical and basal extrusion, mediated by Rho signaling. Analysis of human CRC datasets confirmed that HRAS mutations can act as early events in tumorigenesis but are rarely observed, likely due to extrusion-induced clearance of HRAS-mutant cells, which limits their retention in the tissue. Together, these findings highlight how

different mutations in the same signaling pathway can lead to distinct outcomes in intestinal cells, suggesting that cellular mechanisms such as extrusion could be harnessed to prevent tumorigenesis. In addition to offering insights into intestinal tumorigenesis, this study also raises the possibility of similar mechanisms in other epithelial tissues where HRAS mutations are infrequent.

SRSF2-P95H AND NF1 LOSS CO-OPERATE TO PROMOTE MYELOID-BIASED CLONAL HEMATOPOIESIS VIA JAK-STAT PATHWAY ACTIVATION IN MICE

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Clonal hematopoiesis (CH) is an age-related condition characterized by the clonal expansion of hematopoietic stem cells (HSCs) bearing mutations in certain CH-defined genes. SRSF2 represents one of the most frequently mutated splicing factors. While studies have identified multiple gene mutations co-operating with SRSF2P95H, the interplay between RAS signaling and SRSF2P95H remains unclear. It is known that hyperactive RAS signaling can be caused by the deficiency of NF1. We engineered mice carrying both Srsf2P95H and Nf1 deletions. Peripheral blood (PB) analyses revealed a progressive increase in neutrophil % with a decrease in lymphocyte % in Srsf2/Nf1 mutants. Flow cytometry of bone marrow (BM) showed elevated frequencies of HSC, PreGM, Mac-1+ cells, and neutrophils in Srsf2/Nf1 mutants. Next, we performed BM transplantation (BMT). Longitudinal PB analyses showed a significant decrease in donor chimerism in Srsf2/Nf1 mutants, and BM analysis revealed marked reductions of hematopoietic stem and progenitor cell populations in double mutants; however, Mac-1+ cells maintained relatively higher proportions. Gene set enrichment analysis indicated that both native and BMT Srsf2/Nf1-mutated HSCs exhibited upregulation of JAK-STAT and downregulation of the focal adhesion pathway, a hallmark of aging. Splicing analysis identified multiple aberrant skipping events affecting Jak2 exons 13 and 14 in double mutants. Whole exome sequencing (WES) on aged HSCs revealed an identical Jak2 mutant in Nf1 and Srsf2/Nf1 mice, distinct from that occasionally observed in an age-matched control. We

hypothesized that Srsf2P95H activates the JAK-STAT signaling either through abnormal Jak2 exon skipping or age-related spontaneous Jak2 mutations. To validate this mechanism, we treated BMT mice with DMSO or ruxolitinib, a selective JAK-STAT inhibitor. BM flow cytometry displayed significant improvement in CD45.2+ donor chimerism and HSC frequency, with decreased Mac-1+ cells and neutrophils in ruxolitinib-treated double mutants. Colony formation assays corroborated these findings. This study elucidates enhanced myeloid bias in Srsf2/Nf1 co-mutated HSCs via an unanticipated JAK-STAT-dependent mechanism, suggesting potential therapeutic strategies for patients harboring concurrent SRSF2 and NF1 mutations.

Funding Source: Ministry of Science and Technology of China (2021YFA1100900), CAMS Innovation Fund for Medical Sciences (CIFMS) (2024-I2M-ZD-010), the National Natural Science Foundation of China (92368202).

METABOLIC INFLAMMATION DRIVES ABERRANT STEM CELL FATE TO PROMOTE WOUND-HEALING TUMOR MICROENVIRONMENT IN COLORECTAL CANCER DEVELOPMENT

Ho, **Jennifer Pak Yan**, *Columbia University Medical Center, USA*
Cheng, Chia-Wei, *Columbia University, USA*

Western dietary patterns have emerged as a significant risk factor for colorectal cancer (CRC), with metabolic inflammation speculated to alter the cell-of-origin in CRC. However, the metabolic determinant driving the inflammatory response and cell fate changes remains unclear. Here, we show that metabolic dysregulation differentiates tumor-resident stem cells from tissue-resident stem cells, which can be transcriptionally identified in human CRC and genetically modeled by perturbing rate-limiting metabolic enzymes. In the mouse colon, deletion of the metabolic enzyme HMGCS2 in Lgr5+ stem cells impaired their homeostatic function, leading to inflammatory responses and the emergence of regenerative stem cells typically seen after injury or infection. In APC tumor models, this metabolically-driven regenerative program gives rise to early-onset tumors that exhibit molecular and cellular features reminiscent of human CRC. The resulting cellular reprogramming generates metabolically aberrant stem cells that persist in the tumor microenvironment. Mechanistically, these metabolically aberrant stem cells give rise to cell populations that constitute the pro-tumor wound-healing microenvironment. The evolved niche comprises enterocyte-like SAA1+ tumor cells displaying sugar intake activity and wounding responses, alongside the adjacent FAP+ cancer-associated fibroblasts (CAFs) that expand with over-represented wound-healing programs. Specifically, Ligand-target prediction suggests that SAA1+ tumor cells engage with FAP+ CAFs to secrete growth factors and cytokines (e.g., IGF-1, IL-6, and INHBA/EDF), which are normally induced during wound healing but are co-opted to promote tumor growth in this pathological context. These findings reveal a previously unrecognized metabolic cell-fate determinant and suggest potential implications for how the wound-healing program may influence diet-related CRC development.

Funding Source: NIH R00DK123407 and V foundation.

CHEMOTHERAPY-TREATED BREAST CANCER CELLS ACTIVATE THE WNT SIGNALING PATHWAY TO ENTER A DIAPAUSE-LIKE PERSISTER STATE

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De Oliveira, Willy Abreu, *Stem Cell Institute Leuven, Katholieke Universiteit Leuven, Belgium*
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Qualizza, Alessandra, *Stem Cell Institute Leuven, Katholieke Universiteit Leuven, Belgium*
Scheele, Colinda, *Laboratory of Intravital Microscopy and Dynamics of Tumor Progression (VIB-KU Leuven), Belgium*
Bruna, Alejandra, *Institute of Cancer Research (ICR), UK*
Desmedt, Christine, *Laboratory for Translational Breast Cancer Research, Katholieke Universiteit, Belgium*
Lluis, Frederic, *Stem Cell Institute Leuven, Katholieke Universiteit Leuven, Belgium*

Cancer cells can acquire a reversible, dormant drug-tolerant persister state, mimicking embryonic diapause, to evade therapy pressure. However, the precise mechanisms driving cancer cells into or out of a diapause-like persister cell-state remain largely unknown. Our results demonstrate that Wnt signaling pathway modulation is both sufficient and necessary to determine the diapause-like persister cellular-state. Following chemotherapy, diverse therapeutic agents converge on Wnt signaling-activation to induce de novo diapause-like persister cell-state across various triple-negative breast cancer (TNBC) cell-lines, xenograft, and patient-derived organoid models. Among early persister cells, only transcriptionally Wnt-active persisters, as opposed to Wnt-inactive persisters, exhibit the transcriptional and functional characteristics typical of diapause-like cells, including a negative correlation with MYC transcriptional activity and reversible restricted proliferation. This underscores the Wnt signaling pathway as both an inducer and biomarker of the diapause-like cell-state in both parental (chemo-naïve) and chemotherapy-treated cells. Entry into and exit from a diapause-like cell-state is triggered by the transcriptional regulation of components essential for canonical Wnt ligand-secretion. A combinatorial treatment strategy that inhibits Wnt ligand-secretion alongside chemotherapy effectively targets the early mechanisms underlying the enrichment and acquisition of a diapause-like persister cell-phenotype, potentially benefiting patients with TNBC undergoing systemic chemotherapy.

TITLE AND ABSTRACT NOT AVAILABLE AT THE TIME OF PUBLISHING

Turajlic, Samra, *The Francis Crick Institute, The Royal Marsden Hospital NHS Trust, UK*

TECHNOLOGIES TO STUDY DEVELOPMENT AND PHYSIOLOGY
2:00 PM – 3:30 PM
THEATER 1, LEVEL 1

BLASTOIDS: ELUCIDATING THE MOLECULAR DETERMINANTS AND THE EVOLUTION OF TIMING OF HUMAN BLASTOCYST IMPLANTATION

Rivron, Nicolas, *Laboratory for Blastoid Development and Implantation, Institute of Molecular Biotechnology, Austrian Academy of Science, Austria*

Until now, the principles of human early pregnancy have been largely inaccessible. Recently, we have promoted the self-organisation of stem cells in complete models of mouse and then human blastocysts, which we have named blastoids. Blastoids are morphologically and transcriptionally similar to the blastocyst and contain analogues of all three cell types that eventually develop into the full organism. Here we show that models of gene regulation based on scRNA/ATACseq in combination with functional assays in human blastoids assembled with endometrial organoids demonstrate that local reduction of WNT signalling activity contributes to the activation of a gene regulatory network around the transcription factors GCM1, GRHL1 and TCF7L2 that enables trophoblast attachment to endometrial cells and invasion. Using deep learning models of chromatin accessibility for comparative genomics with apes, we found that a Line1-Alu-Line1-derived enhancer element acquired by primates functionally shifted the timing of GCM1 expression in trophoblasts and became particularly active at the blastocyst stage in great apes, including humans. We propose that this evolutionary innovation has aligned blastocyst competency with endometrium receptivity thereby defining an implantation window enabling deep embedding in utero.

CHROMGEM REVEALS ULTRASTRUCTURAL CHANGES IN CHROMATIN DURING DEVELOPMENT AND DIFFERENTIATION AFTER FERTILIZATION

Giraldez, Antonio, *Yale School of Medicine, USA*
Cross, Stephen, *Yale School of Medicine, USA*
Hoppe, Caroline, *Yale School of Medicine, USA*
Sievers, Fiona, *Yale School of Medicine, USA*
Smith, Zack, *Yale School of Medicine, USA*
Sozen, Berna, *Yale School of Medicine, USA*
Youlten, Scott, *Yale School of Medicine, USA*
Zhong, Liangwen, *Yale School of Medicine, USA*

After fertilization, the maternal and paternal nuclei undergo chromatin reprogramming to mediate genome activation and cell fate specification. How chromatin structure is spatially remodeled during development remains poorly understood, partly due to the lack of methods capable of probing chromatin at nucleosome resolution. To address this, we developed ChromGEM (Chromatin Gold Electron Microscopy), which combines endogenous protein labeling with chromatin tomography to trace chromatin at nucleosome scale in the context of cell and epigenetic states. Using ChromGEM, we quantified changes in chromatin structure during mouse embryonic development. We identified four distinct chromatin structures based on nucleosome density and compaction, revealing ultrastructural features that define developmental potential and epigenetic environments. Additionally, we used ChromGEM to study the silencing of pluripotency genes in the trophectoderm and linked these chromatin changes to Lamin A/C upregulation. This work defines chromatin ultrastructural properties during development and differentiation, uncovering epigenetic states associated with reprogramming and cell fate specification after fertilization.

Funding Source: RO1, R35.

CAPTURING LONG-TERM HIPPOCAMPAL DEVELOPMENT IN VITRO USING A NOVEL STEM CELL-DERIVED 3D BRAIN ORGANOID MODEL SYSTEM

Garone, **Maria Giovanna**, *Stem Cell Medicine, Murdoch Children's Research Institute, Australia*
Nucera, Maria Rosari, *Murdoch Children's Research Institute, Australia*
Leichter, Anna, *Murdoch Children's Research Institute, Australia*
Sawant, Vallari, *Murdoch Children's Research Institute, Australia*
Walkiewicz, Marzena, *Murdoch Children's Research Institute, Australia*
Ball, Gareth, *Murdoch Children's Research Institute, Australia*
Ramialison, Mirana, *Murdoch Children's Research Institute, Australia*
Velasco, Silvia, *Murdoch Children's Research Institute, Australia*

The hippocampus is a brain structure that plays an essential role in memory and learning. Impairments in these cognitive functions are associated with neurodevelopmental disorders, such as intellectual disability and epilepsy. However, the cellular and molecular processes underlying these functions remain largely unknown. Stem cell-derived 3D organoids provide an invaluable opportunity to expand our understanding of the human hippocampus's complex cellular and functional networks in health and disease. By exposing iPSC aggregates to BMP-WNT signalling and adapting the cultures to growth in spinner-flask bioreactors, we have established a new 3D organoid model for long-term hippocampal development in vitro. Single-cell RNA-sequencing of individual organoids cultured at 1, 3, and 6 months showed high organoid-to-organoid reproducibility and a stronger correlation with the foetal hippocampal tissue compared to other human brain regions between 12 and 21 PCW. The gene expression profile of cell types observed in organoids also reveals a region-specific and time-dependent appearance that aligns with those found in developing human hippocampal tissue. Indeed, a significant correlation for radial glia, as well as both excitatory and inhibitory progenitor cells is shown during the early differentiation stage. Conversely, interneurons, astrocytes, oligodendrocytes, pyramidal cells in the Cornu Ammonis, and PROX1+ cells of the dentate gyrus are observed in the later times, demonstrating that the new model resembles the cell composition and developmental time course of human brain development. We also assessed the neuronal connectivity and network dynamics during hippocampal organoid differentiation in 3D space, leveraging a non-invasive and high-throughput microelectrode array. Our findings reveal that synchronised burst firings and local field potentials begin during the early differentiation stage. As time progresses and further cellular complexity is reached, a more intricate neuronal network emerges, recapitulating the functional timeline of embryonic brain development. This new 3D model provides an unprecedented platform to investigate how the hippocampus develops and identify the pathophysiological mechanisms of neurological disorders affecting this brain region.

Funding Source: Human Frontier Science Program (HFSP)-Postdoctoral Fellowship-Long-Term-Fellowships (LTF); LT0024/2022-L. The Novo Nordisk Foundation Center for Stem Cell Medicine, renew (Novo Nordisk Foundation); NNF21CC0073729.

A 3D SPATIAL TRANSCRIPTOME OF PRIMATE EARLY ORGANOGENESIS

Tan, **Jia Ping**, *Westlake University, China*
Liu, Yifang, *Westlake University, China*
Fu, Yuting, *Westlake University, China*

Liang, Langchao, *BGI Research, China*
Wu, Yan, *BGI Research, China*
Hao, Shije, *BGI Research, China*
Liu, Longqi, *BGI Research, China*
Liu, Xiaodong, *Westlake University, China*

Early organogenesis is a pivotal phase of embryogenesis that establishes the foundation for the formation of tissues and organs, driven by highly dynamic spatial and transcriptional changes. Understanding these intricate processes is critical for advancing developmental biology and improving in vitro models, yet spatial transcriptomic data on human early organogenesis remains scarce, particularly due to the technical and ethical constraints associated with studies involving early human embryos. On the other hand, nonhuman primates, such as cynomolgus monkeys, provide an excellent surrogate for studying human development due to their close evolutionary proximity compared to other animal models. In this study, we generated a comprehensive 3D spatial transcriptomic atlas of cynomolgus monkey embryos at Carnegie Stages (CS) 9 and 10, encompassing key events such as cardiogenesis, gut tube development, neurulation, prechordal plate and notochord development as well as somitogenesis. Using spatial transcriptomic profiling, we identified distinct subclusters within ectodermal, mesodermal, and endodermal derivatives associated with these hallmark processes of early organogenesis, highlighting spatially restricted gene expression patterns and signalling interactions. Moreover, our findings extend beyond descriptive analyses by providing actionable insights into tissue interactions. For example, by leveraging the insights gained from studying cellular interactions associated with axial development, we developed a co-culture system integrating neural and mesodermal lineages, resulting in a somito-neuruloid model mimicking axial development. This system autonomously recapitulates somite segmentation and axis elongation without exogenous extracellular matrices, providing a physiologically relevant model for axial development. Collectively, this spatial transcriptomic atlas not only provides unprecedented insights into the molecular and spatial dynamics of primate early organogenesis but also serves as a critical resource for guiding future improvement of in vitro models such as organoids and embryo-like structures.

RE-ENGINEERING THE YAMANAKA FACTORS WITH A PROTEIN LANGUAGE MODEL TO ENHANCE CELLULAR REPROGRAMMING EFFICIENCY AND KINETICS

Larouche, Jacqueline, *Retro Biosciences, USA*
Ueland, Madi, *Applied AI, Retro Biosciences, USA*
Joseph, Kevin, *Retro Biosciences, USA*
Karthikeyan, Swathi, *Retro Biosciences, USA*
Matteson, Tomas, *Retro Biosciences, USA*
Hales, J.J., *Retro Biosciences, USA*
Hallman, John, *OpenAI, USA*
Jaech, Aaron, *OpenAI, USA*
Meinl, Rico, *Retro Biosciences, USA*
Tarkhov, Andrei, *Retro Biosciences, USA*

Reprogramming cells with the Yamanaka factors holds substantial therapeutic potential, but clinical applications have been hampered by low reprogramming efficiency, slow kinetics and

factor delivery challenges, especially in aged and diseased donors. While optimization of media conditions or small molecule supplementation can enhance reprogramming, modifying the amino acid sequences of the reprogramming factors may offer a more direct and versatile approach. Yet, traditional protein engineering methods like directed evolution and chimerization remain largely constrained to minor edits of wild-type proteins due to the combinatorial complexity of the problem. To make larger edits more feasible, we constrained the search space to evolutionarily-plausible sequences by training a protein language model, GPT-4b micro, which can be steered by including co-evolving and interacting sequences in its prompt. We guided the model to generate novel SOX2 and KLF4 variants, modifying up to ⅔ of the wild-type sequences. We screened our variants for improved reprogramming efficiency and speed in human dermal fibroblasts, using polycistronic (OSKM) lentiviral vectors for sustained expression. We first ran pooled reprogramming screens for up to 14 days, identifying the best variants via sorting (SSEA4, TRA-1-60) followed by amplicon sequencing. The top hits were then validated in an arrayed format. Following two rounds of screens (collectively testing 313 variants), our reengineered SOX2 and KLF4 showed two orders of magnitude improvement over wild-type factors, according to alkaline phosphatase (AP) positive colony counts and the expression of early and late pluripotency markers (SSEA4, TRA-1-60, TRA-1-81, NANOG) at day 10. These findings highlight the promise of AI-assisted protein engineering and represent a significant step toward overcoming critical bottlenecks in cellular reprogramming, paving the way for more efficient and scalable production of iPSCs for therapeutic applications.

CELL-TYPE SPECIFIC TAGGING AND TRACING OF METABOLITES IN VIVO
Gould, Alex, *The Francis Crick Institute, UK*

Stable isotope tracing is an important technique for studying metabolism but it has a major limitation. Isotope labelled metabolites have to be administered broadly from exogenous sources, such as the medium for cell culture or an injected/dietary bolus for animals. This makes it difficult to track metabolite transport from one specific cell or tissue type to another. A long-standing challenge in the field, therefore, has been to develop genetically-encoded methods to label metabolites within a specific cell type of a living animal. Here, we harness bioorthogonal reactions of selected xenoenzymes from microbes to add innocuous isotope or other tags to metabolites in a conditional cell-type specific manner. We establish that this methodology can be utilized to tag and trace fatty acids and branched-chain amino acids in vivo in Drosophila and in mice. The zenoenzyme approach is a powerful quantitative technology that enables the intercellular and interorgan exchange of metabolites to be analyzed with unprecedented spatiotemporal resolution.

Saturday, June 14, 2025

PLENARY IV: STEM CELLS FOR HUMAN THERAPY
9:30 AM – 11:00 AM
GRAND HALL, LEVEL 3

FROM PIXELS TO PROFILES: PREDICTING SINGLE-CELL TRANSCRIPTOMICS INFORMATION FROM HISTOLOGY IMAGES

Yang, Jean, *University of Sydney, Australia*

Spatial transcriptomics technologies have enabled the quantification of spatially resolved gene expression, providing new insights into the molecular basis of disease. Predicting spatial gene expression (SGE) from cost-effective haematoxylin-and-eosin-stained (H&E) histology images offers an exciting opportunity to extract additional high-dimensional information from widely available data. Here, we present our recent efforts to leverage sub-cellular spatial transcriptomics data, such as Xenium, to predict spatially resolved gene expression at the single-cell level from H&E images. Applying our method, GHIST, to The Cancer Genome Atlas breast cancer cohort (TCGA-BRCA), GHIST outperforms several state-of-the-art methods in spatial transcriptomics prediction tasks. We then demonstrate how predicted SGE enables downstream analyses linking somatic copy number alterations (CNAs) to differential gene patterning (DP), revealing trans-acting hotspots on chromosomes 1q, 8q24, and 17q11–21. Additionally, we illustrate how GHIST's spatial predictions can be used to explore patterns of cell-type co-localisation and potential intercellular communication within tumour microenvironment, offering a framework for generating hypotheses about disease-associated niches.

Funding Source: This work is supported by NHMRC Investigator Grant APP2017023; Judith and David Coffey Life Lab (CPC); the AIR@innoHK programme of Hong Kong and Chan Zuckerberg Initiative Single Cell Biology Data Insights grant.

MACROPHAGE CELL THERAPY FOR LIVER DISEASE: FROM BENCH TO CLINIC

Forbes, Stuart, *Institute for Regeneration and Repair, The University of Edinburgh, UK*

Following severe or prolonged liver injury, extensive liver scarring and ultimately cirrhosis occurs. Liver transplantation is the only curative treatment for advanced cirrhosis but organ supply limits usage. Strategies to degrade fibrosis and stimulate regeneration are required. Macrophages are involved in scar formation as well as scar resolution in damaged organs and in the liver also have a role in regeneration. In this context we have developed macrophage cell therapy for liver disease as a potential approach. This has moved from proof of concept studies in mice where bone marrow derived macrophages have been tested through to human phase 1 and phase 2 studies where autologous monocyte-derived macrophages have been used (ISRCTN10368050). We will present proof-of-concept for the beneficial effect of macrophage cell therapy on long term clinical outcomes in patients with compensated cirrhosis including long-term efficacy and safety data for this new class of therapy. This data has encouraged further development in patients with other forms of liver disease including acute liver injury. Cell therapy with alternatively activated macrophages (AAMs) have been shown in pre-clinical models to be of benefit in acute liver injury due to paracetamol induced liver damage where their anti-inflammatory features and ability to resolve necrotic material are important in this scenario. Allogenic AAMs have been taken through to phase 1 testing in this scenario (ISRCTN12637839).

REGENERATIVE MEDICINE FOR SPINAL CORD INJURY USING IPS-DERIVED NEURAL STEM CELLS

Nakamura, Masaya, *Orthopaedic Surgery, Keio University School of Medicine, Japan*

Transplantation of iPS Cell-Derived Neural Stem Cells for Subacute Complete Spinal Cord Injury We have previously reported that transplantation of safety-validated iPS-derived neural progenitor cells (iPS-NPCs) into injured spinal cords of immunodeficient mice and monkeys promotes motor function recovery. Based on these results, this clinical study was approved by the Ministry of Health, Labour and Welfare's Health Sciences Council in February 2019. While the manufacturing and quality evaluation of clinical-grade cells had already been completed, the start of the clinical study was postponed due to the spread of COVID-19. However, in October 2021, we initiated a clinical study targeting subacute complete spinal cord injury (2-4 weeks post-injury) and performed the first cell transplantation in December of the same year. Eventually, this clinical trial was completed in November 2024. I will introduce the current status and prospects of this clinical study. Transplantation of Spinal Cord-Type iPS Cell-Derived Neural Stem Cells for Chronic Incomplete Spinal Cord Injury In chronic incomplete spinal cord injury, demyelinated residual axons are the therapeutic target. We established an induction method for spinal cord-type iPS-derived neural stem cells (iPS-NSCs), which have a high differentiation potential into oligodendrocytes, and conducted preclinical studies. As a result, we demonstrated that even in chronic spinal cord injury, where functional recovery was difficult with conventional hindbrain-type iPS-NSC transplantation alone, spinal cord-type iPS-NSC transplantation alone could achieve functional recovery. Based on these findings, our project was selected for AMED's Practical Research Project for Regenerative Medicine, and we plan to conduct a physician-led clinical trial for chronic incomplete spinal cord injury in 2026. I will discuss the preparation status for this trial and our ongoing efforts toward addressing chronic complete spinal cord injury.

REVOLUTIONIZING THE TREATMENT OF ORGAN FAILURE

Qin, Wenning, *Innovation and Process Development, eGenesis, USA*

Organ transplantation is proven to be life-saving for patients suffering from end-stage organ failure. However, globally, the need for organ far exceeds organ supply. Over the years, porcine cells, tissues, and organs have been investigated as an alternative for human transplantation. Owing to the daunting task of making porcine organs compatible with human use, progress has been slow. In this presentation, I will review several technological advancements that are enabling, including an efficacious immune suppression regimen, advent of the gene editing technology, clustered, regularly interspaced, short palindromic repeats (CRISPR), and the use of the decedent model. With favorable transplant data produced from non-human primates, human decedent models, and compassionate uses, xenotransplantation is now advancing to formal clinical studies. It is very likely that in the near future, xenotransplantation may translate and revolutionize the treatment of organ failure.

PLENARY VI: AWARDS & KEYNOTE
1:30 PM – 3:00 PM
GRAND HALL, LEVEL 3

2025 ISSCR OUTSTANDING YOUNG INVESTIGATOR AWARD LECTURE: TOWARD A SPATIOTEMPORAL MODEL OF MAMMALIAN GASTRULATION

Stelzer, Yonatan, *Department of Molecular Cell Biology, Weizmann Institute of Science, Israel*

My research group aims to address the fundamental challenge of understanding how multicellular organisms achieve variation despite cells having identical genetic information. The development of a single fertilized egg into a complete mammalian embryo is an especially beautiful embodiment of this problem. During this process, cellular differentiation is defined by the capacity of the cell ensemble to acquire increasingly specialized internal states. It follows a coordinated and canonical trajectory in which, given sufficient sampling, all possible embryos and cell states can be measured. It also allows embryonic time to be treated as a quantitative metric, providing a powerful framework for modeling developmental dynamics. In recent years, we have worked toward establishing phenomenological quantitative models of the spatiotemporal process of mammalian gastrulation. Building on such models, we strive to dissect epigenetic mechanisms regulating intracellular diversification, as well as signaling mechanisms that shape cell fate decisions and patterning non-cell-autonomously. We are excited by recent breakthroughs in single-cell transcriptomics and epigenomics. However, we believe there is an urgent need to match descriptive single-cell atlases with models and experimental frameworks to derive novel insights into function and regulation. In my talk, I will present recent progress by my group toward these aims.

2025 ISSCR ACHIEVEMENT AWARD LECTURE: MODELING HEMATOPOIETIC AND CARDIOVASCULAR DEVELOPMENT WITH HUMAN PLURIPOTENT STEM CELLS

Keller, Gordon, *McEwen Stem Cell Institute, University Health Network, Canada*

The efficient generation of functional cell types from human pluripotent stem cells (hPSCs) has opened new avenues to study the earliest stage of human development, to model a range of different diseases and to design and develop novel therapies to treat them. The success of these approaches is, however, dependent on our ability to accurately recapitulate the early stages of lineage specification in the differentiation cultures to enable the generation of the target cell of interest. By translating the principles of developmental biology to the hPSC system, we have been able to model human hematopoietic and cardiovascular development in vitro and to establish comprehensive linage maps that define the origin of the different blood and heart cell types. A key finding from these studies was that cell fate decision are made early, at the stage of mesoderm induction. Our current research program is focused on the generation of two additional cell types from hPSCs. The first is functional antibody secreting B cells and the second is vascular progenitors able to revascularize different target tissues in pre-clinical animal models. Updates on these projects will be presented.

KEYNOTE: EX VIVO GENE THERAPY FOR DMD: TOWARDS A UNIVERSAL DONOR CELL
Cossu, Giulio, *Division of Neurosciences, San Raffaele Scientific Institute, Italy*

After encouraging results in preclinical models, we conducted a first in man trial based upon systemic delivery of HLA-matched, donor mesoangioblasts (pericyte derived muscle progenitors) in patients affected by Duchenne Muscular Dystrophy (DMD). The trial proved safe but not efficacious mainly for the very low engraftment of donor cells (< 1%) in dystrophic muscles despite > 1 billion cells injected. Thus, we explored strategies to enhance efficacy despite an engraftment that will be always low in tissues such as muscle where ablation of resident cells is not possible. We reasoned that, being the muscle fibre a multinucleated cell, we may use our transplanted cells as “trojan horses” to introduce the genetic correction also in neighbouring nuclei. Thus, we transduced autologous mesoangioblasts with a lentiviral vector expressing the U7 small nuclear RNA, engineered to skip exon 51 of the dystrophin gene. The U7 snRNA causes exon skipping in the corrected nucleus but also diffuses to neighboring nuclei thus amplifying of about one log the amount of dystrophin production. When transplanted in DMD/NGS mice carrying a humanised, mutated exon 51, the level of dystrophin produced was well above what is considered the therapeutic threshold and mice regained a normal motility. Based on this we are running a phase I trial in non-ambulant patients carrying a skippable mutation of exon 51 (EudraCT number: 2023-000148-47). We have also implemented the transplantation protocol and developed a strategy to target the DMD dilated cardiomyopathy. Even if successful, this would remain an example of “personalised medicine”, with extremely high costs, that are putting at risk the future of many gene and cell therapies. To overcome this problem, we developed universal donor mesoangioblasts, currently being tested for immune privilege and safety.

POSTER ABSTRACTS

Wednesday, 11 June 2025

TRACK: SOMATIC STEM CELLS AND CANCER (SSCC)

Poster Session 1 (ODD)

4:00 PM – 5:00 PM

W1001

ALLOGENEIC IPS CELL-DERIVED GAMMA-DELTA T CELLS DEMONSTRATE CYTOTOXICITY AGAINST PATIENT-DERIVED COLORECTAL CANCER TISSUES VIA LOCAL AND INTRAVENOUS ADMINISTRATION

Futai, Ryoko, *Kobe University, Japan*
Koyanagi, Michiyo, *Kobe University, Japan*
Horikawa, Manabu, *Kobe University, Japan*
Horie, Kazumasa, *Kobe University, Japan*
Aoi, Takashi, *Kobe University, Japan*

Various immunotherapies are being developed to treat malignant tumors. γδT cells, which constitute 3–5% of peripheral blood lymphocytes, have attracted attention for their potent cytotoxic activity and ability to target a broad range of tumors in an MHC-unrestricted manner. We successfully derived γδT cells from induced pluripotent stem cells (iPS cells) and demonstrated their cytotoxic effects against a colorectal cancer cell line in vitro. However, cancer cell lines often lack key tumor characteristics, such as heterogeneity and drug sensitivity. In contrast, patient-derived colorectal cancer organoids (PDCOs) retain many features of the original tumor tissue. This study aimed to evaluate the cytotoxicity of allogeneic iγδT cells against colorectal cancer cell lines and PDCOs in both in vitro and in vivo models. We assessed the cytotoxicity of iγδT cells against the colorectal cancer cell line SW480 and PDCOs in vitro. Next, the cell line and organoids were transplanted subcutaneously into immunodeficient mice. iγδT cells were administered either locally at transplantation or intravenously after confirming tumor formation through IVIS luminescence imaging. Tumor growth in treated groups was compared with untreated groups to evaluate the in vivo cytotoxicity of iγδT cells. In vitro, allogeneic iγδT cells demonstrated over 90% cytotoxicity against the SW480 cell line and 70–90% against the two PDCO lines. In vivo, local administration of iγδT cells suppressed tumor growth by over 80% in mice transplanted with the cell line and by 90–100% in those transplanted with PDCOs. Intravenous administration of iγδT cells to PDCO-transplanted mice resulted in over 70% tumor cytotoxicity. Similarly, intravenous administration after confirming tumor formation achieved comparable cytotoxicity exceeding 70%. Allogeneic iγδT cells showed significant cytotoxicity against patient-derived colorectal cancer tissues in vitro and in vivo. Their efficacy was observed with both local and intravenous administration. These findings suggest that allogeneic iγδT cells hold promise as a novel immunotherapy for colorectal cancer.

W1003

EXTRACELLULAR VESICLES-MEDIATED TRANSFER OF LET-7B/7C PROMOTES THE TRANSIT-AMPLIFICATION OF SPERMATOGONIA IN NEONATAL MOUSE TESTIS

Choy, Kathleen Hoi Kei, *The Chinese University of Hong Kong, Hong Kong*
Xie, Ting, *The Hong Kong University of Science and Technology, Hong Kong*
Fok, Ellis, *The Chinese University of Hong Kong, Hong Kong*
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The self-renewal and differentiation of spermatogonial stem cells (SSCs) play essential roles in spermatogenesis. Extracellular vesicle (EV) is a universal strategy for intercellular communications in stem cell niches. However, the involvement of EVs in regulating the SSCs remains largely unknown. In this study, we have revealed the role of testis EVs isolated from postnatal day 7 (PND7) neonatal mouse testis in guiding spermatogonia into a transit-amplifying state with increased proliferation while retaining their differentiation potential. We profiled the repertoires of proteins and small RNAs by proteomic and small RNA transcriptomic analyses, respectively. We further showed that the EVs secreted by spermatogonial stem/progenitor cells and the Sertoli cells, but not from more differentiated germ cells, conveyed let-7b/7c miRNA cargoes to spermatogonia, which mediates the effect of EVs on spermatognial transit amplification. Together, this study has deciphered an important intercellular communication within the spermatogonial niche mediated by let-7b/7c cargoes of EVs, providing a new insight into the regulation of SSCs and spermatogenesis.

Funding Source: This work was supported in part by grants from the Research Grant Council of Hong Kong (T13N-62S), the Direct Grant of CUHK and the Lo Kwee Seong Start-Up Fund to K.L.F.

W1005

NUCLEOCYTOPLASMIC SHUTTLING OF TET2 PREDICTS THE SURVIVAL OF ADVANCED COLORECTAL CANCER

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TET2 dysfunction has been observed in several types of solid tumours, but the exact role of TET2 in tumour progression is unknown. In this study, via utility of SW480-SW620, which is a unique pair of cell lines derived from the same patient and originated from primary tumour and metastasis tumour respectively, we found by in vivo and in vitro experiments that TET2 translocate from cell cytoplasm to cell nuclear in the late stage of tumour progression. More importantly, nuclear translation of TET2 was associated with increased DNA demethylation activity and tumour

suppressor activity. And we found that epithelial-mesenchymal transition (EMT) is a crucial molecular mechanism regulating cell migration and TET2 nuclear translocation. Further sequencing of individual cells revealed a gradual transition of cells from the inside to the outside of the clone. During migration, TET2 forms negative feedback loops with the EMT and WNT pathways, resembling the clinical progression of colorectal cancer (CRC) patients. Finally, pseudotime points were plotted based on the migration process as a measure of TET2 activity and patient survival in different tumours. Taken together, these data suggest that TET2 is an innate brake of cancer progression and serves as an important therapeutic target in the treatment of solid tumours.

W1007

A CONSERVED CELL SIZE HOMEOSTASIS MECHANISM DETERMINES CELL CYCLE HETEROGENEITY IN BASAL LAYER SKIN STEM CELLS

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The heterogeneity in stem cell cycle underlies many aspects of tissue dynamics, including turnover rate and clonogenicity. A large body of work has identified the intricate and multitudinous ways that extracellular information regulate stem cell behavior. However, the quantitative nature of cell cycle decisions are still not well-understood, especially in vivo. Here, we show that a cell size homeostasis mechanism, first identified in yeast and subsequently in cell cultures, autonomously governs the decision to enter S phase in epidermal stem cells. Cell-extrinsic factors like variation in the cellular microenvironment affects cell growth rates but not the autonomous coupling of cell size to the G1/S transition. We confirm this finding using laser ablation experiments to perturb the cellular microenvironment, and show that the coupling between cell size and the G1/S transition remains invariant despite upregulation of cellular growth rates. Lastly, we show that this cell-intrinsic coupling between cell size and cell cycle progression is conserved to cycling cells in the intestinal epithelium lineage, as well as cycling osteoblast cells in the zebrafish scale. Our work overhauls long-standing models of cell cycle regulation within complex metazoan tissues and reinforces the importance of cell-intrinsic size control as a critical factor regulating cell division in vivo.

Funding Source: NIGMS Company of Biologists.

W1009

ENGINEERING HUMAN IPSC-DERIVED CAR-MONOCYTES/MACROPHAGES CELLS FOR SOLID CANCER IMMUNOTHERAPY

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Cell based cancer immunotherapies are complex living drugs, which has emerged as complementary fourth pillar of cancer treatments alongside surgery, chemotherapy and radiation. However, immunotherapy benefits are mostly confined to liquid cancers and to a limited subset of solid tumor patients. Generalization of immunotherapies have failed to make an impact on gastric cancers. Monocytes and macrophages are the dominant cellular constituents, which are reprogrammed by the tumor to support their growth. However, these macrophages innately engulf cancer cells as a natural defense mechanism. We envision to leverage the potential of these powerful cancer eaters, which are also the natural inhabitants of the solid tumors. We will use our lambda-integrase genome engineering technology (LIGIT) to engineer multiple functional modules in stem cells as a renewable source of tissue macrophages. These functional modules will encompass chimeric antigen receptors (CARs) that will render tumor recognition capability to engineered stem cell derived monocytes and macrophages. Additionally, other functional modules such as brain homing, and tumor kill signals will enable passage of these robust cancer eaters to the tumor site and facilitate killing and inhibition of tumor growth. As such multi-modular functional gene assemblies can only be possible with genetic engineering tools that can integrate DNA of 10-15kb size or more. In this context LIGIT platform offers versatility to achieve such large DNA integrations for innovative cell and gene therapies. Thus, we are well poised to develop first-in-class prototype for robust cell based brain tumor immunotherapies, which can be translatable to brain metastasis and other solid tumors.

Funding Source: A*STAR (Singapore Therapeutics Development Review funding).

W1011

IN VIVO PARTIAL EPIDERMAL REPROGRAMMING ORCHESTRATES SKIN HOMEOSTASIS AND REGENERATION

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Adult stem cells and their niches communicate intricately for tissue maintenance and regeneration. However, effectively coordinating these complex interactions is challenging. Here, we demonstrate that transient dedifferentiation of epithelial stem cell progenies orchestrates beneficial changes within the entire skin's cellular networks to favor repair. We achieved this by inducing a reversible expression of reprogramming factors (Oct-4, Sox2, Klf4, and c-Myc) in the mouse epidermis. This in vivo partial epidermal reprogramming not only affected the reprogrammed cells, but also their microenvironment, including neighboring epithelial cells and T cells, conferring widespread healing characteristics even in the absence of injury. When a wound was introduced, these collective changes accelerated re-epithelialization. Furthermore, the effects extended to dermal healing, leading to reduced scarring and angiogenesis. In conclusion, our work reveals that partial reprogramming of the epidermis influences various cell types within the skin during homeostasis and repair, leading to enhanced cutaneous wound healing.

Funding Source: This work was supported by NRF of Korea grants (NRF-2022R1C1C1011895, NRF-2022M3A9D3016848, NRF-RS-2023-00221112, NRF-RS-2023-00223298, NRF-RS-2025-00560319) and BK21 FOUR Research Fellowship funded by the Korean government.

W1013

T-CADHERIN MARKS AN MSC SUBPOPULATION WITH STEM-LIKE PROPERTIES AND A REGULATORY ROLE IN ADIPOGENIC DIFFERENTIATION

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The heterogeneity of adipose tissue-derived mesenchymal stem/stromal cells (MSCs) has long been a topic of interest. In this study, we performed single-cell RNA sequencing (scRNA-seq) on MSCs isolated from human subcutaneous white adipose tissue (WAT) and cultured under both control conditions and adipogenic induction for 4 days. Our analysis identified a distinct subpopulation of T-cadherin-expressing cells that co-expressed dipeptidyl peptidase-4 (DPP4), a known marker of multipotent progenitors in adipose tissue and other stemness-associated genes (Notch1, Notch3, Wnt family members, N-cadherin, Neuropilin 2, IGFBP4, key MSC markers (CD90, PDGFR), LDLR, adipogenesis regulatory factor and etc.). Upon adipogenic induction, T-cadherin expression declined overall; however, a subset of cells retained high T-cadherin levels and stemness-related genes, such as NANOG, SPN, SOX2, PRMT8. Pseudotemporal trajectory analysis of scRNA-seq data revealed that T-cadherin-expressing cells represented a discrete stem-like subpopulation rather than participating in adipogenic differentiation. Functional assays using lentiviral transduction demonstrated that T-cadherin overexpression significantly reduced MSC proliferation rates. Long-term live-cell imaging over 10 days further revealed a substantial reduction in adipogenic differentiation capacity among T-cadherin-overexpressing MSCs. Interestingly, T-cadherin-expressing cells also suppressed adipogenic differentiation in the surrounding cells, likely through exosome-mediated signaling. Consistently, scRNA-seq analysis revealed an upregulation of exosome markers (CD9, CD63, CAV1) in T-cadherin-expressing cells, implicating their potential role in modulating MSC differentiation. Taken together, our findings identify a distinct T-cadherin-expressing MSC subpopulation characterized by stem-like properties and a regulatory role in adipogenic differentiation, providing new insights into the mechanisms governing adipose tissue homeostasis.

Funding Source: The study was supported by the Russian Science Foundation grant No. 23-11-00205 (https://rscf.ru/en/project/23-11-00205/) and grant No. 19-75-30007 (https://rscf.ru/project/19-75-30007/).

W1015

A BINDING MODE FOR GLUCOSE AND A METHYLTRANSFERASE IS RESPONSIBLE FOR DIFFERENTIATION AND CANCER RESISTANCE

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Methyltransferases are group of enzymes that catalyze the addition of methyl alkyl group to their clients. They play a critical role in cell homeostasis as they are involved in epigenetic, epitranscriptomic, and post-translational modifications. Such events control gene expression, RNA splicing, and protein-protein interactions. Recently, it was found that glucose affects the machinery of methyltransferases. For example, glucose was found to modify epigenomics by inhibiting DNA methyltransferase 1 (DNMT1). It can also mediate oligomerization and catalytic events like what happens in NSUN2. Although glucose binding mode was not revealed yet in methyltransferases-glucose complexes, there is a trend that glucose forms hydrogen bonds with polar and charged amino acid residues, as well as phenyl and indole hydrophobic interactions with its ring. The electrostatic surface analysis of the glucose-binding residues in NSUN2 show that the majority of the region is polar, mainly due to Lysine, Arginine, and Glutamine. Sequence analysis with other human glucose sensitive proteins show conserved amino acids that might play a role in glucose sensing.

W1017

INDUCED PLURIPOTENT STEM CELLS: A RARE OPPORTUNITY FOR STUDYING ULTRA-RARE SMALL ROUND BLUE SARCOMAS

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CIC::DUX4 sarcoma (CDS) is a rare, aggressive malignancy predominantly affecting children and adolescents. Characterized by CIC and DUX4 translocations, CDS accounts for less than 1% of sarcomas and is associated with rapid chemoresistance and poor clinical outcomes, with metastatic cases having a median survival of less than one year. Current treatments, adapted from small round cell sarcoma protocols, have shown limited efficacy. The rarity of CDS, coupled with the absence of robust experimental models, has significantly impeded the development of targeted therapies. To address these challenges, we developed a novel induced pluripotent stem cell (iPSC)-based model that incorporates both the genetic driver (CIC::DUX4) and the epigenetic landscape of cancer-initiating cells. This approach enables the study of permissive cell lineages with embryonic characteristics, creating a microenvironment conducive to malignant transformation. Our model provides a platform to investigate early transformation events, the cell of origin, tumor-immune interactions, stromal contributions, and tumor heterogeneity. We demonstrated that CIC::DUX4 expression in iPSC-derived differentiating cells leads to tumor formation with histological and molecular features consistent with CDS. From these tumors, we derived iCDS cell lines that retain the molecular signature of CDS in vitro and form tumors in vivo. Additionally, CIC::DUX4 was shown to suppress MHC-I presentation, facilitating immune evasion and promoting tumor growth in an immunocompetent environment. Importantly, our model provides

critical insights into the cell of origin for CDS. The tools and findings from this study offer a powerful resource for the CDS research community and provide a versatile platform to study other rare malignancies, particularly those with low mutation burdens and lacking experimental models, such as other small round blue cell sarcomas.

Funding Source: Children's Cancer Research Fund, Department of Defense.

W1019

MATRIX SOFTNESS AND FLUID SHEAR FORCE ACT THROUGH TRPV4 TO PROMOTE OVARIAN CANCER STEMNESS, TUMORIGENICITY AND METASTASIS

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Matrix stiffness and fluid shear force are key mechanical cues in ovarian cancer microenvironment that can impact cancer stemness, tumorigenicity and metastasis. However, the molecular identity of mechanosensor and/or mechanosensitive mediator that can be respond to matrix stiffness and fluid shear force is unclear. TRPV4 is a mechanosensitive Ca2+-permeable channel. In the present study, we found that soft matrix can increase the expression level of TRPV4, subsequently stimulating the growth of ovarian cancer stem cells (OCSC), elevating the expression level of multiple cancer stem cell (CSC) markers, and promoting tumor xenograft growth. Furthermore, fluid shear force can directly stimulate the activity of TRPV4 channels, consequently elevating the expression level of multiple CSC markers, promoting migration of OCSCs in vitro and peritoneal tumor metastasis in vivo. Taken together, TRPV4 is a key mechanosensor (or mechanosensitive mediator) that responds to matrix softness and fluid shear force in ovarian cancer progression.

W1021

THE CELL FATE DYNAMICS OF HEMATOPOIETIC STEM CELLS IN 5-FLUOROURACIL-INDUCED STRESS HEMATOPOIESIS

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Hematopoietic stem cells (HSCs) reside at the apex of the differentiation hierarchy and provide the lifelong supply of all blood cell lineages. While decades of work have revealed HSCs' differentiation pathways and kinetics to maintain homeostasis and ensure regeneration, our knowledge about their immediate cell fates following various stress conditions remains limited. Here, we determined the daily proliferation and survival states of murine HSCs following 5-fluorouracil (5-FU)-induced myeloablation during a 14-day recovery process. We further investigated their differentiation kinetics in this period using complementary approaches of scRNA-Seq and lineage tracing. Using the high expression of endothelial protein C receptor (EPCR) as the primary marker for HSC identification, our work revealed a partial but immediate depletion of the most primitive HSC population following 5-FU treatment. Notably, this partial depletion was not attributed to increased HSC cell death or migration. Instead, it was caused by an abrupt increase in their differentiation, as suggested by the results of a lineage tracing analysis using an inducible EPCR-directed Cre

labeling model. scRNA-Seq and mathematical modeling further revealed that HSC produced the multipotent progenitor (MPP) compartment through an atypical differentiation pathway that is active under the stress condition but latent at the steady state. Unexpectedly, HSC atypical differentiation declined rapidly after this initial surge, and HSCs contributed mainly to the primed HSC compartment and the the megakaryocyte-erythroid biased MPP subtype during the bulk of the recovery period. Collectively, our work uncovers sequential differentiation patterns adopted by HSCs upon extensive myeloablation, which provides a framework to elucidate the molecular mechanisms controlling HSC-mediated regeneration and ultimately helps to design novel strategies to facilitate hematopoietic recovery in myelosuppressed patients.

W1023

TRANSCRIPTION FACTORS TFEC AND MAFB SPECIFY HUMAN IPSC TO BLOOD STEM CELL-SUPPORTIVE SINUSOIDAL VASCULAR ENDOTHELIAL CELLS

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Sinusoidal endothelial cells (ECs) and other perivascular supportive cells constitute a specialized niche microenvironment for the hematopoietic stem and progenitor cells (HSPCs). HSPC niches are found in the fetal liver and the adult bone marrow. The transcriptional programs that orchestrate the HSPC niche functions remain unclear. We elucidated the transcription factor code that specifies the venous sinusoids of the marrow. Using zebrafish, we performed single-cell RNA-sequencing analyses between HSPC niche sinusoidal ECs in the adult marrow and the non-HSPC niche sinusoidal ECs in the adult liver. These results were compared to murine marrow sinusoids. By overexpressing the differentially expressed transcription factors (TFs) tfec and mafbb in the adult zebrafish liver sinusoids, we reprogrammed liver sinusoids to upregulate key genes known for HSPC niche-supportive functions, including mrc1a (log2FC=4.9, p< 0.005), lyve1b (log2FC=3.6, p< 0.005) and dab2 (log2FC=5.0, p< 0.005). These reprogrammed sinusoidal ECs attracted primary runx1+ spi1b+ HSPCs. Transplantation of whole liver cells in the reprogrammed liver achieved functional multi-lineage engraftment in immunocompromised recipients by transplant assay (7/26 in the reprogram group vs. 0/20 in the control not programmed group, p=0.0296). Using this information, we reprogrammed human iPSC into HSPC niche-like sinusoidal ECs by DOX-inducible overexpression of human TFs ETV2, TFEC, and MAFB. These HSPC niche-like sinusoidal ECs significantly upregulated putative HSPC niche-supportive genes, including MRC1

(log2FC=3.6, p< 0.005), STAB2 (log2FC=10.3, p< 0.005), JAG1 (log2FC=1.7 p< 0.005), and CXCL12 (log2FC=5.1 p< 0.005). Co-culture with primary human cord blood-derived CD34+ HSPCs revealed that these HSPC niche-like sinusoidal ECs could better preserve the co-cultured HSPC stem cell properties, shown by higher RNA expression of stem cell marker genes such as CD34 and MECOM, and by a greater number of multipotent colonies in the colony-forming unit (CFU) assay. More importantly, these HSPC niche-like sinusoidal ECs helped the co-cultured HSPCs improved HSPC engraftment compared to generic ECs (p=0.0164). Our findings unveiled the transcription factor code TFEC and MAFB in specifying sinusoidal ECs to have HSPC niche function.

W1025

CRISPR-CAS9 SCREENING IDENTIFIES ROADBLOCK GENES DURING PLURIPOTENCY INDUCTION

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One of the main problems of induced pluripotent stem cell (iPSC) production is that iPSCs often lack the capacity for differentiation when they are reprogrammed partially. Using Sendai virus vector, our lab has isolated homogenous populations of partially reprogrammed iPSCs, termed paused iPSCs, by reducing exogenous KLF4 level, which can resume reprogramming to acquire high pluripotency when the KLF4 level is increased. We had made Rex1-reporter paused iPSCs which can monitor Rex1 expression, which is increased only in fully reprogrammed iPSCs. Using these pluripotency-reporter cells, I tried to find potential roadblock genes that may prevent the reprogramming from acquiring pluripotency to obtain fully reprogrammed iPSCs efficiently. To find the roadblock genes, I introduced Genome-scale CRISPR-Cas9 knockout library into the reporter cells to screen for genes that may be functionally related to acquisition of full pluripotency. I isolated cells that showed higher pluripotency upon introduction of the sgRNA library and identified 43 candidate genes based on the sgRNA sequences integrated into the cells. To validate the relationship between the candidate genes and acquisition of pluripotency, I knocked out or knocked down each of them in the paused iPSCs and confirmed that knockdown of Lnx1 gene, encoding E3 ligase, increased the pluripotency significantly, indicating its potential function as a roadblock gene during acquisition of pluripotency. In addition, Lnx1 downregulation facilitated pluripotency acquisition during Sendai virus-based and Retrovirus-based reprogramming, suggesting that Lnx1 prevented cells from acquiring pluripotency during reprogramming.

Funding Source: JSPS KAKENHI No. JP23H02407 JST SPRING No. HJI06015.

W1027

FUT5 OVEREXPRESSION IN METASTATIC OVARIAN CANCER STEM CELLS PROMOTE IMMUNE ESCAPE BY MODULATING UNCONVENTIONAL DN T CELLS

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Intratumoral heterogeneity is known to drive tumor recurrence and eventual treatment failure. In particular, cancer stem cells (CSCs), a small subset of cells within heterogeneous tumor cells, are capable of adapting to the tumor microenvironment, resisting chemotherapy, and driving metastatic progression. Glycosylation has been implicated in the regulation of cell pluripotency and cancer pathogenesis. However, the role of CSC-associated glycosylation changes in ovarian cancer metastatic progression remains elusive. In this study, we showed that fucosyltransferase 5 (FUT5) is significantly associated with advanced stage (p= 0.0058) and poor overall survival (p= 0.0163) in ovarian cancer patients (HGSOC, n= 53). Multiplex immunofluorescence staining analysis of T cell markers showed that high FUT5 expression in ovarian tumors is negatively linked to an unconventional CD4-CD8- (double negative, DN) T cell subset, IFNγ response, and activation of cytotoxic T cells and NK cells (Ki67+ cells). Unconventional T cells are emerging as important modulators of the anti-tumor immune response within the tumor microenvironment. They possess the unique ability to activate both innate and adaptive immune response through vast production of IFNγ. In an in vivo experiment using a humanized mice model, FUT5 KO in metastatic-CSCs (M-CSCs) suppressed metastatic tumor growth and increased effector cytotoxic T cells. In addition, while the NS control tumors showed no response to anti-PD-1 immune checkpoint blockade (ICB) treatment, activation of unconventional NKT cells sensitized NS tumors to ICB treatment. Together, the study reveals a novel mechanism of immune modulation mediated by M-CSCs in ovarian cancer. Our results on FUT5-dependent attenuation of unconventional DN T cells could offer valuable insights for the development of novel therapeutic strategies aimed at enhancing the prognosis of ovarian cancer patients.

Funding Source: This research is supported by RGC SRFS2223-7S05, HMRF 08192286 and LSCCB under the Health@InnoHK Program launched by ITC, HKSAR.

W1029

HUMAN GASTRIC INTESTINAL METAPLASIA ORGANOIDS DISPLAY GENETIC PROFILES LINKED WITH EARLY NEOPLASTIC GROWTH BEHAVIOUR

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Gastric intestinal metaplasia (IM) is a pre-neoplastic lesion of gastric cancer, characterised by the transformation of normal gastric cells into intestinal cells. Although IM is associated with a higher risk of developing gastric cancer, a representative in vitro model that fully recapitulates the cellular and genetic alterations in IM remains unavailable. This limitation hinders a detailed biological characterisation of IM and restricts the development of predictive tools for assessing gastric cancer risk. This study established a cohort of gastric IM organoids derived from 47 gastric cancer patients and conducted comprehensive molecular profiling and functional characterisation. Transcriptome analysis revealed a distinct gene expression profile for the IM organoids, which co-express gastric

and intestinal lineage-specific markers. WES data analysis demonstrated a higher mutation burden in IM organoids compared to their normal counterparts, noting a frequent chromosome 20 gain. Functionally, IM organoids and normal gastric organoids with chromosomal aberrations exhibited greater cell-matrix independence than copy number neutral organoids. The spheroid assay enriched a subset of cell-matrix independent IM cells. The IM spheroids displayed increased chromosomal aberrations, along with an upregulation of intestinal-specific markers and genes associated with hypoxia and epithelial-mesenchymal transition. In conclusion, this organoid cohort and spheroid model, encompassing genomic, transcriptomic, and clinicopathological data, serve as invaluable resources for investigating the pathways involved in IM pathogenesis and cell-matrix independence. These tools pave the way for a mechanistic understanding of the sequential changes necessary for gastric cancer progression and the future development of early detection methods and targeted therapeutic strategies.

Funding Source: Theme-based research grant (project no.T12-710/16-R) from the RGC of HKSAR General Research Fund (project no. 17108616 and 17107219) from the RGC of HKSAR Health@InnoHK Initiative funded by the Innovation and Technology Commission.

W1031

SUPPRESSING GLUTAMATE DEHYDROGENASE GUIDES HEMATOPOIETIC STEM CELL DIVISIONS INTO SELF-RENEWAL

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Hematopoietic stem cells (HSCs) are capable of both self-renewal and multi-lineage differentiation, which govern the lifelong maintenance of hematopoietic homeostasis. Although HSCs have the potential to expand functional stem cells by self-renewal, their divisions often induce their differentiation in several cases. Thus, it remains unclear how HSCs determine cell fate during or after divisions. To address this question, we firstly focused on the role of Glu catabolism in HSCs, since self-renewing mouse HSCs during hematopoietic regeneration showed greatly lower intracellular Glu level compared to HSCs attenuating stem cell features during ex vivo culture. Typically, HSCs elevated intracellular Glu level via JAK-STAT signaling to promote their proliferation through aminotransferase reactions, which is Glu catabolic pathway. In this context,

we identified glutamate dehydrogenase (GLUD1), an enzyme responsible for another Glu catabolic pathway, as one of the key factors to induce HSC differentiation. Strikingly, GLUD1 inhibition more efficiently maintained stem cell features without impairing their divisional activity, facilitating functional HSC expansion through self-renewing divisions. Crucially, this beneficial effect required robust JAK2-STAT5 signaling. Mechanistically, GLUD1 interacted with STAT5a, an association that was disrupted, when the inhibition of GLUD1-mediated Glu catabolism preserved HSC features. Moreover, when HSC divisions were robustly driven despite low intracellular Glu level in vitro by mimicking BM environments during hematopoietic regeneration, functional HSCs were expanded similarly to those under GLUD1 inhibition. Our findings propose that, particularly after HSC divisions under robust JAK-STAT signaling, GLUD1 acts as a fate determinant in newly generated cells by modulating its interaction with STAT family in response to intracellular Glu levels. Overall, our findings proposed that GLUD1 determines HSC fate after divisions under robust JAK-STAT signaling, highlighting the important link between Glu catabolism and cytokine signaling in HSC fate decisions.

Funding Source: This study is supported with an unrestricted grant from the Chemo-Sero-Therapeutic Research Institute (KAKETUSKEN).

W1033

THE ROLE OF ZNF143 ON ENHANCING STEMNESS AND CHEMORESISTANCE IN MUSCLE-INVASIVE BLADDER CANCER

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Several molecular programs involved in maintaining stem cell properties have been identified as novel molecular signals implicated in oncogenic dedifferentiation, metastasis of cancer, and major contributors to chemoresistance following cancer treatment. Furthermore, the heterogeneity observed in malignant tumors aligns with the diverse differentiation potentials of stem cells. Bladder cancer, a prevalent and deadly malignancy ranking as the sixth most common malignant tumor in men worldwide, recognizes the stemness feature as a poor prognostic factor for cancer malignancy, alongside various clinical and pathological factors. This study aimed to identify the mechanisms underlying the stemness features of muscle-invasive bladder cancer (MIBC), an aggressive form of the disease with limited therapeutic options. We previously reported that phosphorylation of Transcription factor CP2-like protein 1 (TFCP2L1) at Thr177 by cyclin-dependent kinase 1 (CDK1) is a central mechanism in embryonic stem cells (ESCs) pluripotency as well as adult bladder carcinogenesis. Here, we found that Zinc Finger Protein 143 (ZNF143) transcription factor physically interacted with TFCP2L1 and OCT-4 for maintaining naïve pluripotency in murine ESCs. In T24 human bladder cancer cells, the expression level of ZNF143

correlated with cell proliferation capacity. The positive effect of ZNF143 was observed also in tumor sphere formation and limiting dilution colony formation assays, supporting a crucial role of ZNF143 in the stemness features of bladder cancer cells. Additionally, increased ZNF143 expression enhanced glutathione (GSH) dynamics, which is a potential predictive and therapeutic trait for neoadjuvant chemotherapy response in bladder cancer. Indeed, ectopic expression of ZNF143 induced resistance to cisplatin as evidenced by the cell viability and apoptosis assays, demonstrating the significant role of ZNF143 in the response to cisplatin-based chemotherapy in bladder cancer cells. Collectively, our findings suggest that ZNF143 plays a critical role in promoting the stemness properties and chemo-resistance of bladder cancer cells. This study could provide a foundation for developing targeted therapies against ZNF143, potentially improving treatment outcomes for patients with MIBCs.

Funding Source: This research was supported by the National Research Foundation of Korea (NRF-2021R1A2C2005790), the Ministry of Education (RS-2024-00449162), and Asan Institute for Life Sciences, Asan Medical Center (2025IP0083), Seoul, Korea.

W1035

APOPTOTIC VESICLES DIFFERENTIALLY DIRECT GLYCOLYTIC BALANCE TO RESHAPE IMMUNOSUPPRESSION AND ANTI-PD1 THERAPY IN LIVER CANCER

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The clinical efficacy of programmed cell death-1 (PD-1) blockade therapy may be limited by the inclined M2 tumor-associated macrophage (TAM)- and regulatory T cell (Treg)-dominant immunosuppressive tumor microenvironment (TME). However, factors that promote the immune repressive TME remain to be elucidated. Here, we found that apoptotic hepatocellular carcinoma (HCC)-derived apoptotic vesicles (apoVs) were increased in patient tumors following anti-PD-1 therapy, correlated with enhanced infiltration of immunosuppressive cells. Moreover, HCC-apoVs produce Lactate dehydrogenase A (LDHA) to aggravate M2high and Treghigh immunosuppressive phenotypes and aggravate liver cancer. In contrast, mesenchymal stem cell (MSC)-derived apoVs abolish glycolytic tumor metabolite-conferred immunosuppressive phenotypes. Tumor cell- and MSC-derived apoVs displayed distinct properties regulating lactate production and consumption. Mechanistically, MSC-apoVs transfer multiple factors, including miR-34c-5p and nicotinamide phosphoribosyltransferase (NAMPT), to inhibit LDHA-mediated lactate metabolism and promote NAD+ metabolism-initiated glycolysis of macrophages and CD4+ T cells. Furthermore, infused MSC-apoVs can be internalized by the immune cells in the tumor and lymphoid organs. A combination of MSC-apoVs and anti-PD-1 therapy abolishes immunosuppressive TME and potentiates antitumor immunity to boost anti-PD-1 treatment for liver cancer. Our findings reveal an apoV-mediated metabolism-directing effect that can be utilized to rescue tumor metabolite-conferred immunosuppressive TME and provide new therapeutic strategies to evoke the antitumor effect of PD-1 blockade.

Funding Source: This work was supported by grants from the National Natural Science Foundation of China (82170924 to X.K.), Pearl River Talent Recruitment Program (2019ZT08Y485, 2019QN01Y138, 2019JC01Y182 to S.S. and X.K.).

W1037

AVOIDANCE OF FRATRICIDE AND IMPROVED EFFICACY OF CD33-MSLN CAR-iNK CELLS IN TREATING ACUTE MYELOID LEUKEMIA

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CAR-NK cell therapy has been investigated for treating acute myeloid leukemia (AML). CD33 is a well-established therapeutic target for AML. Conventional CD33 CAR-NK cells are prone to fratricide due to endogenous expression of CD33 on expanded NK cells. Mesothelin (MSLN), a tumor differentiation antigen, is expressed in approximately 36% of pediatric AML cases and 14% of adult AML cases. In this study, we designed a novel CD33-MSLN loop CAR (Loop CAR) and evaluated its anti-tumor efficacy in human umbilical cord blood-derived NK (UCB-NK) cells and human pluripotent stem cell-derived NK (hPSC-iNK) cells. The Loop CAR exhibited superior cytotoxicity against dual-antigen-positive tumor cell lines and primary AML cells. To further optimize this approach, we established a hPSC-derived cell line via knockout of endogenous CD33 gene (CD33 KO) and integration of the Loop CAR construct. Using organoid induction technology, we generated mature CD33KO-Loop CAR-iNK cells, characterized by expression of endogenous CD16. This innovative strategy effectively mitigated NK cell fratricide and significantly enhanced CD33-mediated specific cytotoxicity. Moreover, the CD33 KO-Loop CAR-iNK cells demonstrated superior tumor-killing activity in AML xenograft mice and significantly prolonged survival of the treated animals. Our findings provide evidence supporting that hPSC-derived CD33 KO-Loop CAR-iNK cells have translational potential for treating AML.

W1039

CASPASE3/GSDMD COUPLED SUB-APOPTOSIS RECONSTRUCT NUCLEAR ARCHITECTURE TO GUIDE STEM CELL NEUROGENESIS

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Stem cell neurogenic differentiation is crucial for physiological and pathological process of nervous system, but the underlying mechanisms regulating differentiation are elusive. The neurogenetic cells undergo dramatic cellular morphological changes. However, the specific mechanisms

regulating these cellular architecture remodeling and how it contribute to neurogenic differentiation are unclear. Here, we showed that neuro-differentiated stem cells displayed apoptosis-like features, including cell membrane changes, nuclear morphology alterations, and heterochromatin accumulation. RNA sequencing of these neuro-differentiated cells revealed an early apoptotic profile, confirmed by the expression of various apoptotic proteins such as activated Caspase 3, increased levels of Bcl-2, and decreased expression of GSDMD. Conversely, proteins associated with neural differentiation increased following sub-apoptotic stimulation, which accelerated neural differentiation. Inhibition of Caspase 3 resulted in decreased neural differentiation of the stem cells, whereas the knockdown and knockout of GSDMD led to enhanced neural differentiation. Notably, caspase 3-/- mice exhibited lower behavioral activity, while gsdmd-/- mice demonstrated increased behavioral activity. Mechanistically, we showed that nuclear remodeling was detected in both apoptotic cells and Neuro-differentiated stem cells, and the enhanced heterochromatin accumulation and increased H3K9me3 expression are responsible for Caspase3 and GSDMD-mediated neurogenic differentiation. Therefore, injection of siGSDMD plasmid supply with sub-apoptotic induction in the hippocampus notably improved the cognitive impairment of aging mice. This study reveals a previous unrecognized sub-apoptotic state that controls the neurogenic differentiation of stem cells and provides potential targets for developing therapeutic approaches of neurodegenerative diseases.

W1041

COMPARATIVE ANALYSIS OF CLINICAL -GRADE HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS FROM EARLY AND LATE PASSAGES

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Adipose-derived mesenchymal stem cells (ASCs) offer an ideal source for cell-based regenerative therapies due to their easy accessibility and multipotency features. The present study evaluated the impact of in vitro expansion on the morphology, growth, presence of surface markers, lineage differentiation and the expression of molecular markers related to immunomodulation, proliferation, senescence, and aging of clinical-grade human ASCs obtained from early (2-3) and late (9-10) passages. The adherent cells of ASCs cultured in the serum-free media (SFM) exhibited a fibroblast-like morphology. However, the cells with robust proliferative ability were more abundant in cultures of early passages. Cells expressed the mesenchymal markers (CD44, CD73, CD90, CD105) but not the hematopoietic markers (CD34, CD45, HLA-DR), and no difference in expression was observed between the early and late passages. But, late ASCs displayed an enlarged senescent-like morphology, and reduced clonogenic capacity when compared to early ASCs. Upon induction, ASCs were differentiated into osteogenic, adipocytic and chondrogenic lineages. However, early ASCs had higher propensity towards the formation of osteocytes, adipocytes and chondrocytes as evidenced by cytochemical staining and the expression of lineage specific molecular markers. The expression levels of markers assessed by ELISA and qPCR related to immunomodulation, such as interleukin (IL)-1 beta, IL-6, TNF-alpha (pro-inflammatory cytokines), IL-10, TGF-beta and IL-12 (anti-inflammatory cytokines) showed variations with the

duration of the ASCs expansion. The markers pertaining to the proliferation (SIRT1 and Ki67) were downregulated in late ASCs. In contrast, the molecular markers implicated in senescence and aging (p53, p21 and p16) showed the higher levels of expression in late ASCs. In conclusion, there was no significant difference in the expression of phenotypic markers among the passages of clinical-grade ASCs. However, the biological and molecular characteristics of ASCs cultured in SFM varied as the number of passages increased. Therefore, further optimization and assessment of cell performance are required in large scale culture system before using clinical-grade ASCs of late passages for therapeutic applications.

Funding Source: Nitte (Deemed to university).

W1043

DEFINING A NEW ROLE OF VITRONECTIN IN REGULATING MIGRATION OF HUMAN PLACENTA-DERIVED MSCS

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For successful transplantation therapy, it is a prerequisite that MSCs migrate and home in adequate numbers to the site of tissue damage marked by adverse microenvironment conditions. Thus, it is pivotal to identify novel factors and their mechanism of action in regulating the adhesion and migration of MSCs under different microenvironment stress conditions. Our study in human placenta-derived MSCs (PL-MSCs) under nutrient stress demonstrated an increase in cell spread area, increased adhesion, and reduced migration of the cells. Correspondingly, an increase in the total number and size of focal adhesions (FAs), along with prominent stress fibers, were observed. Furthermore, the FAs in nutrient-stressed MSCs were more stable, exhibiting slower turnover and longer lifespan. Vitronectin (VTN), an ECM glyocprotein, was upregulated under nutrient stress condition. Knockdown of VTN under nutrient stress condition led to a significant reduction in the total number and size of FAs along with their faster turnover and shorter lifespan. Subsequently, a reversal in the cell spread area and the adhesion and migration properties of the PL-MSCs were noted. Additionally, our findings indicated that VTN, acting upstream, influenced the phosphorylation of myosin light chain (MLC), promoted the maturation and stability of FAs, and generated organized stress fibers. Overall, our study identifies a new role of VTN as a critical regulator of adhesion and migration in PL-MSCs under nutrient stress condition.

W1045

DISRUPTED FEEDING CIRCUITRY IN ALZHEIMER'S DISEASE MOUSE MODEL LINKS EXCESSIVE EATING TO IMPAIRED ADULT NEURAL STEM CELL ACTIVITY

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Excessive eating behavior has been observed as a precognitive symptom prior to the onset of classical Alzheimer's disease (AD) symptoms such as memory loss/cognitive decline. Currently, it is unknown how the abnormal food intake behavior directly links to AD pathophysiology. Interestingly, hyperphagia and perturbed neurogenesis have been reported in adult 3xTg-AD mice much earlier than the onset of AD pathological hallmarks. In addition, hypothalamic arcuate nucleus (ARC) satiety-activated proopiomelanocortin (POMC) neurons are reported to send long

range axonal innervation to the ventral subdomain subventricular zone (vSVZ) and regulate vSVZ Nkx2.1 marked neural stem cell (NSC) activity in the rodent model. In this regard, it is important to determine the direct link between abnormal feeding behavior and perturbed neurogenesis in 3xTg-AD mice. Intriguingly, our food consumption study revealed drastic increases in the daily intake of food in the 3xTg-AD mice, and we found reduced ARC axonal inputs to contact Nkx2.1+ vNSCs in the 3xTg-AD mice via viral-mediated axonal tracing technique. Associated with this, we identified reduced activation rate of Nkx2.1+ quiescent NSCs (qNSCs) in the vSVZ of 3xTg-AD mice as well. Importantly, administering leptin, a neuropeptide involved in feeding regulation, for 7 days can successfully increase ARC POMC+ neuronal activity and specifically rescue deficits in the activation rate of vSVZ Nkx2.1+ qNSCs in 3xTg mice. Thus, the current study reveals that abnormal feeding behavior in 3xTg-AD mice is associated with perturbed ARC to SVZ neural circuits. This disrupted feeding circuit impairs regionally distinct SVZ Nkx2.1+ NSC activation, potentially contributing to reduced neuroregenerative plasticity/increased vulnerability in AD. Our study provides the basic understanding on how abnormal food intake behavior dampen neuronal vulnerability, contributing to AD pathogenesis before the onset of pathological hallmarks.

Funding Source: CIHR.

W1049

EFFECT OF MITOPHAGY IN THE TUMOUR MICROENVIRONMENT ON GLIOBLASTOMA STEM CELLS

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Glioblastoma stem cells (GSC) are cells in glioblastoma (GBM) with self-renewal capabilities and ability to initiate tumours and differentiate into multiple lineages, contributing to tumour heterogeneity and poor prognosis of GBM. Mitophagy is a type of autophagy that targets mitochondria, removing unhealthy or unnecessary mitochondria. Tumour microenvironment (TME) is the microenvironment that surrounds the tumour, which plays an important role in various aspects of the tumour, such as tumour behaviour, proliferation and death. The effects of mitophagy in the TME on GSC have not been studied extensively in current literature. Mitophagy activation in the TME has been discovered in our study to cause significant changes in gene expression and reduce tumorigenicity of GSCs. This study may provide insight into alternative treatment possibilities and pathophysiology of GBM.

W1051

ENGINEERED CRO-CD7 CAR-NK CELLS DERIVED FROM PLURIPOTENT STEM CELLS AVOID FRATRICIDE AND EFFICIENTLY SUPPRESS HUMAN T-CELL MALIGNANCIES

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T cell malignancies are highly aggressive hematological tumors with limited effective treatment options. CAR-NK cell therapy targeting CD7 has emerged as a promising approach for treating T-cell malignancies. However, conventional CAR-NK cell therapy faces the challenges of cell fratricide due to CD7 expression on both malignant cells and normal NK cells. Additionally, engineering CARs into human tissue-derived NK cells demonstrates heterogeneity, low transduction efficiency, and high manufacturing costs. The human pluripotent stem cells (hPSCs) were genetically modified by knocking out the CD7 gene and introducing the CD7 CAR expression cassette to generate CD7 KO-CD7 CAR-hPSCs. These modified hPSCs were subsequently differentiated into CD7 KO-CD7 CAR-iNK cells using an efficient organoid induction method. The cytotoxicity of CD7 KO-CD7 CAR-iNK cells against CD7+ tumor cells was evaluated. Furthermore, we overexpressed the CXCR4 gene in CD7 KO-CD7 CAR-hPSCs and derived CXCR4-expressing CD7 KO-CD7 CAR-iNK (CRO-CD7 CAR-iNK) cells. The dynamics of CRO-CD7 CAR-iNK cells in vivo were tracked, and their therapeutic efficacy was assessed using human T-cell acute lymphoblastic leukemia (T-ALL) xenograft models. The CD7 KO-CD7 CAR-iNK cells derived from CD7 KO-CD7 CAR-hPSCs effectively avoided fratricide, demonstrated normal expansion, and exhibited potent and specific anti-tumor activity against CD7+ T-cell tumor cell lines and primary T-ALL cells. CXCR4 overexpression in CRO-CD7 CAR-iNK cells improved their homing capacity and extended their persistence in vivo. The CRO-CD7 CAR iNK cells significantly suppressed tumor growth and prolonged the survival of T-ALL tumor-bearing mice. Our study provides a reliable strategy for the large-scale generation of fratricide-resistant CD7 CAR-iNK cells with robust anti-tumor effects from hPSCs, offering a promising cell product to treat T-cell malignancy.

W1053

EPITHELIAL WNT SECRETION DRIVES NICHE ESCAPE OF DEVELOPING GASTRIC CANCER

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WNT signaling plays a crucial role in cancer development, with APC as the primary tumor suppressor in colon cancer. Even though WNT signaling is still essential for maintaining the gastric epithelium and driving tumor progression, mechanisms of WNT self-sufficiency in gastric cancer remain unclear, as APC and CTNNB1 mutations are relatively rare. Using human and mouse gastric organoids and in vivo mouse models, we found that mesenchymal WNTs maintain normal gastric epithelium, while KRAS activation induces epithelial WNT secretion, enabling WNT self-sufficiency. Single-cell multi-omics revealed that KRAS-driven MAPK signaling activates SMAD2/3 and unlocks the WNT7B locus, generating WNT7B+ niche cells within the epithelium. In human gastric cancer, HER2-KRAS-MAPK activation or WNT2 amplification drives epithelial WNT production. Our findings identify epithelial WNT secretion as a key mechanism of WNT self-sufficiency in gastric tumorigenesis. Unlike APC or CTNNB1 mutations in colon cancer, this process is targetable, offering potential therapeutic opportunities

Funding Source: This research was supported by the National Research Foundation of Korea (NRF) funded by the Korea Government (MSIT)(No.RS-2024-00431505) and SCORPION program_FWF DOC72 (Doi :10.55776/DOC72).

W1055

ESTABLISHMENT OF A SCREENING SYSTEM FOR THE THERAPEUTIC EFFICACY OF IMMUNE CHECKPOINT INHIBITORS USING A CO-CULTURE MODEL OF CANINE URINE-DERIVED BLADDER CANCER ORGANOIDS AND IMMUNE CELLS

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Canine bladder cancer has a high prevalence of high-grade cases with a poor prognosis and innovative therapies are needed. The number of studies to determine the efficacy of immunotherapy for canine cancers is gradually increasing. Still, the number of successful cases is small, and the causes of resistance to treatment are largely unknown. Therefore, we established an evaluation system to screen the efficacy of immune checkpoint inhibitors using urine-derived canine bladder cancer Org and immune cells co-culture model. Three-dimensional canine bladder cancer Org were prepared from cancer stem cells in urine samples of bladder cancer-diseased as described before1 and their PD-L1 expression level was evaluated. Peripheral blood mononuclear

cells (PBMCs) from healthy dogs were isolated using Ficoll methods and treated with concanavalin A (Con A) for 72 hours to activate T cells. Next, activated PBMCs are co-cultured with bladder cancer Org for 72 hours in the presence of the immune checkpoint inhibitors PD-1 and PD-L1, and changes were reported. Con A treatment resulted in PBMC accumulation and proliferation, and IFN-γ expression and the ratio of CD8-positive cells was increased. Additionally, the level of PD-L1 expression in canine bladder cancer Org with and without IFN-γ stimulation was different among patient-derived organoids. Furthermore, the cell viability of canine bladder cancer Org co-cultured with immune cells and treated with PD-L1 inhibitor was decreased in some canine bladder cancer organoids. In this study, we succeeded in establishing a co-culture model of canine bladder cancer Org and immune cells and evaluated the therapeutic effect of PD-L1 inhibitors. In the future, we plan to use canine PD-L1 inhibitor, caninized mouse model, and the organ-on-a-chip system to elucidate the mechanism of therapeutic resistance.

W1057

GENOME-WIDE SCREENING IN HUMAN EMBRYONIC STEM CELLS TO IDENTIFY GENES INVOLVED IN THE P53 PATHWAY

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The tumor suppressor protein p53, mutated in half of human cancers, plays a crucial role in cellular responses to DNA damage and genome stability, making it a key target for advancing cancer treatment and diagnosis. To identify genes and pathways critical for resistance to p53 upregulation, we performed a genome-wide CRISPR-Cas9 loss-of-function screen using Nutlin-3a, an inhibitor of the p53-MDM2 interaction that induces p53 accumulation and apoptotic cell death. Our findings revealed three enriched pathways: heparan sulfate glycosaminoglycan biosynthesis, diphthamide biosynthesis, and the Hippo pathway, with our study further confirming the interaction between the p53 and Hippo pathways. Notably, we discovered that the E3 ubiquitin-protein ligase TRIP12 is essential for the transcriptional activation of specific pro-apoptotic p53 target genes, with RNA-seq analysis in TRIP12 knockout cell lines revealing selective effects on p53's transcriptional activity and highlighting its role in modulating p53-dependent gene expression. By uncovering novel pathways and mechanisms regulating p53 function, our study expands the understanding of p53 biology and provides valuable insights that may guide the development of innovative therapeutic strategies for combating cancer.

W1059

HAPLOINSUFFICIENCY OF HNRNP A1 RESCUES AGING PHENOTYPES AND RESTORES INTESTINAL LINEAGE MARKERS IN SIRT6-DEFICIENT MICE

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Aging is a multifaceted process modulated by an intricate network of molecular pathways. Sirtuin 6 (Sirt6), a member of the sirtuin protein family, is essential for maintaining genome stability, metabolic homeostasis, and regulating aging. Sirt6-deficient mice displayed significant reduction in lifespan, surviving no longer than 4 weeks of age. In this study, we address the knowledge gap surrounding the interplay between Sirt6 and HnRNP A1, a multifunctional RNA-binding protein

implicated in cellular stress responses and aging. Our novel approach explores the hypothesis that haploinsufficiency of HnRNP A1 can rescue the severe aging phenotypes associated with Sirt6 deficiency. Our findings reveal that haploinsufficiency of HnRNP A1 significantly extends the lifespan of Sirt6 knockout (KO) mice, enabling them to survive beyond 200 days compared to the typical lifespan of less than 4 weeks observed in Sirt6 KO mice. Furthermore, HnRNP A1 haploinsufficiency rescues intestinal structure and restores the expression of intestinal stem cell markers LGR5 and DCAMKL1, which are otherwise downregulated in Sirt6 KO mice. These results suggest that HnRNP A1 heterozygosity serves as a compensatory mechanism, ameliorating the adverse phenotypic effects associated with Sirt6 deficiency. In addition, the loss of intestinal stem cells markers Lgr5 and Dcamk1 were highlighting a critical role for HnRNP A1 in mitigating Sirt6-related intestinal deterioration. These results suggest that HnRNP A1 heterozygosity serves as a compensatory mechanism, ameliorating the adverse phenotypic effects associated with Sirt6 deficiency. Our work advances the field by elucidating a potential molecular interaction between Sirt6 and HnRNP A1, providing insights into the compensatory pathways that modulate aging phenotypes. These findings could inform the development of novel therapeutic strategies targeting the Sirt6-HnRNP A1 axis to address age-related diseases and promote healthy aging.

W1061

IDENTIFY A SURFACE MARKER FOR PROLIFERATING ISLET CELLS IN ADULT PANCREAS

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Inducing beta cell proliferation is theoretically a straightforward and effective strategy to increase the absolute beta cell mass and develop new regenerative approaches for pancreatic islets. However, little is known about these proliferating beta cells, and the challenge of isolating them remains unresolved. In this study, we have identified a surface marker indicative of proliferating cells within adult murine and human pancreatic islets. Through single-cell RNA sequencing analysis, we identified a population characterized by high proliferative activity. Among the signature genes of this population, we focused on a gene encoding a surface protein. Fluorescence-activated cell sorting (FACS) analysis confirmed that this protein labels proliferating cells, which represent approximately 0.6% of islet cells in adult mice and 2% in neonatal mice. Immunostaining further indicated that these cells are specifically in the G2/M phase of the cell cycle. To further investigate the division behavior and progeny cell fates of these proliferating cells, we engineered CreER mice. In vivo lineage tracing revealed that cells labeled shortly after tamoxifen administration rapidly divided into two Nkx6.1-low immature beta cells. After a two-month tracing period, 90% of these cells matured into Nkx6.1-high beta cells. Additional clonal analysis indicated that a subset of these proliferating islet cells possess multipotent capabilities. Notably, the surface marker is conserved in humans, as it also identifies proliferating cells in human pancreatic

neuroendocrine tumors. Collectively, our findings unveil a surface marker that identifies proliferating islet cells during both homeostasis and in the context of endocrine tumors. This discovery provides novel insights into the processes of islet cell proliferation, differentiation, and maturation.

W1063

IL-1 SIGNALING INTERFERES WITH NICHE ENVIRONMENT, CAUSING ALTERATION IN EPIDERMAL STEM CELL HETEROGENEITY

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The skin serves as a primary defense by altering cellular behavior in response to infection or external stressors, which triggers an inflammatory response. While the immunological aspects of skin inflammation have been studied, the crosstalk between the stem cell niche and inflammatory signals remains poorly understood. Here, we utilize a mouse model of 12-O-tetradecanoyl-phorbol-13-acetate (TPA)-induced acute skin inflammation to investigate how inflammation impacts epidermal stem cell heterogeneity and tissue remodeling. The topical application of TPA induces inflammation in mouse skin, as indicated by a significant increase in epidermal thickness, hyperproliferation, and elevated levels of pro-inflammatory cytokines. By lineage tracing, we demonstrate that Dlx1+ slow-cycling stem cell clones persist and exhibit high resistance to inflammation. In contrast, Slc1a3+ fast-cycling stem cell clones are progressively lost by differentiation, resulting in a reduction in epidermal compartment size in the skin. The decoy receptor IL-1R2, which is highly expressed in the slow-cycling stem cell compartment, suggests a potential link between the observed imbalance in epidermal stem cell heterogeneity and IL-1 signaling during inflammation. The intradermal injections of IL-1a or IL-1b induce similar stem cell alterations in stem cell populations as observed in the TPA model. Conversely, overexpression of IL-1R2 mitigates these changes, indicating a dependence on IL-1 signaling. Transcriptome analysis reveals that inflammatory signals may interfere with normal niche signaling, leading to an impairment of the epidermal stem cell heterogeneity during TPA-induced inflammation. These results provide new insights into the cellular dynamics of epidermal stem cells during skin inflammation and highlight IL-1 as a critical immune signaling that disrupts the heterogeneous epidermal stem cell populations under pathological tissue remodeling in inflammatory skin.

Funding Source: AMED, 24bm1123052, AMED-PRIME, AMED (21gm6110016), Grant-in-Aid for Scientific Research (B) (24K02035, 20H03266), The Sumitomo Foundation, Takeda Science Foundation, Naito Foundation, Koyanagi Foundation (all to A.S.), MEXT.

W1065

INVESTIGATING THE ROLE OF EXTRACELLULAR MATRIX STIFFNESS IN THE AGING OF OLIGODENDROCYTE PROGENITOR CELLS

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Ageing causes deterioration of tissue regeneration capacity due to declined function of stem cells and progenitor cells, such as oligodendrocyte progenitor cells (OPC). With age, OPCs gradually lose their proliferation and differentiation capacities, resulting in accelerated myelin loss and cognitive decline which are associated with the development of neurodegenerative diseases. OPCs reside in extracellular matrix (ECM), and interestingly, the mechanical properties of ECM in the brain change with age, contributing to the dysfunction of brain cells. However, the question of how stiffness regulates OPC functions remains largely unknown. Recent studies have identified various modulators that regulate OPC functions during aging. However, the intricate interplay between mechanical and intrinsic modifications in regulating the molecular and cellular functions of OPCs remain poorly understood. To bridge this knowledge gap, we have developed a hydrogel model that recapitulates the age-related mechanical changes of brain ECM. Our findings indicate stiffness has a profound impact on the cellular functions of OPCs. Coupling with comprehensive data mining analyses, we found ageing impairs ECM-related functions in the brain of aged mice, and adversely affects OPC functions. We also identified a specific mechanosensitive ion channel target which is functionally expressed in OPCs and exhibits increased expression in response to higher stiffness level. Notably, either overexpression or enhanced functionality of this channel leads to age-related changes in OPCs, indicating its potential role in the aging process at the cellular level. The study advances the understanding of the influence of mechanical properties of ECM to OPC functionality. The identification of mechanosensitive ion channel paves the way for novel therapeutic strategies which aim at enhancing OPC functions within the context of ageing. By targeting the mechanical properties of the ECM or modulating mechanosensitive pathways, it is possible to rejuvenate OPC and restore remyelination capacities in age-related neurological disorders, thereby profoundly impacting on myelin repair and overall brain health.

W1067

LINKING MORPHOLOGY AND STEMNESS IN GLIOBLASTOMA STEM CELL: A LESSON FROM NEURODEVELOPMENT

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Glioblastoma (GBM) is one of the deadliest brain tumors, with limited therapeutic options due to its extreme heterogeneity, largely driven by glioblastoma stem cells (GSCs). GSCs exhibit self-renewal, plasticity, and therapy resistance, making them key players in tumor progression and recurrence. Targeting GSCs is an unmet clinical challenge requiring a deeper understanding of the mechanisms underpinning their stemness. GBM hijacks neurodevelopmental pathways, and GSCs

share transcriptomic similarities with neural stem cells of the developing brain, called basal or outer radial glia (bRG). Those cells were shown to exhibit a morphological heterogeneity which is thought to regulate their proliferation and migration. Similarly, our recent work revealed that also GSC morphology is tightly linked to their function. These findings prompted us to conduct an in-depth examination of the GSC morphology. By employing a cohort of patient-derived 2D and 3D in vitro model system, we identify cell morphology as a new layer of GSC heterogeneity. While bRG cells have been morphologically categorized based mainly on the number of their cellular protrusions, GSCs exhibited further inter- and intra-patient morphological heterogeneity. This is particularly pertinent to the length and thickness of the protrusions, tendency to form cell-cell networks, and cell flatness. The analysis of these features, coupled with the measure of their in vitro clonogenic ability, allowed us to identify different morphoclasses with specific self-renewal potentials. We further performed a transcriptomic profiling and revealed morphoclass-intrinsic molecular traits. The gene expression profile of morphoclasses associated with higher clonogenic potential was enriched in pathways linked to tumor growth and aggressiveness. Moreover, the enriched pathways play fundamental roles in neurodevelopment, including the maintenance of bRG cells, the promotion of neuronal process growth, and their morphogenesis. Hence, this study highlights that GSC morphology and stemness are intricately linked, likely through neurodevelopmental morphoregulatory pathways. This provides rationale for a potential future targeting of morphoregulators to therapeutically target GSCs and disrupt GBM progression.

W1069

MECHANOSENSING ACTIVATES PIEZO1 CHANNEL AND REGULATES THE FUNCTION OF BONE MARROW PERI-ARTERIOLAR OSTEOLECTIN+ CELLS AND OSTEOGENESIS

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Bone's ability to adapt to mechanical stress is a fundamental principle in orthopedics, stated by Wolff's Law, which posits that bone formation and strength are directly related to the degree of mechanical loading they experience. We have discovered that the mechanosensitive ion channel Piezo1 plays a crucial role in the osteogenic differentiation of bone marrow-derived LepR+Osteolectin+ cells (Osteolectin+ cells), a process integral to bone formation. Specifically, we have shown that exercise-induced mechanical stimulation activates Piezo1, thereby promoting bone formation and reinforcing the relevance of Wolff's Law in the context of peri-arteriolar stromal cells. Despite Piezo1's broad expression across LepR+ cells, including peri-sinusoidal LepR+Oln– cells, the precise reason for the dependency of peri-arteriolar Osteolectin+ cells on Piezo1 for their function is not fully understood. To address this, we developed Piezo1-GCaMP mice, which feature a genetically encoded fluorescent probe allowing for non-invasive optical monitoring of Piezo1 activity. Our studies revealed a significant increase in Piezo1 activation in peri-arteriolar LepR+Osteolectin+ cells, compared to peri-sinusoidal cells, highlighting the selective role of mechanosensing in these cells. Importantly, we observed that weight-bearing bones, which are more responsive to mechanical loading, have a higher frequency of Osteolectin+ cells and are more affected by the conditional deletion of Piezo1 in LepR+ cells, leading to a loss of bone mineral density and cortical bone thickness. Our ongoing work is focused on uncovering the molecular mechanisms downstream of Piezo1 that contribute to these observations, with a hypothesis that peri-arteriolar Osteolectin+ cells exhibit increased mechanosensitivity due to enhanced matrix protein deposition and local stiffness near arterioles. This research underscores the critical importance of Piezo1-mediated mechanosensing in peri-arteriolar Osteolectin+ cells for bone formation, particularly in weight-bearing bones, which have a higher mechanosensitivity and

a greater abundance of Osteolectin+ cells.

W1071

MICROTUBULE-ASSOCIATED PROTEIN 2 (MAP2) PROMOTES CANCER CELL PLASTICITY IN HEPATOCELLULAR CARCINOMA THROUGH INTEGRIN DYSREGULATION

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Integrins are adhesion molecules that mediate mechanical and chemical signal transduction for supporting cell survival and proliferation. Dysregulated integrin signaling empower tumor cells with abilities to drive cancer cell plasticity, including stemness, epithelial plasticity, metastatic reactivation and resistance to therapies. However, the interplay of cancer cell plasticity and integrin signaling in hepatocellular carcinoma (HCC) remains poorly understood. Through lineage-tracing and lineage-ablation studies, our team has previously shown HCC cells genetically marked by Prom1/CD133 to represent an important functional subset in HCC tumors, displaying a dedifferentiated status with stem cell traits. In this study, transcriptome profiling revealed specific downregulation of integrin signaling in ‘HCC’ CD133+ cells isolated from NRAS+AKT protooncogenes-driven HCC, but not epithelial-specific ‘normal’ CD133+ cells isolated from the regenerating liver. One of the most differentially upregulated genes identified in the CD133+ HCC cell profiling, MAP2, demonstrated the ability to suppress integrin expression. MAP2 overexpression is frequently observed in HCC, and correlated with aggressive clinical features. Functionally, MAP2 promoted cancer stemness and proliferation, and conferred resistance to sorafenib. Mechanistically, MAP2-induced F-actin polymerization activates YAP, which transcriptionally suppresses expression of integrin α genes to ablate integrin β1-mediated cell adhesion. Pharmacological inhibition of MAP2 using Estramustine Phosphate (EMP), which is reported to inhibit the interaction of MAP2 with actin filaments, attained a synergistic effect in suppressing tumor initiation and growth of HCC cell lines, HCC patient-derived organoids and NRAS+AKT protooncogenes-driven HCC mouse model when used in combination with sorafenib. In summary, our findings reveal the pivotal functional role and clinical significance of MAP2 in mediating cancer cell plasticity to drive HCC, and that MAP2 inhibition represents a potential novel therapy for the disease targeted at its stemness roots.

W1073

MODELLING BETTER GLIOBLASTOMA TREATMENT: NOVEL NANO-ELECTRO-CHEMOTHERAPY USING PIEZOELECTRIC NANOPARTICLES AND LOW-INTENSITY ULTRASOUND

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Glioblastoma (GBM) is a highly aggressive and common brain tumor with resistance to conventional therapies, leading to poor outcomes and a median survival of less than 15 months. The ability to resist apoptotic signals, evade the immune system, and high recurrence and adaptability of GBM cells further underscore the urgent need for transformative therapeutic strategies. To tackle these challenges, we propose a novel polytherapy combining delivery of optimal low-intensity ultrasound (LIUS) with piezoelectric nanoparticles (PNPs) for direct electrotoxicity and enhancing chemotherapy efficacy. PNPs generate localized electric fields upon ultrasound activation, amplifying reactive oxygen species (ROS) production in cells, oxidative stress, and apoptosis, as wells as altering cytoskeletal dynamics, compromising GBM cell structure and motility. Our work demonstrates pre-clinical proof-of-concept for nano-electro-chemotherapy, including unique direct electrotoxicity and affirming augmented chemotherapy of GBM. The approach synergizes mechanical, chemical, and electrical stimuli to also overcomes resistance mechanisms inherent to GBM. Notwithstanding the potential for treating other solid tumors, this innovative polytherapy represents a promising step toward developing more effective, targeted, and less invasive treatments for GBM. By integrating advanced nanotechnology with ultrasound-based delivery systems, we provide a foundation for further preclinical and clinical investigations with the aim of improving cancer patient outcomes.

Funding Source: Funding to support the research for this manuscript was provided by the Arto Hardy Family, Tour de Cure, and Tregaskis Foundation.

W1077

NOVEL POSTOPERATIVE TREATMENT STRATEGY FOR OSTEOSARCOMA: INTEGRATED APPROACH OF PHOTOTHERMAL IMMUNOTHERAPY WITH BONE REGENERATION BASED ON INJECTABLE HYDROGEL

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Osteosarcoma is the most common primary malignant bone tumor. Current treatments, including surgery and chemotherapy, have not significantly improved survival rates, and there is a high rate of recurrence. Surgery often results in large bone defects, and existing reconstruction methods are inadequate. This study proposes a new treatment approach that combines photothermal immunotherapy with autologous bone repair, utilizing a novel injectable thermosensitive hydrogel loaded with the immunomodulatory drug JQ1, the photothermal agent indocyanine green (ICG), and the bone repair agent nano-hydroxyapatite (nHAp). In a postoperative mouse model of osteosarcoma, the combination of photothermal immunotherapy demonstrated significant tumor suppression, while neither treatment alone effectively inhibited tumor recurrence. Tumor samples were analyzed using single-cell RNA sequencing (scRNA-seq), revealing a unique immune microenvironment characterized by low lymphocyte counts and a predominance of myeloid cells, particularly tumor-associated macrophages (TAMs) with immunosuppressive properties. Pseudotime analysis traced the differentiation of Ly6c+ monocytes into M1 and M2 macrophages. The results indicated that photothermal therapy enhanced monocyte infiltration, while JQ1 promoted differentiation into M1 macrophages. The hydrogel containing nHAp exhibited significant osteogenic effects, with the inclusion of nHAp upregulating the expression of osteogenic genes and promoting osteogenic differentiation in MC3T3 cells. In a femoral defect model, micro-CT and histological analyses supported that nHAp significantly increased new bone formation, mineral density, and trabecular density at the defect site, effectively facilitating bone repair in mice. This study provides a novel and efficient postoperative treatment method for osteosarcoma, integrating photothermal immunotherapy and bone regeneration, and evaluates the therapeutic effects at the molecular level, offering new insights for the treatment of osteosarcoma.

W1079

POTENTIAL SENOLYTIC EFFECTS OF PARP1 INHIBITION ON HUMAN PLURIPOTENT STEM CELLS

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Telomeres are repetitive sequences located at the ends of chromosomes, protecting genomic integrity. In most somatic cells where telomerase is inactive, telomeres shorten with each cell replication. Critically short telomeres lead to proliferation arrest, senescence, or cell death. Poly(ADP-ribose) polymerase 1 (PARP1) is a major sensor of DNA breaks and plays important roles in DNA repair. In a previous study, we demonstrated that PARP1 inhibition elongated telomeres in telomerase deficient human induced pluripotent stem cells (hiPSCs), implicating that PARP1 inhibition mediated telomere elongation is not through the activation of telomerase. Here, we hypothesize that PARP1 inhibition has senolytic effect, i.e., eliminating cells of shorter telomeres, on hiPSCs, thus leading to the observed longer telomeres in surviving cells. In this study, we treated wildtype hiPSCs with PARP1 inhibitor for three days. Our data showed that such treatment did not activate telomerase activity, but significantly reduced the cells' proliferation rate and caused the S-phase arrestment. Markers of senescence, including P16, P21, and IL-6, are upregulated, while the apoptosis-related marker BCL2 demonstrated a downregulation trend in the inhibitor treated cells. Importantly, the telomere lengths are longer after the inhibitor treatment. These data suggest that PARP1 inhibition may selectively eliminate cells with critically short telomeres.

Funding Source: National Science and Technology Council, Taipei, Taiwan, R.O.C. Grant number NSTC 113-2313-B-002-011.

W1081

REGIONALIZATION OF THE ESOPHAGEAL EPITHELIUM

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The basal cells of the esophageal epithelium have traditionally been considered a homogeneous population. However, work from our group suggest that organoid forming efficiency as well as ability to generate basal clones in vivo differ along the proximal-distal esophageal axis. Combining single-cell sequencing with spatial transcriptomics, we identified location-specific differences in the transcriptome of proximal and distal basal cells. In addition, the distal esophageal epithelium displayed circumferential transcriptional heterogeneity, correlating to the folding of the esophageal tube. To validate these findings, we employ two Cre-dependent reporter mouse lines that selectively mark either folded or extended distal epithelial regions. Genetic labeling and tracing of basal cells in folds and extensions will reveal differences in progenitor cell behavior in vivo. Additionally, our spatial transciptomics characterization allowed us to map back specific subpopulation of fibroblasts to esophageal cross sections, identifying region-specific fibroblast-epithelial basal cell interactions. Bioinformatic analysis revealed local differences in predicted fibroblast-basal cell signaling, with Wnt and TGF-β pathway activity enriched in extended regions. These findings provide new insights into the spatial regulation of progenitor cell behavior in the esophageal epithelium.

W1083

SINGLE-CELL ANALYSIS REVEAL KEY PLAYERS IN TRANSFORMATIONS OF MESENCHYMAL STROMAL CELLS INTO CANCER-ASSOCIATED FIBROBLASTS AND METASTATIC-ASSOCIATED FIBROBLASTS IN THE BREAST CANCER

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Breast cancer is a leading cause of mortality in adult women, particularly when it metastasises. Cancer-associated fibroblast (CAF) in the tumor microenvironment (TME) significantly influences cancer spread and metastasis. CAF originate from fibroblasts and mesenchymal stromal cells (MSCs) within the TME and transform into metastatic-associated fibroblasts (MAFs). Research on CAFs often focuses on MSC behavior at the primary tumor sites post-stimulus, and few studies evaluate their fate and roles at the metastatic sites. By utilizing single-cell RNA sequencing (SC-RNA seq.) data of human breast cancer, we aim to clarify the pathways underlying the conversion of MSCs to CAFs and CAFs into MAFs. Data from primary breast cancer and secondary lymph node metastatic patients were retrieved and analysed from the GEO dataset. Clustering and annotation were performed to identify MSCs, CAFs, and MAFs in both primary and secondary tumor sites. Our data show that inflammatory CAFs (iCAFs) and matrix CAFs (mCAFs) were the predominant subtypes at the metastatic site. Trajectory analysis pinpointed perivascular MSCs (pMSCs) as the origin of both iCAFs and myofibroblastic CAFs (myoCAFs). Following cell-cell interaction analysis, the receptor-antigen interactions within the TME were anticipated. Upon performing a pseudo-bulk differential expression analysis, two metastatic biomarkers (NPY1R and CCDC102B) were shown to be exclusively expressed by inflammatory MAFs (iMAFs), implying potential targets for inhibiting breast cancer metastasis. Our data identify novel targets that may impede the conversion of MSCs into CAFs and CAFs into MAFs, which potentially impacts breast cancer diagnosis, prognosis, and treatment strategies.

Funding Source: Grant #46721 from the Egyptian Science and Technology Development Fund, Cairo, Egypt.

W1085

STRETCHING IN STEM CELL GYM MODULATES MITOCHONDRIAL DYNAMICS AND ENERGY METABOLISM TO INDUCE SMOOTH MUSCLE DIFFERENTIATION IN MESENCHYMAL STEM CELLS

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The smooth muscle cells (SMCs) located in the vascular media layer are continuously subjected to cyclic stretching perpendicular to the vessel wall and play a crucial role in vascular wall remodeling and blood pressure regulation. Construction of bioengineered blood vessels requires regeneration of functional SMC layer to withstand the different magnitudes of blood pressure. However, the quantity of SMCs isolated from natural vessels is insufficient, and their proliferative capacity is limited. Mesenchymal stem cells (MSCs) are promising tools to differentiate into SMCs. We are inspired by the notion that stretching exercises in gyms can stimulate muscle cell metabolism,

improve muscle contractility, and thereby accelerating muscle repair processes. Therefore, we design a stem cell stretching program, like training the stem cell in the gym, which induces the expression of SMC markers α-SMA and SM22 in MSCs. These cells exhibited contractile ability in vitro and facilitated vascular structure formation in the Matrigel plug assay in vivo. The contraction of SMCs requires remodeling of their energy metabolism. However, the underlying mechanism remains to be revealed. Our study shows that stretch training promotes glycolysis, oxidative phosphorylation, and mitochondrial fusion, thereby contributing to MSCs differentiation. Yes-associated protein (YAP) affects mitochondrial dynamics, oxidative phosphorylation, and glycolysis to regulate stretch-mediated differentiation into SMCs. Additionally, Piezo-type mechanosensitive ion channel component 1 (Piezo1) impacts energy metabolism and MSCs differentiation by regulating intracellular Ca2+ levels and YAP nuclear localization. Therefore, our findings indicate that YAP can integrate stretch force and energy metabolism signals to regulate the differentiation of MSCs into SMCs. It provides a scientific foundation for future vascular tissue engineering with MSCs.

Funding Source: BNSF [L242125, L234072, L242042]. National Natural Science Foundation of China [32171310, 12472315, U20A20390, 12332019]; The Fundamental Research Funds for the Central Universities.SKLOD2024OF01.

W1087

THBS1+ MONOCYTIC MYELOID-DERIVED SUPPRESSOR CELLS THAT TRIGGER CD8+ T CELL EXCLUSION AND DYSFUNCTION IN CTNNB1-MUTATED HCC

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Despite the increasing use of immune-checkpoint blockade (ICB) as standard care in hepatocellular carcinoma (HCC), the multifaceted heterogeneity of HCC and its diverse immune microenvironment remain major challenges to the efficacy of ICB, with the treatment benefiting only

a minority of HCC patients. CTNNB1 mutations, the most common driver mutations in HCC, play a crucial role in regulating cancer cell plasticity, tumor progression, and drug resistance. Recent findings have also highlighted the link between CTNNB1 and T cell exclusion and as a consequence poor ICB response. Our current analysis of public human HCC patient data unveiled distinct immune profiles between CTNNB1-mutated and non-CTNNB1-mutated patients. Through comparing clinically relevant murine models of CTNNB1-mutated and non-CTNNB1-mutated HCC, we observed that CTNNB1-mutated tumors exhibit enhanced β-catenin activation, reduced CD8+ T cell infiltration, and their compromised mobility and proliferation capabilities as compared to non-CTNNB1-mutated tumors. scRNA-seq analysis of these mouse tumors also demonstrated a decrease in effector CD8+ T cells and an increase in monocyte population in CTNNB1 mutation-driven HCCs. Notably, these enriched monocytes expressed signatures of classical monocytes and displayed elevated levels of THBS1 in CTNNB1-mutated HCC, showcasing immunosuppressive characteristics. The enrichment of monocytes was likewise consistently observed in CTNNB1-mutated HCC patients. A high THBS1+ monocytic myeloid-derived suppressor cell (mMDSC) signature not only predicted poor survival and correlated positively with advanced tumor stages but also indicated a poor response to ICB in CTNNB1-mutated HCC patients. In summary, the presence of THBS1+ monocyte-like cells emerges as a promising predictive marker for immunotherapy response in CTNNB1-mutated HCC patients, offering valuable insights for future therapeutic strategies.

Funding Source: RGC Research Fellow Scheme RGC Research Impact Fund (R7022-20) RGC Theme-Based Research Scheme (T12-705-24-R).

W1089

THE SYNERGISTIC RELATIONSHIP BETWEEN ORAL AND TONSILLAR MICROBIOMES AND THEIR ROLE IN PEDIATRIC TONSILLAR HYPERTROPHY

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Oral microbes can spread throughout the gastrointestinal system and are linked to multiple diseases. This study analyzed the microbial communities in the saliva and tonsils of Korean children undergoing tonsillectomy due to tonsil hyperplasia (n = 29) using 16S rRNA gene sequencing. The microbial communities of saliva and tonsils showed high similarities. Treponema, the causative bacterium of periodontitis, had a significant positive correlation in both saliva and tonsils. Prevotella in saliva and Alloprevotella in tonsils were negatively correlated, while Treponema 2 showed a strong positive correlation. Additionally, the study evaluated the correlation between the tonsil microbiome and tonsillar hypertrophy. Children with obesity and tonsillar hypertrophy had similar microbiome compositions. Obesity and tonsillar hypertrophy demonstrated a strong correlation with the Proteobacteria to Firmicutes ratio, and alanine aminotransferase levels increased with obesity and tonsillar hypertrophy, indicating a possible association of the tonsil

microbiome with liver metabolism. These findings highlight the significance of the tonsil microbiome and suggest the need for tonsil regulation, particularly during childhood.

Funding Source: This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (RS-2023–00210100).

TRACK: DISEASE MODELING AND DRUG DISCOVERY (DMDD)

W1091

A VERSATILE TOOLBOX OF HUMAN IPSC-DERIVED MICROGLIA FOR DISEASE MODELLING, CRISPR SCREENS AND MULTICELLULAR IN VITRO MODELS FOR NEURODEGENERATION DRUG DISCOVERY

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Microglia, the resident macrophages of the brain, are essential for neural homeostasis, regulating neurogenesis, synaptic remodelling, and serving as first responders to injury or infection. Dysregulated microglial function is implicated in neurodegenerative diseases such as Alzheimer's disease (AD). Despite their critical role in disease progression, existing in vitro models fail to replicate the complexity of microglia, limiting advances in drug discovery. Therefore, new tools are needed to model disease, generate gene knockouts, and track cellular responses in co-culture systems. In this study, we used opti-ox™, a deterministic cell programming technology, to generate scalable human-induced pluripotent stem cell (hiPSC)-derived microglia from both male and female genetic backgrounds. These derived microglia express key markers, including CD45, P2RY12, CD11b, CD14, IBA1, and TREM2, and exhibit robust phagocytic activity, uptaking pHrodo labelled BioParticles and Amyloid-beta1-42 as well as pro-inflammatory cytokine secretion, with distinct responses based on genetic background. To advance AD modelling, we engineered hiPSC-derived microglia with AD-relevant mutations, such as TREM2 (R47H) and APOE (C112R), known to alter microglial function. In response to the time-intensive nature of CRISPR-compatible cell line development, we created CRISPRko-Ready ioMicroglia, which constitutively express Cas9, enabling high-throughput CRISPR screening and reducing workflow duration from months to days. Proof-of-concept experiments in these cells demonstrated efficient single-gene knockouts and successful pooled CRISPR screens. Additionally, we generated GFP-expressing ioMicroglia for live-cell imaging, antibody-free sorting, and tracking of the cells within complex multicellular neurobiology co-culture systems. Our cells were benchmarked against the HMC3 immortalised cell line, and exhibited superior phenotypic and functional characteristics, including more accurate gene expression profiles, enhanced phagocytosis and expression of key microglial markers. Our model offers a physiologically relevant tool for investigating AD mechanisms, providing critical advancements for drug discovery and neurodegeneration research.

W1093

ENGINEERED NEURAL STEM CELL-DERIVED EXTRACELLULAR VESICLES DELIVERING WNT7A-K190A: A NOVEL THERAPEUTIC PLATFORM FOR BLOOD–BRAIN BARRIER RESTORATION

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The blood–brain barrier (BBB), a highly specialized neurovascular structure indispensable for preserving cerebral homeostasis, exhibits significant impairment across diverse neurological pathologies; however, its therapeutic targeting persists as a formidable challenge due to the inherent complexity of its multicellular architecture and dynamic regulatory networks. Although the Wnt/β-catenin signaling pathway orchestrates the development and maintenance of the BBB, the clinical translation of Wnt-based interventions remains elusive. To overcome these limitations, we engineered a neural stem cell-derived extracellular vesicle (EVs-NSC) platform functionalized with Wnt7a-K190A—an engineered BBB-tropic ligand (designated EVs-WK). In vitro mechanistic studies demonstrated that EVs-WK had three main effects: they enhanced BBB integrity, promoted synaptogenesis through β-catenin-mediated reinforcement, and suppressed neurotoxic activation of astrocytes by preserving EV-embedded miR-124-3p. In murine intracerebral hemorrhage (ICH) models, administering EVs-WK significantly reduced hematoma expansion and accelerated motor recovery. Cross-species validation using humanized EVs (hEVs-WK) confirmed the conserved therapeutic efficacy of this approach, as shown by the mitigation of LPS-induced barrier dysfunction and downregulation of inflammatory pathways. Overall, this modular EV platform combines BBB restoration with neurovascular unit repair, thus overcoming critical translational barriers in neurological therapeutics through targeted-controlled activation of Wnt signaling.

Funding Source: This work was supported by grants from the Ministry of Science and Technology of China (2018YFA0107900, 92168103, 32171417, 82001140, 2019CXJQ01, 2023TQ0072, and 2023M740676), the National Natural Science Foundation of China.

W1095

PAEONIFLORIN COMBINED WITH NEURAL STEM CELL TRANSPLANTATION FOR PARKINSON'S DISEASE: SYNERGISTIC EFFECTS OF CELL THERAPY AND INFLAMMATION REGULATION

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This study investigates the therapeutic potential of combining paeoniflorin (PF), a neuroprotective and anti-inflammatory compound derived from traditional Chinese medicine, with neural stem cell (NSC) transplantation for Parkinson's disease (PD) treatment. PD, characterized by progressive dopaminergic neuron loss in the substantia nigra, lacks therapies that modify disease progression. While NSC transplantation aims to replace degenerated neurons, its efficacy is limited by inflammatory microenvironments that impair graft survival and differentiation. In vitro, embryonic rat midbrain-derived NSCs differentiated into functional dopaminergic neurons. PF pretreatment significantly enhanced neuronal viability under inflammatory stress, suppressed pro-inflammatory cytokine release, and inhibited key inflammatory signaling pathways (TLR4/MYD88/NF-κB and NLRP3 inflammasome). In vivo, PF co-administered with NSCs in a neurotoxin-induced PD model markedly improved striatal dopaminergic neuron survival compared to NSC-only transplantation, while concurrently reducing neuroinflammation. These findings demonstrate that PF synergizes with NSC therapy by mitigating inflammatory damage and enhancing graft survival and maturation.

The study establishes a novel combinatorial strategy that addresses critical barriers in PD cell therapy, offering a promising translational approach to slow disease progression through dual neuroprotection and immunomodulation.

W1097

ANGIOGENESIS PROVIDES A FUNDAMENTAL SUPPORT FOR HOMING OF EXOGENOUSLY INFUSED ALLOGENEIC AD-MSCS TO THE INJURED BRAIN TISSUE POST ACUTE ISCHEMIC STROKE

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Mesenchymal stromal cells (MSCs) are mainstay of cell therapy for currently unmet disease conditions. MSCs need homing to the injured tissue in order to promote tissue regeneration. However, the mechanism by which MSCs home to their action site remains elusive. This study was undertaken to specifically address this fundamental issue for MSCs clinical application. Rats were subjected to a permanent middle cerebral artery occlusion (pMCAO) surgery to produce an acute ischemic brain iry. Allogeneic AD-MSCs were infused via tail vein at 6, 24, or 36 hrs post the pMCAO surgery. The best recovery of brain structure and function, as judged by balance beam test, left forelimb placement test and 2,3,5-triphenyltetrazolium chloride (TTC) staining of injured brain tissue, from the acute ischemia was found at the time of AD-MSCs administration at 24 or 36 hrs, not 6 hrs, post the surgery. This time period was well correlated with the peak time period of self-defense motivated angiogenesis in the injured brain tissue, as determined by co-staining of CD31 and BrdU. The infused AD-MSCs were found to be condensed in the highly angiogenesis area. In this area, AD-MSCs were colocalized with newly generated vascular endothelial cells. Prevention of endothelial cell regeneration blocked the homing of AD-MSCs to the injured brain tissue and diminished the therapeutic effect of AD-MSCs on acute ischemic stroke. This study thus confirmed that the therapeutic efficacy of MSCs depends on the homing of these cells to the injured site, and found that MSCs homing is angiogenesis-dependent. Therefore, promotion of angiogenesis prior to MSCs administration would significantly enhance the efficacy of MSC therapy.

W1099

COLLAGEN MATRIX IMPROVES CARDIOMYOCYTE CONTRACTILITY AND MATURITY COMPARED TO FIBRIN IN 3D ENGINEERED HIPSC CARDIAC TISSUE STRIPS

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Human ventricular cardiac tissue strips (hvCTS), composed of pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) in a 3D hydrogel matrix, provide in vitro human models for preclinical drug screening. Although collagen and fibrin are common hydrogels for cardiac tissue engineering, a quantitative comparison of these matrix materials has not been systematically examined. This study aims to compare the effects of collagen vs. fibrin matrix on hvCTS structure and the contractility and phenotype of the resident hPSC-cCMs. hvCTS fabricated using hPSC-CMs mixed with either a collagen type I (COL) or fibrin (FIB) hydrogel base were measured for contractility and excitation threshold from day 7-14 post-fabrication using an automated screening system. Developed force and maximum capture frequency were significantly higher, whereas passive tension and excitation threshold were significantly lower, for COL-hvCTS compared to FIB-hvCTS. The contraction rise time for COL-hvCTS was significantly shorter than FIB-hvCTS. The inotropic dose responses to isoprenaline and nifedipine were comparable for both hvCTS types when developed force was normalized to baseline. Gene ontology enrichment analysis of single cell-RNAseq data revealed that pathways related to oxidative metabolism were over-represented in COL-hvCTS, whereas pathways related to motility and adhesion were over-represented in FIB-hvCTS. Scanning electron microscopy (SEM) revealed a larger pore size distribution in the cell-free COL than the FIB matrix material, which is consistent with significantly faster medium permeation through COL- compared to FIB-layered Transwell plates. Hence, our results suggest hPSC-CMs in COL-hvCTS exhibit improved contractility and electrical coupling and are developmentally more mature compared to FIB-hvCTS. The superior contractility and maturity in COL-hvCTS could be attributable to the larger pore size and enhanced permeability in the collagen hydrogel base, facilitating nutrient diffusion and cell connectivity in hPSC-CMs. Such COL-hvCTS, with improved tissue contractility and maturity, could offer enhanced sensitivity for drug screening and disease modeling applications and to accelerate translation to clinical trials as our group has previously shown.

W1101

DISENTANGLING NETWORK DYSFUNCTION PHENOTYPES IN LITHIUM-RESPONSIVE BIPOLAR DISORDER AND SCHIZOPHRENIA

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Our lab has shown that the post translational modification of Collapsin Response Mediator Protein 2 (CRMP2), a cytoskeleton modulator that serves as a master regulator of neural network formation and function, is dysregulated in both schizophrenia (SZ) and lithium-responsive bipolar disorder (LiR-BPD). CRMP2 activity, pivotal to dendrite morphometrics and function, is dependent on its phosphorylation state, which, under normal circumstances, is in dynamic equilibrium. Increasingly, researchers are identifying large-scale brain network dysfunction in psychiatric disorders like these with neurocognitive symptoms which may contribute to the complex symptomatology. SZ is a debilitating mental disorder characterized by disruptions in thought processes, perception, emotional responsiveness, and social interactions. BPD is a chronic mental disorder characterized by oscillations between depressive and manic episodes. Both SZ and BPD patients have an elevated risk for premature mortality. By differentiating LiR-BPD and SZ patient induced pluripotent stem cells (iPSC) into cortical neurons, our lab has identified imbalances between phosphorylated (inactive) and non-phosphorylated (active) CRMP2 and found that this imbalance promotes neuronal hypersynchrony. This leads to neural network hypofunction by promoting overly-synchronous circuits and impairing the transfer of patterned information. We have shown elevated active CRMP2 and reduced total CRMP2 in SZ relative to healthy patients. In contrast, in LiR-BPD, we have seen elevated inactive CRMP2, an imbalance that was normalized by lithium treatment. Elevated active CRMP2 was associated with hypoactive neural networks while elevated inactive CRMP2 was associated with hyperactive neural networks in calcium imaging and microelectrode array. Interestingly, both conditions exhibited hypersynchrony. As the final pathway of many neurocognitive disorders is neural network dysfunction that results from the linking of hypofunctioning neurons, CRMP2 may be a feasible drug target for improving network function. Elucidating how CRMP2 regulates the molecular machinery underlying network dysfunction may unlock powerful alternatives to treat network hypofunction across neurocognitive disorders.

Funding Source: This research was funded by the Pharmacological Sciences T32 Training Grant.

W1103

MODELLING INJURY SPECIFIC LUNG REPAIR USING HIPSC-DERIVED IMMUNE COMPETENT ALVEOLAR ORGANOIDS

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Lung alveoli are comprised of two epithelial cell types; Type I alveolar epithelial cells (AT1s) that facilitate gas exchange, and type II alveolar epithelial cells (AT2s), a progenitor cell that secretes pulmonary surfactant and is responsible for alveolar homeostasis and wound repair. In response to normal cell turnover, AT2s can self-renew, or differentiate in AT1s to restore normal epithelial cell composition. In response to acute injury, AT2s also have the capacity to dedifferentiate into basal cells to facilitate more wide-spread tissue repair. Alveolar regeneration and repair is a tightly

controlled process involving interactions with stromal, endothelial and immune cells. During chronic injury, mechanisms of alveolar wound repair can become dysregulated leading to the appearance of aberrant basaloid cells, myofibroblasts and pro-inflammatory immune cells that collectively cause inflammation, fibrosis and a failure to re-establish normal alveolar epithelial composition and architecture resulting in functional decline. The mechanisms driving this dysregulated wound healing are not well understood. Here we have developed hIPSC-derived immune competent alveolar organoids that contain AT2s, macrophages, fibroblasts, endothelial and dendritic cells to better understand mechanisms of normal and pathogenic wound healing. Using scRNA-seq and models representative of normal homeostasis, acute (particulate matter exposure) and chronic (pulmonary fibrosis) injury we identify signatures of normal and pathogenic wound healing, including the production of basal cells, myofibroblasts, M1 & M2 polarised macrophages and rare aberrant basaloid cells. These different cell types appear in an injury dependant manner, confirmed via mapping to the human lung cell atlas, reflecting known repair mechanisms in the lung, as well as associated gene expression, cell-signalling and inflammatory cytokine profiles. Additional analysis such as cell-cell interactions and disease ontology further confirm that our models replicate alveolar disease and wound repair. Our approach provide new opportunities to understand mechanisms driving normal and pathogenic wound repair and identify novel interventions that may reduce the impact of respiratory disease.

Funding Source: MRC, NC3Rs.

W1105

ORGANOID FIBROSIS MODEL: ACCELERATING THE RESEARCH AND DEVELOPMENT OF THERAPEUTICS FOR FIBROTIC DISEASES

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Organoids are structures comprised of multiple cell types that are spatially organized similarly to an organ and recapitulate at least some specific organ functions. Several types of organoids have been described, derived both from adult tissue and from pluripotent stem cells. This technology will likely have a major impact on the study of developmental biology, organ physiology and function, and disease modeling. The lung bud organoid (LBO) model described in the current protocol displays branching morphogenesis, proximodistal specification and evidence of early alveologenesis both in vivo and in vitro. Their development reaches a stage equivalent to the second trimester of human development. LBO-derived branching structures in Matrigel contain type 2 alveolar epithelial cells (AT2) with abundant lamellar bodies. Pulmonary fibrosis model can be induced by bleomycin treatment. The LBOs generated by this study therefore fulfill the definition of true organoids, and will be useful for studying human lung development and potentially for modeling human lung disease.

Funding Source: Novoprotein Scientific Inc.

W1107

RECAPITULATING HEREDITARY ANGIOEDEMA USING A PERSONALIZED EXPANDED POTENTIAL STEM CELL (EPSC) PLATFORM: DEMONSTRATING SIGNALS OF LIVER DISEASE AMONG HEPATOCYTES DIFFERENTIATED FROM PATIENT EPSC

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Hereditary angioedema (HAE) is mostly caused by deficiency or dysfunction of C1-esterase inhibitor (C1-INH), caused by the mutations of SERPING1 – which is predominantly expressed in hepatocytes. The exact pathomechanism of SERPING1 mutations has not been fully elucidated, and previous studies have primarily focused only on circulating C1-INH. Evidence showed HAE to be more of a metabolic liver disorder, but studies on patient hepatocytes have been limited by difficulty in acquiring relevant samples. In this study, we utilized our expanded stem cell (EPSC) platform to derive hepatocytes from individual HAE patients with different type I or type II HAE mutations. Using this personalized disease model, we found patient EPSCs showed comparable pluripotency and trilineage differentiation capability as healthy controls. All EPSCs could be further differentiated into hepatocytes which showed typical polygonal and binuclear hepatic characteristics and expressed critical hepatocyte markers. However, patient EPSC-derived hepatocytes showed morphological abnormalities compared to controls, including ballooning, displaced nuclei, lipid deposition, and other apoptosis-like features. Hepatocytes from type I HAE patients demonstrated < 50% expression of SERPING1 at both RNA and protein levels, while those from type II HAE patients showed equal expression, compared to controls. Furthermore, patient-derived hepatocytes demonstrated retention of C1-INH within the cytoplasm, significantly more in type II than type I patients. Moreover, bulk RNA sequencing showed that patient-derived hepatocytes significantly upregulate collagen-containing extracellular matrix and ZFP41, common hallmarks of chronic liver disease and hepatocellular carcinoma. At last, repaired patient_EPSC-derived hepatocytes showed normalized expression and secretion of SERPING1 together with a decrease of ZFP41 and amelioration of fibrosis. In conclusion, our patient EPSC-derived hepatocytes were able to accurately model SERPING1 dysfunction and we confirmed that HAE is indeed a metabolic liver disorder – with hepatocytes demonstrating retention of C1-INH in their cytoplasm and expression of markers typical of chronic liver disease. Furthermore, we showed the possibility of curing the disease by genetic editing.

W1109

CHARACTERIZATION AND VALIDATION OF HUMAN IPSC-DERIVED NOCICEPTORS FOR PAIN MODELING

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Chronic pain is a leading health crisis linked to reduced quality of life, mental disorders, opioid dependence, and suicide. Despite its prevalence, effective long-term therapeutic options remain elusive, as prolonged opioid treatments are highly addictive and often fatal. Preclinical drug screenings using animal models lead to high clinical trial failure rates due to genomic differences between animal and human tissues. Although human cadaver tissue offers some advantages, the

limited availability and viability restrict its use. As such, human induced pluripotent stem cells (hiPSCs) and the derivatives present a scalable, physiologically relevant alternative. In this study, we developed a robust method to differentiate hiPSCs into nociceptors (NOCs) that are highly pure and functionally mature. Immunocytochemistry reveals that the cultures are >95% positive for Peripherin and β-III Tubulin, >80% positive for Brn3A, and >90% positive for NaV1.7 and NaV1.8. Electrophysiological characterization using multi-electrode arrays (MEAs) demonstrates that these NOCs are spontaneously active by day 14 and reach a stable baseline by day 25, achieving 95% active electrode yield (AEY). Functionally, these NOCs exhibit a significant increase in mean firing rate (MFR) and AEY in response to capsaicin (TRPV1 agonist, 100 nM and 67 nM). Conversely, both MFR and AEY significantly decrease in response to Huwentoxin (NaV1.7 inhibitor, 30 nM) and A803467 (NaV1.8 inhibitor, 300 nM and 150 nM). These findings demonstrate that our hiPSC-derived nociceptors are a powerful and physiologically relevant model for nociception research. Their scalability, high purity, and rapid maturation make them an ideal candidate for integration into high-throughput screening platforms, advancing the discovery and development of novel analgesics.

Funding Source: BrainXell.

W1111

CHOROID PLEXUS ORGANOIDS AS A MODEL FOR INVESTIGATING GLUCOSE TRANSPORT IN GLUT1 DEFICIENCY SYNDROME

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Glucose is the primary energy source for the brain, transported via solute carrier transporter family members. SLC2A1 (GLUT1) is a crucial glucose transporter at the blood-brain barrier (BBB) and cerebrospinal fluid-blood-brain barrier (CSF-BBB), facilitating glucose uptake into the brain and cerebrospinal fluid (CSF). GLUT1 plays a critical role in maintaining glucose homeostasis within the CSF, ensuring adequate energy supply to the brain. Mutations in SLC2A1 cause GLUT1 Deficiency Syndrome (GLUT1DS), leading to seizures, microcephaly, and other neurological symptoms. The standard treatment, a ketogenic diet, has limited efficacy and long-term adverse effects, necessitating alternative therapeutic approaches. This study aims to develop a choroid plexus organoid model to investigate glucose transport in GLUT1DS. We generated choroid plexus organoids from patient-derived GLUT1DS iPSCs and an isogenic corrected control. Organoids were characterized by morphological and metabolic differences using biochemical assays, glucose uptake studies, and omics-based analyses. GLUT1DS choroid plexus organoids exhibited early cyst formation, reduced cerebrospinal fluid (CSF) production, and lower glucose levels compared to isogenic controls. Metabolic profiling confirmed altered glucose metabolism, which will be further validated using ^{13}C-glucose uptake assays. Our study establishes a clinically relevant choroid

plexus organoid model to investigate glucose transport deficits in GLUT1DS. This model provides a platform for mechanistic studies, offering new insights into therapeutic strategies for GLUT1DS.

Funding Source: Wallenberg Academy Fellow grant, RED Postdoctoral Fellowships – SciLifeLab Campus Solna, Stockholm, Sweden.

W1113

CRISPR-ENHANCED IPSC DIFFERENTIATION AND ENRICHMENT FOR THE ASSEMBLY OF NOVEL BIOPRINTED RETINAL MODELS IN AMD RESEARCH

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Age-related macular degeneration (AMD) is a leading cause of vision loss, yet current preclinical models fail to replicate the complexity of human retinal tissue. Traditional animal models and 2D in-vitro cultures lack the spatial organization necessary for modeling key cellular interactions, limiting their relevance for disease research and therapeutic screening. To address this, we have developed a multilayered 3D bioprinted retinal model by integrating enriched retinal progenitor populations into engineered constructs designed to mimic the human retina. We first established iPSC reporter lines with fluorescent tags to track and purify photoreceptor and bipolar cell progenitors, enabling the development of efficient cell purification strategies. These purified progenitor cells were maintained in culture as retinal spheroids, preserving their viability and differentiation potential until their use in bioprinting. Furthermore, we systematically tested various bioink formulations incorporating biologically relevant components of the retinal extracellular matrix, identifying an optimal composition that supports the structured deposition of retinal cells onto mature retinal pigment epithelium (RPE). Using this optimized bioink and bioprinting parameters, we successfully generated stable 3D scaffolds that remained intact for at least 20 days, maintaining an overall cell viability of >70%. Retinal cells bioprinted on top of RPE demonstrated superior survival, stable proliferation, and a more homogeneous distribution within the scaffold compared to those printed directly onto plastic substrates. These results highlight the importance of a biomimetic microenvironment in promoting the stability and functionality of bioprinted retinal constructs. Future efforts will focus on incorporating additional neuronal layers, such as retinal ganglion cells, enhancing synaptic connectivity, and integrating endothelial cells beneath the RPE to model the blood-retinal barrier. This work establishes a robust foundation for the development of physiologically relevant retinal models, providing a powerful tool for AMD research, drug discovery, and preclinical testing.

Funding Source: This research was funded by the European Commission (HORIZON-MSCA-2021-PF-01, iRETINA, Grant 101062218) and the Regional Government of Andalusia, Spain

(I+D+i in Biomedicine and Health Sciences 2022, Project PI-0044-2022).

W1115

ENGINEERING APPROACHES TO ENHANCE THE THERAPEUTIC POTENTIAL OF EXTRACELLULAR VESICLES FROM MESENCHYMAL STEM CELLS

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Extracellular vesicles (EVs) derived from mesenchymal stem cells (MSCs) hold great promise as therapeutic agents due to their low immunogenicity and ability to deliver bioactive molecules. However, the endogenous cargo of MSC EVs is not always sufficient to confer therapeutic effects. Additional payloads and modifications may enhance the potency and specificity of MSC EVs, allowing for effective therapeutic action at lower doses. In this study, we generated immortalized MSCs from umbilical cord tissue and scaled up MSC culture using a 3D culture system with the CelCradle® Benchtop Bioreactor. This system facilitates cell growth on macroporous carriers or biomesh scaffolds suspended in a dynamic culture environment, enabling efficient cell attachment, proliferation, and nutrient exchange. These optimized conditions support high-yield MSC expansion and EV production. Among the three immortalized MSC lines evaluated, MSCs expressing hTERT with P53 knockdown exhibited the highest MSC EV yield. We further tested several methods for loading nucleic acids into MSC EVs and labeling the EVs with fluorescent dyes. Our results demonstrated that REG1-mediated plasmid loading into MSC EVs enabled successful delivery and gene expression. Additionally, MemGlow was identified as the most effective fluorescent dye for labeling MSC EVs without aggregation, facilitating further studies on EV uptake and biodistribution. We are also working on the conjugation of MSC EVs with proteins using click chemistry for the addition of protein payloads and targeted delivery. This is a post-isolation modification method that does not require genetic engineering. It is a gentle yet efficient method for EV modification that can be scaled up for GMP production.

Funding Source: Singapore Agency for Science, Technology and Research EVANTICA program.

W1117

HISTONE LACTYLATION REGULATING VISTA+MDSC TO MEDIATE IMMUNE ESCAPE OF INTRAHEPATIC CHOLANGIOCARCINOMA

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The immune escape mechanism of intrahepatic cholangiocarcinoma (iCCA) is still unclear, and there is a lack of effective intervention strategies in the clinic. The project applicant previously

found through analysis of clinical samples and single-cell data that myeloid-derived suppressor cells (MDSC) in the iCCA microenvironment highly expressed the immune checkpoint VISTA, and its enrichment was associated with poor prognosis in patients, and enhanced the immunosuppressive activity of regulatory T cells (Treg) through the VISTA/PSGL-1 axis. Metabolomics analysis showed that abnormal lactate accumulation in the tumor microenvironment drives MDSC to VISTA^{+} phenotype transformation by inducing histone H4K12 lactylation (H4K12la). Based on the previous work, this study intends to reveal the key modifying enzymes that regulate H4K12la and clarify the key transcription factors involved in VISTA transcription, so as to systematically analyze the regulatory mechanism of the H4K12la-VISTA/PSGL-1 axis on the MDSC-Treg immunosuppressive network. This study will also use patient-derived organoids, humanized mice and other models to evaluate the synergistic efficacy of targeting key regulatory molecules in combination with PD-1 monoclonal antibodies, providing a scientific basis for the development of new iCCA immunotherapy strategies based on the "H4K12la-VISTA+MDSC-Treg" axis.

Funding Source: This work was supported by the Key Research and Development Plan of Zhejiang Provincial Department of Science and Technology (No. 2024C03051), and the National Key Research and Development Program of China (2021YFA1100500), the Major Research.

W1119

OSTEOBLAST AND OSTEOCLAST DIFFERENTIATION IN GAUCHER DISEASE: INSIGHTS INTO BONE ABNORMALITIES

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Gaucher disease (GD) is a lysosomal storage disorder caused by GBA1 mutations, with ~75% of patients experiencing skeletal complications that significantly impact morbidity and mortality. Understanding the pathophysiological mechanisms underlying GD-related bone abnormalities is crucial for developing effective therapeutic strategies. This study aimed to investigate the roles of osteoblasts and osteoclasts in GD-related skeletal pathology using patient-derived (NC) and GBA1-corrected (COR) cell lines. Osteoblast differentiation, assessed by alkaline phosphatase (ALP) and alizarin red (AR) staining, showed no significant differences between NC and COR, suggesting that osteoblast dysfunction may not be a primary factor in GD-related skeletal abnormalities. However, osteoclast differentiation, evaluated using tartrate-resistant acid phosphatase (TRAP) and F-actin staining, revealed larger and more numerous osteoclasts in NC compared to COR, suggesting that enhanced osteoclast activity may contribute to bone complications in GD. These findings highlight the importance of osteoclast dysregulation in GD-related skeletal pathology. To elucidate the molecular mechanisms underlying osteoclast dysregulation in GD, RNA sequencing (RNA-seq) is needed to identify key regulatory factors. This approach may reveal critical pathways contributing to skeletal pathology and provide potential therapeutic targets. Future research should validate these findings through functional studies to confirm the role of candidate genes in osteoclast differentiation and activity. A deeper understanding of these mechanisms will enhance the potential for novel therapeutic strategies to improve bone health in GD patients.

Funding Source: This research was supported by the Bio and Medical Technology Development Program of the National Research Foundation (NRF) and funded by the Korean government (MSIT) (No. RS-2023-00261905, 2022M3A9E4017151).

W1123

A NOVEL FORM OF ANGIOPOIETIN 1 AS A PRIMING AGENT TO ENHANCE THE THERAPEUTIC EFFICACY OF MOBILIZED HUMAN PERIPHERAL BLOOD STEM CELLS IN TREATING ISCHEMIC CARDIOVASCULAR DISEASE

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Ischemic diseases lead to necrosis and dysfunction of the organ, while current treatment options have limited efficacy to induce tissue regeneration. Autologous cell therapy has shown potential to augment tissue recovery, but its therapeutic efficacy still needs further improvement, and its safety should be guaranteed before clinical application. Here, we evaluated the efficacy of using Angiopoietin-1 (Ang1)-primed human peripheral blood stem cells (mobPBSC) for cell therapy in ischemic diseases. Also, we established a priming strategy which can be applied to the clinic for patients diagnosed with myocardial infarction. First, we developed a stable form of Ang1 by genetic engineering, named FVA3-Ang1. FVA3-Ang1 priming conditions were tested on human umbilical vein endothelial cells, showing that a 1- hour priming at 400ng/mL could induce maximal angiogenesis potential. Genetic ontology analysis of 1-hour primed FVA3-Ang1 mobPBSC revealed no significant change in gene expression, while the CD31 and CXCR4 protein expression increased. This implied that 1- hour priming did not modify the cell characteristics, however could augment the angiogenic potential of FVA3-Ang1 primed mobPBSC. In a mouse hind-limb ischemia model and a myocardial infarction model, injection of FVA3-Ang1 primed mobPBSC to the infarcted area showed superior engraftment and tissue regeneration compared to non-primed mobPBSC injection. As to evaluate the efficacy and safety of FVA3-Ang1 primed mobPBSC in human patients diagnosed as acute myocardial infarction, we are conducting a clinical trial which will treat those patients with FVA3-Ang1 primed mobPBSC injected into the culprit coronary artery after standard care.

Funding Source: This research was supported by the Bio and Medical Technology Development Program of the NRF and funded by the Korean government (MSIT) (No. RS-2022-NR067329).

Clinical Trial ID: MAGIC cell-6 trial, NCT06364150.

W1125

CHRONIC FOS ACTIVATION AS A DRIVER OF MUSCLE STEM CELL DYSFUNCTION IN DUCHENNE MUSCULAR DYSTROPHY

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Duchenne muscular dystrophy (DMD) is a currently incurable muscle-wasting disease that typically leaves children wheelchair bound by 10 and usually dead by their 20s. DMD is caused by the loss of Dystrophin, a structural protein that maintains skeletal muscle integrity. Although Dystrophin is lost at birth, severe muscle loss does not occur until later in adolescence, coinciding with the decline in muscle stem cell (MuSC) function. Human DMD patient progression and DMD mouse model data suggests that preserving muscle-forming ability through MuSCs is key to preserving muscle mass in DMD patients. This raises two unresolved questions: (1) what are the molecular driver(s) of MuSC dysfunction in DMD; and (2) how can therapies be developed to reverse MuSC dysfunction, prevent muscle loss, and prolong DMD patient lives? Our laboratory recently identified a stress-response pathway, FOS, which is transiently expressed in healthy adult MuSCs and is necessary for stem cell activation and regeneration of skeletal muscles. However, when FOS was

continuously expressed in muscle progenitor cells, these cells failed to differentiate into muscle ex vivo and we identified a gene expression profile consistent with loss of stem cell differentiation, identity, and muscle fiber stability, phenotypes that resemble the pathology of DMD. In essence, we recreated a DMD-like phenotype ex vivo by shifting FOS from transient to continuous expression. Consistent with this model, our preliminary data shows that FOS is continuously expressed in 30-40% of DMD MuSCs before and after injury in vivo when compared to healthy MuSCs in a D2.mdx mouse model, suggesting that FOS is chronically activated in a subset of DMD MuSCs. Based on our findings, I will test the hypothesis that chronic FOS activation is a driver of MuSC dysfunction and leads to muscle wasting and weakness in DMD mice; and that restoring proper FOS activation in DMD MuSCs can reverse stem cell dysfunction and DMD muscle pathology. By targeting FOS, our work aims to clarify its role in DMD pathology and identify FOS as a therapeutic target, with the potential to improve muscle function and extend patient lifespans.

Funding Source: California Institute for Regenerative Medicine (CIRM) EDUC4 Fellowship.

W1127

DECIPHERING CELLULAR METABOLISMS OF GLIA IN APOE4 CARRIERS OF ALZHEIMER'S DISEASE BRAIN USING HIPSCS TO IDENTIFY SMALL MOLECULE TARGETS FOR THERAPEUTICS

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The ε4 allele of the APOE gene (APOE4) is the strongest genetic risk factor for late-onset Alzheimer's disease (AD). We previously investigated the effects of APOE4 on brain cell types derived from population and isogenic human induced pluripotent stem cells, post-mortem brain, and APOE targeted replacement mice. Global transcriptomic analyses reveal human-specific, APOE4-driven lipid metabolic dysregulation in astrocytes and microglia. Thus, we further focused on investigating the downstream mechanisms by which APOE4 leads to dysfunction in human astrocytes to identify druggable targets. We found downregulation of lysosomal gene expression as a major transcriptomic deficit of APOE4 astrocytes derived from AD patient tissue and isogenic human iPSCs. This transcriptomic profile is associated with major shifts in the synthesis and catabolism of lipids and proteins in astrocytes. Homozygous APOE4 (APOE 44) astrocytes display reduced lysosomal proteolysis, lipid catabolism, macroautophagy, and endosomal microautophagy. We demonstrated that APOE 44 astrocytes exhibit mTORC1 dysfunctions that lead to increase of lipid synthesis via enhancing SREBP2 signaling. Finally, mTORC1 inhibition rescued lysosome, lipid, and inflammatory phenotypes in APOE 44 astrocytes, suggesting mTORC1 signaling and autophagy-lysosome pathways may be promising therapeutic targets for APOE4 carriers.

Funding Source: NIH NIA R01AG082362, R01AG083941.

W1129

DERINAT PROMOTES ANAGEN ENTRY BY INHIBITING CA²⁺ SIGNALING

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Ca2+ is a ubiquitous intracellular signal regulating various stem cell types and niches. Our previous study indicated that Ca2+ signaling governs hair follicle (HF) morphogenesis and regeneration. Furthermore, treatment with Derinat, a transient receptor potential canonical channel (TRPC) inhibitor, altered hair growth patterns in BALB/c-nu and C57BL/6 mice by prolonging the anagen phase of the hair cycle. However, the specific mechanisms by which Ca2+ signaling influences HF stem cells and their niche to control the hair cycle phase remain unclear. This study elucidated the role of Ca^{2+} signaling in regulating the hair cycle, revealing that Ca^{2+} signals originating from TRPCs affect HF stem cells and their niche, thus controlling the phase of the hair cycle. Vibrissae follicles (VFs) from mice were analyzed across the hair cycle for Ca2+ signaling, ROS accumulatinon, and DNA damage (TUNEL). Additionally, C57BL/6 female mice were treated with Derinat during telogen, from P49 (encompassing late catagen to telogen) to P59. Hair growth, cell proliferation (BrdU incorporation), TUNEL, and expression of bulge markers (K15), secondary hair germ (SHG) markers (P-cadherin), as well as CaMKII and β-catenin levels, were then evaluated. Our results indicated that Ca^{2+} signaling and intracellular ROS levels differ across the hair cycle phases of VFs. Specifically, middle and late catagen phases exhibit higher levels of Ca^{2+} signaling and intracellular ROS compared to the anagen phase. DNA damage was only observed in late catagen VFs. We further investigated the effect of the TRPC inhibitor, Derinat, on HFs. Our findings demonstrated that Derinat treatment during the telogen phase prematurely initiates the transition to the anagen phase. This is likely due to the blockage of Ca^{2+} signaling by Derinat, which activates HF stem cells in the bulge and SHG, as indicated by increased BrdU incorporation. Furthermore, we found that CaMKII and β-catenin signaling pathways are involved in Derinat-regulated hair cycle transition. Notably, Derinat treatment did not cause any alterations in body weight. Inhibition of TRPC-mediated Ca^{2+} signaling by Derinat promotes premature anagen entry by activating hair follicle stem cells and modulating CaMKII and β-catenin pathways.

Funding Source: This work was funded by the National Science and Technology Council (NSTC) of Taiwan (NSTC 111-2314-B-400-042-MY2 and NSTC 113-2314-B-400-021-MY3).

W1131

DEVELOPING CELL BASED BIOASSAYS FOR CLINICAL TRIALS- THE USE OF PATIENT DERIVED INDUCED NEURONS TO STUDY AUTOPHAGY IN THE FELL-HD TRIAL

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder, caused by CAG expansions in the huntingtin gene (HTT), which results in the production of mutated huntingtin protein (mHtt). HD is incurable and typically presents in mid-life and progresses to death over a 20-year period. Autophagy, a lysosomal degradation pathway ensuring cytoplasmic homeostasis is dysfunctional in HD, and thus contributes mHTT protein accumulation. Preclinically, it has been shown that felodipine can upregulate autophagy and clear protein aggregates in cells, including neural cells in HD. Thus, a phase II clinical trial was undertaken (Fell-HD) to assess the tolerability and feasibility of testing this drug in patients with early stage HD while also looking for any signal of efficacy. Given we cannot look at autophagy in the living human brain, we sought to do this using induced neurons (iN) directly reprogrammed from skin fibroblasts from Fell-HD participants. iNs keep the genetic and aging signatures of the donor bypassing any stem cell or neuroprogenitor phase during conversion. We converted 7 control and 18 Fell-HD patient derived fibroblasts to iNs with the same conversion efficiency and purity. DNA methylation array and analysis in iNs showed accelerated aging in some patients. Moreover, most HD-iNs showed a less elaborate neuronal morphology and increased HTT expression using qPCR. We used 0.1 □M and 1 □M Felodipine treatment for 24h to assess its effects. After 28 days of conversion followed by Felodipine treatment iNs were counterstained using neuronal and autophagy markers to determine neuronal morphology and subcellular autophagy changes using high-content automated microscopy. Additionally, HTT measurements were again performed using qPCR after treatment. Our results showed that Felodipine enhanced autophagy in only a subset of patients while having no obvious adverse effects on HD-iNs. Lastly, we compared and correlated our preclinical results with FELL-HD trial outcomes - the patient's cognitive and motor scores - and found some correlation to clinical response. In summary, this project using an in vitro preclinical iN model offers a new approach for looking at pathways targeted by drugs that cannot be studied in the living human brain and opens a new dimension in testing agents in clinic.

W1133

ESTABLISHMENT OF A RAT MODEL SUITABLE FOR HUMAN-RAT XENOTRANSPLANTATION AND HUMANIZED LIVER

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Severe immunodeficient rats have served as a better model for transplantation, however, this tool cannot provide an appropriate xenogeneic regenerative microenvironment. The primary limitation is interspecies immune rejection, particularly is the clean of human cells by macrophages if with human donors. Macrophages recognize and phagocytose foreign cells via surface receptors, leading to the elimination of invasive allogeneic cells. Therefore, it is essential to avoiding from the recognition by host macrophage to prevent macrophage-mediated immune attack on human cells during xenotransplantation. To address the issue, we knocked in the human signal regulatory protein alpha (hSIRPA) gene sequence into wild-type rats. CD47 is the key molecule in the "Don't Eat Me" signaling pathway, which interacts with the SIRPα receptor on the surface of macrophages, inhibiting their phagocytic function and thereby preventing the clearance of human-derived cells. With PCR assay, it is confirmed that the stable inheritance and expression of the hSIRPA gene in both homozygous and heterozygous rats and with flow cytometry, it is validated that macrophage-mediated phagocytosis of human red blood cells, whose surface express hCD47 in vitro and in vivo. The results showed that the wild-type had the highest phagocytic rate comparing with the heterozygotes and the homozygotes. Furthermore, it was crossbred homozygous hSIRPA rats with severe immunodeficient rats, established a novel rat model that is suitable for human-rat xenotransplantation and to generate humanized liver called FRG-pluS. This model effectively addresses the influence of macrophages and overcomes interspecies transplantation barriers, creating a microenvironment that is friendly to human-derived cells. The FRG-pluS rat model exhibits thymic hypoplasia, lacks T, B, and NK cells, and is compatible with human cells. During in vivo transplantation, it demonstrated an affinity for human cells, establishing a preconditioning protocol for human liver cell transplantation in large animal models. This significantly enhances the potential for organ transplantation, drug screening, and disease modeling.

W1135

HUMAN MSCS AMELIORATE EXTRAOCULAR MYOPATHY IN THYROID EYE DISEASE

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Thyroid eye disease (TED) is an autoimmune condition affecting the orbit and extraocular muscles (EOM) that may lead to orbital disfigurement, double vision, and even vision loss. The main processes involved in TED are inflammation, glycosaminoglycan accumulation, adipogenesis, and myofibrogenesis in the EOM tissue. These changes lead to the pathological characteristics of TED: orbital tissue expansion, remodeling, and fibrosis. While the clinical symptoms of myopathy in TED are diverse and difficult to treat, much of the research has focused on fat tissue, leading to a lack of understanding regarding myopathy. Human mesenchymal stem cells (hMSCs), which are

important mediators of therapeutic effects in anti-inflammation and regenerative medicine. Human MSCs were injected into the left orbit of TED animal models, and their effects on EOM volume and fibrosis were compared between TED and hMSCs-treated TED using tissue staining. Additionally, single-cell RNA sequencing was performed on the EOM of TED animal models. As a result, the groups treated with hMSCs showed a reduction in EOM volume and fibrosis. In the TED EOM, treatment with hMSCs resulted in a decreased proportion of Type II myofibers, which was found to be mediated by the regulatory effects of Six1 and Eya1 on myofiber differentiation. Myofiber differentiation involves a synergistic regulatory relationship between Six1 and Eya1 during the transformation of adult myofibers. Balanced expressions of the Six1 and Eya1 co-factors in Type II myofibers can induce their transition to Type I myofibers. Furthermore, subclustering analysis of fibroblasts identified five major signaling pathways involved in the pathogenesis of TED. Examination of receptor-ligand (R-L) interactions within these pathways revealed that hMSC treatment led to a reduction in R-L activity, resulting in a decreased expression of TED-associated signaling pathways. This study explored the use of hMSCs as a therapeutic agent for TED myopathy and identified genes that modulate both TED myopathy and myofibrosis. By identifying the target genes modulated by hMSCs, this research provides a potential role of hMSCs as a treatment for TED myopathy.

Funding Source: This research was supported by grants from the National Research Foundation of Korea (NRF): 2021R1A2C2010523 and RS20233938.

W1137

IGF1 DRIVES NEURAL PRECURSOR EXPANSION AND INHIBITORY NEURON SPECIFICATION VIA MTOR TRANSLATIONAL CONTROL: INSIGHTS INTO TUBEROUS SCLEROSIS

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The balance of excitatory and inhibitory (E/I) neurons is essential for proper mammalian central nervous system (CNS) function, as disruptions in this balance are implicated in autism spectrum disorders (ASDs) and other neuropsychiatric conditions. For example, macrocephaly-associated autism is characterized by neural overgrowth resulting from excessive proliferation of FOXG1+ neural progenitor cells (NPCs), which skews the proportions of excitatory and inhibitory neurons. The development of the CNS requires precise regulation by intrinsic and extrinsic signaling molecules. A recent meta-analysis of autism trio/dyad exome sequences identified damaging mutations in components of the insulin-like growth factor 1 (IGF1) signaling pathway. IGF1, produced by endodermal support cells, plays a critical role in promoting the proliferation and maintenance of human neural precursor cells (hNPCs) through the mechanistic target of rapamycin (mTOR) pathway. This pathway regulates protein synthesis and cellular growth. Overexpression of IGF1 in the developing mouse brain leads to increased cortical volume without altering brain morphology; however, the neuronal composition of the expanded cortex has not been systematically explored. IGF1 signaling activates the PI3 kinase/AKT pathway, resulting in phosphorylation of the tuberous sclerosis complex (TSC), composed of TSC1 and TSC2 subunits, which in turn regulates mTOR activity. Ribosome profiling of hNPCs treated with the mTOR

inhibitor Torin 1 identified 155 translational targets of mTOR signaling, including GSX1, a homeobox transcription factor essential for inhibitory neuron development. To investigate the role of mTOR-mediated regulation of GSX1, a GSX1-knockout human pluripotent stem cell line was generated. Additionally, human pluripotent stem cell lines with loss-of-function mutations in TSC1 or TSC2 were developed to phenotypically recapitulate tuberous sclerosis (TS), a syndromic form of autism associated with macrocephaly and decreased inhibitory neuron subtypes. Current and future studies aim to elucidate how mTOR-mediated regulation of GSX1 influences inhibitory neuron fate and contributes to the development of E/I balance, providing deeper insights into the molecular mechanisms underlying CNS disorders such as ASDs and TS.

Funding Source: NIH/NIA/Alzheimer's Disease Research Center Developmental Award (P30 AG066507).

W1139

INDUCTION OF PROINFLAMMATORY CYTOKINE RELEASE FROM HUMAN PLURIPOTENT STEM CELL-DERIVED MACROPHAGES BY EXOSOMAL ORF3A DERIVED FROM SARS-COV-2-INFECTED CELLS

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The emergence of SARS-CoV-2 variants remains a major global health concern. In severe COVID-19, exacerbated proinflammatory cytokine release is often associated with acute respiratory distress syndrome and poor prognosis. It is pivotally important to understand the mechanism that drives this pathogenic event. SARS-CoV-2 ORF3a is capable of inducing inflammasome activation and IL-1β secretion. Macrophages are thought to be major producers of proinflammatory cytokines. However, it remained debated if macrophages are susceptible to infection with SARS-CoV-2. The relevance of proinflammatory cytokine release from macrophages to SARS-CoV-2 biology and pathogenesis remains to be established. We hypothesized that certain secretory viral factors might be responsible for proinflammatory response in macrophages. In this study, we identified secretory ORF3a as a key player in proinflammatory cytokine release in macrophages derived from human pluripotent stem cells. In overexpression system, we detected secretory ORF3a in culture medium. Exosomal ORF3a was verified by Western blotting and immunoelectron microscopy. Exosomal ORF3a was capable of transferring to neighboring cells. Feeding ORF3a-containing exosomes derived from SARS-CoV-2-infecetd cells to PMA-differentiated macrophages triggered proinflammatory cytokine release. Taken together, our demonstration of exosomal ORF3a-mediated proinflammatory cytokine release lent support to the notion that SARS-CoV-2 could modulate host exosome biogenesis to facilitate pathogenic inflammation. Our findings also have implications in identifying biomarkers for novel diagnostic and therapeutic strategies to mitigate cytokine storm in severe COVID-19.

Funding Source: Supported by RGC (C7142-20GF and T11-709/21-N) and HMRF (22211042).

W1141

MESENCHYMAL STEM CELLS FROM PERINATAL TISSUES PROMOTE DIABETIC WOUND HEALING VIA PI3K/AKT ACTIVATION

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Diabetic foot ulcers (DFUs) represent a major complication of diabetes, often leading to poor healing outcomes with conventional treatments. Mesenchymal stem cell (MSC) therapies have emerged as a promising alternative, given their potential to modulate various pathways involved in wound healing. While diverse MSC sources have been explored, consensus is lacking on optimal donor specifications. This study evaluates and compares the therapeutic potential of MSCs derived from perinatal tissues—human umbilical cord MSCs (hUCMSCs), human chorionic villi MSCs (hCVMSCs), and human decidua basalis MSCs (hDCMSCs)—in a diabetic wound healing model. In our study, keratinocyte proliferation, re-epithelialization, collagen deposition, and angiogenesis were assessed through immunofluorescence and histological analyses. Our research results demonstrated that MSCs from perinatal tissues, particularly hUCMSCs and hCVMSCs, significantly enhanced diabetic wound healing and promote keratinocyte proliferation and vascular regeneration in db/db mice, whereas hDCMSCs were less effective. Mass spectrometry revealed a conserved set of proteins involved in extracellular matrix (ECM) organization and wound healing, with the PI3K/AKT signaling pathway playing a central role in these processes. Additionally, The PEGDA/SA/Col-I hydrogel supported the viability and function of MSCs, offering a promising scaffold for the treatment of DFUs. In another rat model of wound healing, the combined therapy of local MSC-encapsulated hydrogels with systemic administration of stem cell secretome demonstrated a stronger repair function compared to local hydrogel-stem cell encapsulation treatment alone.". These findings highlight the potential of specific perinatal MSCs and optimized hydrogel formulations in advancing diabetic wound care, offering new strategies for clinical treatment of diabetic wound healing.

Funding Source: Hong Kong Innovation and Technology Commission (MHP/024/19 and ITS/448/18) National Key Research and Development Program of China (2019 YFE0198400).

W1143

MODELLING TUMOUR MICROENVIRONMENT BY ASSEMBLOIDS FUSION OF IMMUNE VASCULAR ORGANOIDS AND PATIENT DERIVED ORGANOIDS

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Macrophages, vasculature, and fibroblasts form oncofetal niches that determine immune cell behavior and responses to immunotherapy in the tumor microenvironment (TME). How they spatially contribute to TME is still poorly understood, but the lack of immune and stromal cells in cancer organoids hamper this line of research. Therefore, we endow immune stromal compartment in cancer organoids. We established scaffold-free assembloids that fused hPSC-derived vascular immune organoids (VIOs) with either patient-derived cancer organoids (PDOs) or cancer spheroids. scRNA-seq showed a heterogenous population of macrophages and fibroblasts in the VIOs. We next characterized spatial TME features in assembloids by whole-mount

immunofluorescent staining. We observed macrophage shifting from tissue-resident into cancer-infiltrating behaviour in colorectal assembloids. We observed angiogenic switch of vasculature growing towards cancer core in liver assembloids. We also observed perivascular niches and pairs of intravasating macrophage and cancer for metastasis. We further characterized extracellular matrix by Masson's trichrome staining. We observed collagen density was elevated by agonistic CD40 myeloid immunotherapy while provoking macrophage phagocytosis and infiltrations. To resolve the oncofetal niches, we are performing Visium-HD spatial transcriptomic on lung PDO assembloids. We will investigate how do the oncofetal interactions determines anti-HER2 CAR-T efficiency.

W1145

REVERSAL OF CONTRACTILE DEFECTS IN ENGINEERED HUMAN TISSUE MODELS OF HEART FAILURE WITH PRESERVED EJECTION FRACTION (HFPEF) LEADS TO FIRST-IN-HUMAN GENE THERAPY CLINICAL TRIAL

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Heart failure with preserved ejection fraction (HFpEF), is a global health problem lacking disease-modifying therapeutic options, reflecting a lack of predictive models for preclinical drug testing. Aligned with FDA Modernization Act 2.0, we aimed to create the first in vitro human-specific mini-heart models of HFpEF, and to test the efficacy of a candidate gene therapy to improve cardiac kinetics and correct the disease phenotype. To achieve these aims, healthy human pluripotent stem cell-derived ventricular cardiomyocytes were used to bioengineer beating human ventricular cardiac tissue strips (hvCTS) and pumping cardiac organoid chambers (hvCOC), and contractile function of these mini-heart models was tested using commercial cardiac screening systems designed specifically for such applications. When conditioned with transforming growth factor-β1 and endothelin-1, these mini-heart models exhibited signature HFpEF disease phenotypes of significantly elevated diastolic force and tissue stiffness, and slowed contraction and relaxation kinetics, with no significant deficit in systolic force or ejection fraction versus unconditioned controls. Bioinformatic analysis of bulk RNA sequencing data from HFpEF mini-heart models and patient ventricular samples confirmed downregulation of SERCA2a of the calcium signalling pathway as a key differentially expressed gene, representing a novel therapeutic target for HFpEF.

After dosage optimization in vitro, AAV-mediated expression of SERCA2a abrogated the disease phenotype and improved the cardiac kinetics in HFpEF mini-hearts. These findings contributed IND and FTD applications approved by the FDA for an ongoing first-in-human gene therapy clinical trial for HFpEF. We conclude that such human-based disease-specific mini-heart platforms, created from human pluripotent stem cells, are relevant for target discovery and validation that can facilitate clinical translation of novel cardiac therapies.

W1147

SINGLE CELL ANALYSIS OF HUMAN IPSC-DERIVED NEURONS CARRYING THE ALZHEIMER DISEASE-ASSOCIATED APPV717I MUTATION AFTER LONG-TERM ENGRAFTMENT IN THE ADULT MOUSE FOREBRAIN

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Alzheimer's disease (AD) is the most frequent form of dementia affecting millions of people without a cure, and disease mechanisms are still not fully understood. Here, we applied a human-to-mouse xenotransplantation approach to assess histological alterations and changes in gene expression in human induced pluripotent stem cell (iPSC)-derived AD neurons at 2 and 12 months post injection into the mouse brain in comparison to transplanted control neurons. To this end, we differentiated human iPSCs carrying the familial AD APPV717I mutation into neurons, which demonstrated enhanced Aβ42 production, elevated phospho-tau, and impaired neurite outgrowth in vitro. After injection into the forebrain of immunocompromised mice, APPV717I or isogenic control neural progenitor cells differentiated into NeuN-positive neurons representing about 90% of cells in both APPV717I and control grafts at 2 months post injection. 12 months after injection however, APPV717I grafts were significantly smaller and contained an increased number of phospho-tau-positive neurons. We performed comparative single-nucleus RNA-sequencing of microdissected APPV717I and control grafts at 2 and 12 months post injection, and found shifts in the cellular composition of grafts with an enrichment of cell death pathways in APPV717I neurons at 12 months post injection, which were not seen in control neurons at that time point or in APPV717I neurons 2 months after injection. These data give important insights into transcriptional dysregulation in human APPV717I neurons linked to cellular vulnerability in vivo and provide a unique opportunity to study potentially beneficial effects of therapeutic compounds in this xenograft disease model.

W1149

THE DELETERIOUS IMPACTS OF INTERFERON-GAMMA AND NEUROTOXIN QUINOLINIC ACID ON CELLULAR HEALTH OF SVZ NEURAL STEM CELLS AND CONSEQUENCES FOR REGENERATION

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Current treatments for Multiple Sclerosis (MS) reduce the autoimmune-driven relapses, but are ineffective at preventing neurological disability arising in the progressive phase, where brain cells die. The Kynurenine Pathway (KP) contributes to MS pathogenesis by it's dysregulation in cells like proinflammatory monocytes, producing neurotoxins including Quinolinic acid (QUIN) that our group showed potently kills brain cells, particularly oligodendrocytes. Circulating blood/CSF QUIN correlates with increased MS severity. The KP is activated by the proinflammatory cytokine interferon-gamma (IFN-g), upregulating rate-limiting enzymes including indoleamine-2,3-dioxygenase (IDO-1), however whether this pathway is active in adult subventricular zone (SVZ) mouse neural stem cells (mNSCs) is unknown. IFN-g reduces proliferation of other stem cells, and could compromise repair mechanisms. We further hypothesised QUIN might damage the cellular health of mNSCs, and have similar sequelae. QUIN can be taken up by amino acid transporters and localise to lysosomes, where it can produce reactive oxygen species (ROS) via the Fenton reaction. A Muse cytometer was used to measure cell-health in neurospheres grown from the subventricular zone, passaged and plated as monolayers, over 24-72 hour timepoints; analysis used an ANOVA. IFN-g significantly increased oxidative stress in mNSCs (72 hour control 14.26±3.28% versus IFN-γ 26.03±4.17%, *p0.0183). IFN-g significantly increased G0/G1 NSCs, signifying reduced proliferation (72hr control 56.54±2.44% versus IFN-g 67.09±1.60%, *p0.037) – this was also observed in significantly reduced neurosphere size. QUIN dose-dependently significantly increased activated caspases, even at the 24 hour timepoint - control (36.45%±3.09%) vs 3uM QUIN (70.92%±6.82%), demonstrating QUIN activates the apoptosis cell death pathway. Live cell imaging was then used to assess lysosomes and their Fe2+-iron ion content, linked with ROS production, demonstrating substantial pools of iron in NSCs and reduced confluence in 50nM/250nM-treated cells (14 hour imaging). In conclusion, reducing IFN-g and QUIN production by other cells could reduce cell death, potentially improve the regenerative capacity of NSCs and in turn be effective in treating the neurodegeneration in MS.

Funding Source: We acknowledge funding of the Peter Duncan Neurosciences Unit.

W1151

ADSC-CM HAVE SCAR-SUPPRESSING EFFECT IN HUMAN KELOID TRANSPLANT MOUSE MODEL

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Keloids and hypertrophic scars are diseases specific to human skin that occur due to delayed inflammatory responses during the wound healing process, activating fibroblasts which produce excessive collagen. This process is often triggered by mechanical stress or infection. Current non-invasive treatments include the application of steroid impregnated tapes or local injection of steroids to suppress inflammation. However, steroids have adverse effects that cannot be ignored. In addition, since the maximum dosage is determined, the range of treatment in one session is limited for extensive scars. We have previously demonstrated and reported that human adipose-derived stem cell concentrated conditioned medium (ADSC-CM) suppress the expression of inflammation-related genes and collagen production genes in fibroblasts and further suppress the contractile ability of collagen disks by fibroblasts. In this study, we have conducted experiments to observe the effects of ADSC-CM on hypertrophic scar tissue in vivo by using a nude mouse model transplanted with human keloid tissue. Collagen gene expression was compared by qPCR to evaluate the effects of ADSC-CM. Immunohistochemical staining was performed to evaluate the activation of fibroblasts. ADSC-CM treated scar tissue showed tendency of collagen gene expression downregulation. The therapeutic potential of ADSC-CM in the treatment of keloids and hypertrophic scars may be promising.

W1153

ANALYSIS OF MICRORNA-MEDIATED GENE REGULATION IN VARIOUS CELL LINES UPON EXPOSURE TO LDL AND LATEX PARTICLES

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MicroRNAs (miRNAs) are critical regulators of gene expression involved in various biological processes, including angiogenesis, inflammation, cell proliferation, migration, and invasion. The present study aims to investigate the differential expression of miRNAs and their associated pathways in the EA.hy926 cell line exposed to low-density lipoproteins (LDL) and latex particles. The study was conducted using EA.hy926 cells subjected to three conditions: Control, LDL treatment, and Latex particles (Beads) treatment. MicroRNA expression was analyzed and compared between groups, and significant changes in expression were identified. Additionally, related genes, affected pathways, and predicted targets were investigated through literature analysis and experimental validation. The expression of miR-126 was downregulated upon exposure to both LDL and latex particles. The associated genes include IL-17A, caspase-3, and survivin, with the affected signaling pathway being PI3K/AKT/mTOR. Predicted targets of miR-126 are LRP1, LRP1B, LDLRAD2, STARD4, SEC14L1, and MSR1. Direct targets of miR-126 identified are LRP11 and VEGFB. In contrast to miR-126, the expression of miR-616 was upregulated upon

exposure to LDL. The associated genes include MMP2, MMP9, and TIMP2, influencing processes such as cell proliferation, migration, and invasion. Predicted targets of miR-616 include TLR1, TNFSF14, TNFRSF11B, LILRB4, and ILDR2. However, no significant direct targets for miR-616 were identified in the present study. The study reveals distinct patterns of miRNA expression and their involvement in key signaling pathways associated with LDL and latex particle exposure. Downregulation of miR-126 and its involvement in the PI3K/AKT/mTOR pathway suggests potential modulation of angiogenesis and inflammation. The upregulation of miR-616 indicates enhanced cell migration and invasion pathways. Further experimental validation is required to confirm the predicted targets and elucidate their roles in these processes. The differential expression of miRNAs in response to LDL and latex particles highlights their potential regulatory roles in cellular processes. Understanding these mechanisms could provide new insights into the molecular basis of lipid metabolism and inflammation in various cell types.

Funding Source: This work was supported by the Russian Science Foundation (Grant No. 22-65-00089).

W1155

BULK AND SINGLE CELL TRANSCRIPTOMIC ANALYSIS OF HUMAN IPSC-DERIVED SENSORY NEURONS AS A MODEL FOR NON-OPIOID PAIN THERAPEUTIC DRUG DISCOVERY

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With the recent FDA approval of Suzetrigine, Vertex's selective pain inhibitor targeting NaV1.8 sodium channels, there is renewed biopharma focus on non-opioid based pain drugs. The desire to improve predictability is fueling interest in new human-relevant models, with a strong focus on induced pluripotent stem cell (iPSC)-derived peripheral sensory neurons. These cells have the potential to advance pain research, however, a deeper understanding of how they recapitulate relevant expression and function of non-opioid pain targets is needed. In this study, baseline transcriptomic characterization of a novel human iPSC-derived sensory neurons, iCell® Sensory Neurons (FUJIFILM Cellular Dynamics), was performed and compared to human dorsal root ganglion (hDRG) and alternative published iPSC-derived sensory neuron protocols. The bulk RNAseq data demonstrated that iPSC-based methods yielded cells that clustered distinctly from hDRG. Within this grouping of iPSC-derived sensory neurons, subclusters were identified, suggesting that variations in differentiation protocols can impact the resulting sensory neuron populations. Importantly, iCell Sensory Neurons showed the highest expression of non-opioid pain-relevant genes (i.e., SCN9A, SCN10A, TRPV1, PEIZO2, and P2RX3) which was corroborated with single cell RNA-seq data showing these genes were expressed in the majority of the population. In addition, these sensory neurons also displayed accentuated expression of neural maturation markers, calcium handing genes, sodium channels, and potassium channels, as compared to cells from other iPSC differentiation protocols. These genomic data correlate with functional data in cell-based assays, including robust calcium influx responses to sensory-specific molecules (i.e., capsaicin or Yoda2) and relevant electrophysiology (low spontaneous activity and sensory-induced

activity) on MEA. In summary, iPSC-derived sensory neurons have a transcriptome profile relevant for human in vitro modeling of pain and neuropathy. Importantly, iCell Sensory Neurons express premier non-opioid pain targets within a population that is enriched in ion channels and intracellular signaling pathways that correlate with advanced sensory function.

Funding Source: Fujifilm Cellular Dynamics.

W1157

CONSTRUCTION OF TISSUE DERIED KIDNEY-DERIVED ORGANOIDS AND DISEASE MODELING

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The development of kidney organoids holds significant implications for both fundamental research and clinical applications in renal diseases. Despite remarkable advances in kidney organoid research in recent years, several challenges still exist. Through systematic screening of small molecules and recombinant proteins, we identified critical roles of retinoic acid (RA), glial cell line-derived neurotrophic factor (GDNF), and fibroblast growth factor 20 (FGF20) in enabling long-term in vitro expansion of kidney organoids. Immunofluorescence staining and single-cell RNA sequencing analyses confirmed the concurrent differentiation of proximal tubule and collecting duct lineages within these organoids. Leveraging this culture system, we generated polycystic kidney disease (PKD) organoids carrying PKD1 or PKD2 mutations using renal tissues from autosomal dominant polycystic kidney disease (ADPKD) patients. Single-cell transcriptomic profiling validated that PKD organoids recapitulated key molecular features of native PKD tissues. Furthermore, comparative analysis revealed cellular heterogeneity between PKD1- and PKD2-mutant organoids, underscoring their capacity to mirror the phenotypic diversity of patient-derived tissues. Based on these findings, we established a high-throughput drug screening platform using cystic structure diameter as a quantifiable endpoint. Our study presents a groundbreaking adult kidney-derived organoid culture system capable of sustaining in vitro expansion. By integrating patient-specific tissues, we further developed a robust platform for drug discovery in polycystic kidney disease and identified a promising therapeutic agent. These advancements enrich the foundational understanding of adult renal cell differentiation into organoids, provide a reliable model for studying adult kidney diseases, and demonstrate substantial scientific and clinical translational potential.

Funding Source: The National Natural Science Foundation of China (32122031 to Huili Hu and T2321004 to Huili Hu).

W1159

DECIPHERING THE MOLECULAR BASIS OF HEREDITARY ULNAR DEFICIENCY INDUCED BY COMPLEX STRUCTURAL VARIANTS

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Congenital limb defects are among the most prevalent categories of congenital anomalies, primarily manifesting as longitudinal deficiencies in isolated or syndromic forms. Non-syndromic limb defects often hinder effective prenatal diagnosis due to unclear pathogenic mechanisms. Most congenital limb defects are genetically driven and associated with mutations or chromosomal abnormalities. These abnormalities, including deletions, duplications, inversions, and translocations, can disrupt gene regulatory networks, thereby altering the expression of genes critical for limb development. The complexity of these anomalies poses significant challenges in generating suitable cellular and animal models. Current knockout and knock-in technologies struggle to recapitulate complex structural variants (SVs), and interspecies developmental divergence may further limit the applicability of these models. In this study, we analyzed a large family pedigree comprising individuals with bilateral ulnar longitudinal deficiency. Whole-exome sequencing (WES) failed to identify clinically significant pathogenic variants in the affected individuals. However, BioNano optical genome mapping revealed complex SVs, including duplications and inversions, within the 21q22 region of all affected individuals across the pedigree. Initial Hi-C analysis demonstrated that these SVs perturb the three-dimensional (3D) genome organization and disrupt certain topologically associating domains (TADs). Third-generation sequencing is currently being employed to investigate the transmission of variants between parents and offspring. Moreover, patient-derived and normal induced pluripotent stem cells (iPSCs) have been differentiated into induced mesenchymal stem cells (MSCs), with subsequent osteogenic and chondrogenic differentiation planned to compare phenotypic disparities across developmental stages. This investigation aims to elucidate how TAD disruption in the 21q22 region underlies non-syndromic ulnar longitudinal deficiency at the molecular level. These findings will enhance mechanistic insights into non-syndromic limb defects and provide a framework for modeling SV-associated diseases.

Funding Source: National Key Research and Development Program (2024YFC2707002). Innovation Program of Shanghai Municipal Education Commission (2023ZKZD16) and National Natural Science Foundation of China (82071262).

W1161

DECREASED COMPLEXITY AND ACTIVITY OF HUMAN CORTICAL NEURONS DERIVED FROM PATIENT WITH DYRK1A MUTATION

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Dual specificity tyrosine-phosphorylation regulated kinase 1A (DYRK1A), a phosphorylation kinase, is localized within the central nervous system and is linked to various neurodevelopmental disorders, including developmental delays, intellectual disability (ID) and autism spectrum disorders (ASD). Haploinsufficiency of DYRK1A leads to ASD-related phenotypes in mice and human,

however, the key pathological mechanisms remain unclear, particularly in human. Here, we generated human induced pluripotent stem cell (iPSC) lines from two patients with de novo missense mutations and differentiated these lines into cortical neurons. We found that DYRK1A-mutant neurons exhibited small soma size with increased soma circularity. Besides, DYRK1A-mutant neurons showed decreased dendritic branching and immature synaptic formations. Transcriptomic analysis revealed reduced expression of multiple ion channel genes, particularly calcium channels, in DYRK1A-mutant neurons. Electrophysiological results showed that DYRK1A-mutant neurons exhibited an increased action potential half-width and decreased calcium currents. Overall, these findings indicate that DYRK1A deficiency disrupts calcium channels, suggesting calcium channels modulators as potential therapeutics for diseases with DYRK1A deficiency.

Funding Source: National Natural Science Foundation.

W1163

DEVELOPMENT OF HUMAN IPSC-DERIVED HEPATOCYTES FOR DRUG DISCOVERY, TRANSLATIONAL RESEARCH AND TOXICITY TESTING

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Hepatocytes comprise over 80% of the liver mass and are responsible for most of its functions, including lipid and glucose metabolism, storage of macronutrients, secretion of plasma proteins, detoxification and xenobiotic metabolism. Due to this vast spectrum of functionality, in vitro hepatocytes have been used in both toxicology research and to study debilitating genetic diseases and life-threatening conditions, such as alpha-1-antitrypsin deficiency and MAFLD. Despite the growing burden of liver diseases, treatments remain limited due to challenges with traditional models; e.g. primary human hepatocytes are scarce and difficult to maintain, immortalised cell lines lack key liver functions and animal models present interspecies differences. Here, we used opti-ox™, a deterministic cell programming technology, to generate consistent, scalable human induced pluripotent stem cell (hiPSC)-derived hepatocytes and demonstrated their suitability for both research and disease modelling (ioHepatocytes Discovery Research), and toxicology (ioHepatocytes Toxicology). ioHepatocytes display expected cobblestone morphology with distinctive nuclei and well-defined borders. Cells express key pan-hepatocyte markers including ALB, HNF4A, ASGR1 and SERPINA1, and present a transcriptomic signature similar to primary human hepatocytes. Additionally, ioHepatocytes show a high degree of consistency when derived from iPSCs with opti-ox. ioHepatocytes Discovery Research secrete albumin, store glycogen and accumulate lipids as well as forming bile canaliculi in vitro, demonstrating suitability for modelling metabolic syndromes. ioHepatocytes Toxicology show expression of phase I, II and III drug metabolism genes and have functional cytochrome P450 enzymes (CYP3A, CYP2B6 and CYP1A2). Finally, when challenged with compounds known to cause drug induced liver injury, ioHepatocytes Toxicology shows a similar toxic response to primary human hepatocytes, indicating prospective use for ioHepatocytes Toxicology in the prediction of hepatotoxicity. ioHepatocytes

offer a highly consistent, functional human hepatocyte model, with enhanced scalability to mitigate some of the pitfalls and challenges faced with existing technologies.

W1165

EFFICIENT PRODUCTION OF CARDIOMYOCYTES IN 2D EN 3D: WNT AND INSULIN ENHANCE PROLIFERATION AND INHIBIT MATURATION OF HUMAN IPSC-DERIVED CARDIOMYOCYTES VIA TCF AND FOXO SIGNALING

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Embryonic signaling pathways exert stage-specific effects during cardiac development, yet the precise signals for proliferation or maturation remain elusive. To uncover the cues for proliferation, we performed a combinatory cell cycle screen for Insulin and glycogen synthase kinase-3 (GSK-3) inhibition in spontaneously beating human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). Our analysis for proliferation, and subsequential downstream sarcomere development, gene expression analysis and molecular interventions identified a temporal interplay between Insulin/Akt/FOXO and CHIR99021/Wnt/GSK-3/TCF signaling. Combined pathway activation led to proliferation of immature hiPSC-CMs with low sarcomere and mitochondria content, while in the absence of pathway activators, cardiomyocytes rapidly exited the cell cycle and fetched higher organization of sarcomeres and mitochondria. Our data demonstrates two important pathways which enhance proliferation and inhibit maturation, and provides molecular mechanistic understanding of these cell fate decisions in immature hiPSC-CMs. Moreover, this technology allows for expansion of hiPSC-CMs 2-dimensional (2D) large culture flasks and 3-dimensional (3D) stirrer flasks for the production of hundreds of millions of cells. This facilitates a desired scale up for drug testing and large heart tissue generation.

Funding Source: Dutch Heart Foundation.

W1167

GENE TARGETING OF NOS1AP IN HUMAN IPSC-DERIVED CARDIOMYOCYTES FROM PATIENTS WITH LONG QT SYNDROME TYPE 1

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Long QT Syndrome Type 1 (LQT1) is a heritable cardiac disorder that can affect individuals of all ages. While often asymptomatic, it can lead to life-threatening arrhythmias, particularly under stress or intense physical activity. The condition is typically marked by prolonged action potential duration (APD) caused by mutations in the KCNQ1 gene, which encodes a cardiac potassium channel. Nitric Oxide Synthase Adaptor Protein (NOS1AP) – a chaperone involved in NO signalling in both cardiomyocytes (CMs) and sympathetic neurons – is a common variant in QT prolongation. Overexpression of NOS1AP shortens APD by accelerating repolarisation in guinea-pig ventricular myocytes. Here, we investigated the therapeutic utility of NOS1AP in a human model of LQT1, by differentiating induced pluripotent stem cells (iPSCs) into CMs for use at day 30-35 and transducing them with an adenoviral vector encoding NOS1AP marked by mCherry fluorescence, or the empty vector. Structural properties were assessed via scanning ion conductance microscopy and confocal microscopy, while functional analyses include optical mapping and multielectrode array (MEA) recordings. First, western blot confirmed overexpression of NOS1AP in CMs. Preliminary results show that LQT1-CMs exhibit significantly reduced cell area and decreased membrane stiffness compared to healthy CMs, reflecting the fragility of the disease phenotype. NOS1AP caused an increase in membrane stiffness of LQT1-CMs, comparable to that of healthy CMs. Confocal imaging revealed an inversion of alpha and beta-tubulin expression in LQT1-CM, signifying disruption of the microtubule complex; which were reversed to levels comparable to healthy CMs with NOS1AP. Optical mapping showed increased calcium transient amplitudes in LQT1-CMs, and MEAs demonstrated prolonged field potential duration and RR intervals (decreased beat rate). Notably, NOS1AP treatment shortened MEA electrograms in LQT1-CMs, approaching those of healthy controls. This study highlights key structural and functional features of hiPSC derived LQT1-CMs. We further show that NOS1AP gene transfer in a human model of LQT1 syndrome may provide a therapeutic opportunity to restore a normal electrophysiological phenotype.

Funding Source: British Heart Foundation.

W1169

HSP70 AND MIR-22-3P IN MESENCHYMAL STEM CELL-DERIVED EXTRACELLULAR VESICLES: MODULATING TH2 IMMUNITY IN ATOPIC DERMATITIS

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Atopic dermatitis (AD) is a chronic inflammatory skin disorder characterized by immune dysregulation, primarily driven by T helper 2 (Th2) cytokines, leading to severe pruritus, eczematous lesions, and impaired skin barrier function. Despite advancements in targeted

therapies, achieving long-term efficacy with minimal side effects remains challenging. Mesenchymal stem cell (MSC)-derived extracellular vesicles (EVs) have emerged as promising therapeutic agents due to their immunomodulatory and regenerative properties. To enhance their therapeutic potential, we primed MSCs with interferon-γ (IFN-γ) to generate IFN-γ-stimulated MSC-derived EVs (IFN-γ-MSC-EVs), which exhibit enhanced anti-inflammatory and immunoregulatory functions. Using both in vivo and in vitro AD models, we demonstrated that IFN-γ-MSC-EVs effectively mitigated AD pathology by reducing inflammatory and mast cell infiltration while downregulating Th2 cytokine receptors, including IL-4Rα and IL-13Rα1, along with their associated downstream signaling pathways. Notably, IFN-γ-MSC-EVs significantly alleviated key AD symptoms, including pruritus, transepidermal water loss (TEWL), and skin thickening, while systemically suppressing Th2-related markers in serum. Mechanistically, IFN-γ-MSC-EVs were enriched with immunomodulatory proteins and miRNAs, particularly heat shock protein 70 (HSP70) and miR-22-3p, which contributed to the suppression of Th2 cytokine receptor expression and downstream signaling, ultimately restoring immune balance and promoting skin homeostasis. These findings highlight that IFN-γ-MSC-EVs not only attenuate Th2-driven immune responses but also enhance skin regeneration. Our study suggests that IFN-γ-MSC-EVs represent a promising stem cell-derived therapy for AD and warrant further investigation to evaluate their long-term clinical efficacy.

W1171

HUMAN TROPHOBLAST ORGANOID-BASED INFECTION MODELS REVEAL DIFFERENTIAL RESPONSE TO HEPATITIS E VIRUS GENOTYPES

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Hepatitis E virus (HEV) infection during pregnancy leads to high maternal mortality and significant adverse fetal outcomes resulting from its cross-placenta transmission. These cases vary across HEV genotypes, especially between genotype 1 (HEV1) and 3 (HEV3). However, since the lack of physical-relevant infection models, the underlying pathologic and virologic mechanisms of different HEV subtypes on placenta remain largely undefined. Here, we developed trophoblast stem cell (hTSC)-based cell and organoid infection models to HEV1 and HEV3, and characterized their infectivity to trophoblast derivatives. We revealed that hTSCs and syncytiotrophoblasts (STB) were permissive for HEV1/3 infection, and the infection was more robust on organoids than on cell models. Based on these models, we found that although HEV3 was more infectious to trophoblast cells than HEV1, post-infection transcriptome analysis indicated more dramatic transcriptional changes upon HEV1 infection. For example, the downregulated KRT3, SLIT3, COL14A1, HOXD8, and PDGFRB suggested disruption of spiral artery remodeling process and upregulated GCM1 suggested a promotion of syncytialization. Trophoblast organoid-based infection models enabled evaluating the antiviral properties of drugs, like sofosbuvir and ribavirin, against HEV infection to placenta. In summary, based on the HEV-infected trophoblast organoid model, we dissect differential placental response to HEV1 and HEV3, and provides a robust platform for developing the antiviral drugs available during pregnancy.

Funding Source: National Natural Science Foundation of Youth Fund (NSFC82301883).

W1173

INNOVATIVE IN VITRO TERATOGENICITY MODELS: FROM MOUSE TO HUMANS

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Cutting-edge in vitro teratogenicity models represent a significant advancement in drug safety assessment, aimed at reducing the reliance on animal testing while enhancing the predictivity and relevance of human-based assays. Conventional animal embryofetal development (EFD) testing requires the use of a large number of animals and is very time-consuming, making it feasible for only a limited number of drug candidates at a late stage in drug development. In contrast, alternative in vitro models, while promising, face their own challenges. Data from model compounds is mostly based on animal studies, and most recent in vitro alternative models are human-based, which creates a "translational gap" from animal EFD data to human in vitro models. Traditional in-vitro teratogenicity testing relies heavily on the mouse embryonic stem cell test (EST), a validated assay for identifying potential teratogens. However, recent developments using human-based models, such as hiPSC-derived, offer promising alternatives that closely mimic early human embryonic development. Here, we present a comprehensive comparison of the mouse EST and these novel human-based assays, such as the Teratox and gastruloid assays. Validation data are presented using a range of reference compounds known for their teratogenic or non-teratogenic effects. These compounds were tested across all models to evaluate and compare their performance and predictive accuracy. Human-based models align better with known human teratogenic outcomes, showing superior translational potential. Specifically, the Teratox assay exhibited higher sensitivity and specificity in detecting teratogenic effects compared to the mouse EST. In conclusion, these innovative in vitro teratogenicity models represent a shift from traditional animal-based methods to human-relevant assays. These models not only offer ethical benefits but also provide more relevant biological insights, potentially leading to safer and more effective pharmaceuticals. This transition promises improved drug development processes, better safety profiles, and safer therapeutic options for patients.

W1175

INTEGRATION OF IPSC-DERIVED MICROGLIA INTO CEREBRAL ORGANOID FOR STUDYING NEUROIMMUNOLOGY

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Microglia is tissue-resident macrophage that exist in the central nervous system (CNS). Microglia is involved not only immune responses in CNS, but also contributes to the development and functional regulation of neurons and other glial cells. Microglia is influenced by genetic predisposition and micro-environments, and their dysfunction is a key characteristic of neurological diseases. However, unlike pheriperal blood mononuclear cells, microglia is difficult to isolate

securely. Animal model-derived microglia and cell lines only partially recapitulate human microglial characteristics due to genetic differences. In contrast, human iPSC-derived microglia provide a stable supply of cells with specific traits and replicate the microglial development process, closely resembling embryonic hematopoiesis. Therefore, we described an effective differentiation method of microglia using human iPSC and integration into cerebral organoids. Firstly, embryonic bodys (EB) were produced using human iPSC with SCF, BMP4, VEGF and 20uM ROCK inhibitor. To differentiate the hematopoietic lineage, we induced primitive macrophage progenitor (PMP) using IL-3 and M-CSF. PMP cells were expressed myeloid immune cell markers (CD11b, CD14, CD45). To induce the maturation of microglia, we cultured PMP cells in the presence of IL-34 along with low concentrations of GM-CSF and M-CSF. After maturation step, cells express typical microglia markers (IBA-1, CX3CR1, TMEM119, P2RY12). Moreover, we investigate whether iPSC-derive microglia exhibit functional properties. In the phagocytosis assay, we observed that iPSC-derived microglia internalized fluorescence latex beads in a time dependent manner after treatment. Furthermore, LPS treatment shifted microglia to an inflammatory phenotype, marked by decreased CD206 and elevated HLA-DR expression. To generate the neuro-immune organoids, microglia was co-cultured with human cerebral organoid. Notably, iPSC-derived microglia successfully integrated into the cerebral organoid and exhibited proliferation. Taken together, we established an iPSC-derived microglia differentiation protocol with functional characteristics. These microglia is expected to be applicable to the study of microglial function and their role in neuroimmunology.

W1177

INVESTIGATING RETINAL TAU PATHOLOGY IN FRONTOTEMPORAL DEMENTIA

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Frontotemporal dementia is an early-onset neurodegenerative disorder characterized by progressive neuronal loss, often associated with hereditary mutations in the MAPT gene, which encodes the tau protein. Patients with Alzheimer’s disease and FTD exhibit retinal defects, including changes in retinal thickness and the accumulation of hyperphosphorylated tau aggregates, which correlate with disease severity. The retina's accessibility for non-invasive imaging makes it an ideal model for studying disease progression and evaluating potential therapeutic interventions in real-time. A key gap in current research is the limited understanding of tau's role in neurodegeneration. The link between neurodevelopment and neurodegeneration has already been proposed for iPSC-derived cortical cultures and mouse models. To address this gap, we explored the involvement of tau in retinal development and maturation using two isogenic iPSC lines differing in the presence of the MAPT IVS10+16 mutation, which is linked to FTD. We generated 2D retinal cultures and 3D retinal organoids to investigate the effects of this mutation. Our findings reveal that tau-mutant retinal cultures, at later differentiation stages, exhibit abnormal tau isoform expression, increased tau phosphorylation, and toxic tau aggregates, indicative of early pathological tau accumulation. Functionally, we observed impaired synaptic maturation, reduced network synchronization, and elevated markers of cellular stress. At earlier differentiation stages, tau-mutant cultures displayed delays in neural rosette formation, impairing differentiation, and maturation of retinal progenitor cells, accompanied by a reduced number of mitochondria. This developmental delay extended to retinal organoids, where optic vesicle structures were smaller, and the neuronal retina exhibited disorganized morphology. These results demonstrate that the MAPT IVS10+16 mutation disrupts human retinal neurogenesis, leading to both developmental deficits and early tau pathology. By establishing an iPSC-derived retinal model for tauopathy research, this study provides a novel platform to investigate the neurodevelopmental aspects of

FTD and related disorders, offering a fresh perspective on tauopathies before the onset of full-blown neurodegeneration.

W1179

METABOLIC PROFILING OF VENTRALIZED NEUROECTODERM TO UNDERSTAND HIGH REDOX POTENTIAL OF HUMAN MIDBRAIN DOPAMINERGIC CELLS

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Midbrain dopaminergic (mDA) cells represent a distinct cell type important in motor control. mDA cells are highly metabolically active and very sensitive to redox states compared to other neural cell types, but the nature of this sensitivity is not well understood. We hypothesize that mDA cells are programmed to allow high redox states early in development, prior to differentiation of unique dopaminergic cell features. To test this hypothesis, we compared the metabolic profile of ventral neuroectoderm, an early mDA progenitor cell state, to neuroectodermal cells, which both underwent dual-SMAD inhibition and differed solely in their exposure to sonic hedgehog (SHH), an essential ventralizing morphogen. iPSCs were induced from six control human lines and cells were treated in induction media for 7 days. Metabolic profiling was conducted using two complementary liquid chromatography-mass spectrometry (LC-MS/MS) approaches, ion-pairing reversed-phase LC-MS/MS and hydrophilic interaction liquid chromatography, for a comprehensive coverage of central carbon metabolism. We found that metabolites from the transsulfuration pathway were significantly upregulated in ventralized neuroectodermal cells. The transsulfuration pathway produces sulfur-containing metabolites, such as glutathione and taurine, which can act as antioxidants and regulate cellular redox balance. RNA-seq analysis of these two cell types at this developmental timepoint showed that a subset of genes encoding enzymes of the transsulfuration pathway were upregulated in ventralized neuroectoderm. This suggests that ventralized neuroectoderm is equipped with a distinctly active transsulfuration mechanism early in development, likely to reduce oxygen free radicals from high mitochondrial ATP production. Dopaminergic cell redox states may thus be programmed much earlier than previously thought and may allow for the differentiation of unique dopaminergic cell features such as extensive branching, dopamine production, and calcium flux.

W1181

MULTI ORGANOID CULTURE SYSTEM FOR RECAPITULATING LIVER PANCREAS INTERACTION IN OBESITY

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The prevalence of obesity-related metabolic disorders, such as metabolic dysfunction-associated steatotic liver disease (MASLD) and type 2 diabetes (T2DM) is rising. Understanding the metabolic pathways underlying these diseases is crucial for drug development, which requires advanced in

vitro models of key organs involved in metabolism, particularly the liver-pancreas axis. While existing liver-pancreas axis models are useful for studying glucose regulation, they fall short in replicating an in vivo-like liver-pancreas axis due to the slow transport of metabolic substrates and suboptimal organ function in mixed media cultures. To address these limitations, we developed a multi-organoid device (MOD) that enables convective transport of metabolites between separated organoids. This device significantly enhances metabolic substrate transport compared to passive diffusion-based systems and improves organoid function. To model MASLD on this platform, liver organoids were treated with palmitate (PA), a fatty acid, which induced characteristic lipotoxicity, lipid accumulation, and increased secretion of Fetuin A (FetA), a key signaling molecule. Moreover, our device successfully replicated MASLD-induced T2DM, characterized by FetA-mediated β-cell apoptosis, impaired pancreatic glucose sensitivity, glucose intolerance, and hyperinsulinemia. Additionally, the widely used T2DM drug metformin exhibited clinically relevant efficacy within the system, underscoring its potential for drug screening applications. Overall, this MOD provides a robust platform for modeling the interactions between MASLD and T2DM and holds promise for the development of effective therapeutic strategies for metabolic diseases.

Funding Source: Supported by NRF-2022R1C1C1008610 and KFRM (24A0105L1, 22A0105L1-11).

W1183

NADPH OXIDASE 4 DEFICIENCY ENHANCES IL-12 PRODUCTION AND TH1 RESPONSES IN MYCOBACTERIUM TUBERCULOSIS INFECTION THROUGH BONE MARROW-DERIVED DENDRITIC CELLS

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Mycobacterium tuberculosis (Mtb) infection induces oxidative stress, necessitating host regulatory mechanisms to maintain redox balance. The NADPH oxidase (NOX) family modulates reactive

oxygen species production, yet while NOX2 has been extensively studied in Mtb infection, the role of NOX4 remains unclear. Given the importance of dendritic cells (DCs) in orchestrating adaptive immune responses, we investigated how NOX4 influences DC function and its subsequent effects on host immunity. Using NOX4-deficient (Nox4-/-) and wild-type (WT) mice infected with Mtb, we found that Nox4-/- mice exhibited reduced bacterial burden and milder lung pathology, accompanied by increased DC infiltration and a higher frequency of interferon-gamma (IFN-γ)-producing CD4+ T cells. Ex vivo experiments revealed that while T cells from WT and Nox4-/- mice exhibited comparable IFN-γ production when stimulated directly, Mtb-infected bone marrow-derived DCs (BMDCs) from Nox4-/- mice significantly enhanced IFN-γ production in WT T cells. Further analysis demonstrated that NOX4 deficiency led to increased IL-12 production in DCs through enhanced activation of IRF1, mediated by the AKT/GSK-3β signaling pathway. These findings suggest that NOX4 negatively regulates IL-12 production in Mtb-infected DCs, thereby suppressing Th1-mediated immunity. Notably, since BMDCs are derived from hematopoietic stem and progenitor cells (HSPCs), our results highlight the potential for modulating NOX4 in stem cell-derived DCs to enhance immune responses. This could open avenues for utilizing stem cell-based immunotherapies to improve host defense against tuberculosis and optimize vaccine efficacy. Given the critical role of DCs in immune regulation, targeting NOX4 may offer a novel strategy for harnessing stem cell-derived immune cells to combat infectious diseases.

Funding Source: This work was supported by the NRF grants funded by MSIT [RS-2023-00208115] and the Ministry of Education [RS-2023-00246091].

W1185

NOVEL MECHANISM OF CYFIP2 INVOLVEMENT IN THE PATHOGENESIS OF DEVELOPMENTAL EPILEPTIC ENCEPHALOPATHY

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Developmental and epileptic encephalopathy (DEE) is a severe neurodevelopmental disorder in children, characterized by high genetic heterogeneity. Despite advances, the mechanisms underlying DEE remain complex and poorly elucidated. In this study, we identified a novel de novo CYFIP2 nonsense mutation, c.2473C>T (p.Arg825Ter), in a DEE65 family, presenting milder phenotypes compared to other gain-of-function (GoF) mutations, suggesting a distinct mechanism. CYFIP2 is crucial for actin polymerization, yet its loss-of-function (LoF) effects are largely unexplored. To address this, we generated induced pluripotent stem cells (iPSCs) from the Arg825 patient and used CRISPR to create heterozygous and homozygous p.Arg87Cys cell lines, representing the most severe DEE65 mutation, in wild-type iPSCs. Additionally, to elucidate the mechanism of DEE65 caused by the Arg825 mutation, we performed CRISPR-mediated correction in Arg825 iPSCs to obtain an isogenic control iPSC line. Analysis showed that CYFIP2 expression was decreased in Arg825 iPSCs but remained at wild-type levels in Arg87 iPSCs. All lines were

differentiated into neural progenitor cells, forebrain neurons, and excitatory cortical projection neurons. Multi-electrode array analysis revealed that the Arg825 neurons exhibited reduced epileptiform activity compared to the Arg87 neurons. Arg825 neurons also showed reduced SYN1 synaptic signaling and lower dendritic spine density compared to Arg87 neurons, consistent with patient phenotypes. RNA-seq analysis indicated that the Arg825 mutation impairs axon development and causes transcriptional dysregulation. Overexpression studies in HEK293T cells demonstrated that the Arg825 mutation significantly reduces CYFIP2 interaction with WAVE1 and increases nuclear localization, indicating a potential genetic compensation mechanism affecting mRNA transcription. Overall, this study developed human iPSC models of CYFIP2 mutations, revealing a mechanism where nonsense mutations lead to mRNA truncation, disrupting the assembly of the WAVE1 regulatory complex and actin polymerization in DEE. Future research will employ this iPSC platform for deeper mechanistic insights and drug screening, offering new avenues for prenatal diagnosis and personalized treatment of DEE.

Funding Source: This study was supported by National Key Research and Development Program (2024YFC2707002), Innovation Program of Shanghai Municipal Education Commission (2023ZKZD16) and National Natural Science Foundation of China (82071262).

W1187

PRENATAL MEHG EXPOSURE LEADS TO ABERRANT CHOLINERGIC NEURON DEVELOPMENT, POTENTIALLY CONTRIBUTING TO ASD-LIKE BEHAVIOURS

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Aberrant neurodevelopment is a core deficit of autism spectrum disorder (ASD). Previously, we showed that adult mice prenatally exposed to non-apoptotic MeHg exhibited key ASD characteristics, including impaired communication, reduced sociability, and increased restrictive, repetitive behaviours, whereas, in the embryonic cortex, prenatal MeHg exposure caused premature neuronal differentiation. However, it remains unknown regarding underlying cellular mechanisms that contribute to prenatal MeHg-induced ASD. To determine underlying cellular mechanisms unbiasedly, we performed bulk RNA-seq analysis using cortical tissues from adult mice prenatally treated with 0.2ppm MeHg and revealed aberrantly increased cholinergic synaptic function. We further confirmed the finding by showing increased expression of choline acetyltransferase (Chat) in postnatal day 7 cortical tissues followed by increased expression of acetylcholine transporter (Slc5a7) in adult cortical tissues of mice receiving prenatal treatment of 0.2ppm MeHg. Intriguingly, we also observed an increased number of acetylcholine neurons (Chat+ cells) in the prefrontal cortex (PFC) of adult mice prenatally treated with 0.2 ppm MeHg. To ask about the embryonic origin of the increased Chat+ neurons in the prefrontal cortex, we performed embryonic day 12 (E12) dorsal and ventral neural precursor cultures and showed that non-apoptotic 25nM MeHg treatment in culture can increase the genesis of Chat+ neurons from both dorsal cortical precursors and ventral neural precursors. Overall, these results suggest that prenatal MeHg exposure increases cholinergic neuron production from embryonic neural precursors, consequently leading to dysregulated cholinergic neuron communication in adulthood, potentially contributing to ASD phenotypes.

Funding Source: The project is funded by the Natural Sciences and Engineering Council of Canada, the Canadian Institute of Health Research and the Canada Research Chair Program.

W1189

RAPID AND CONSISTENT GENERATION OF HUMAN IPSC-DERIVED OLIGODENDROCYTE-LIKE CELLS USING OPTI-OX TECHNOLOGY

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Oligodendrocytes (OLs) are the myelinating cells in the central nervous system. By ensheathing axons, OLs enhance the action potential conduction velocity. OLs arise from oligodendrocyte precursor cells (OPCs) during pre- and postnatal development. The death of OLs and the impairment of differentiation of OPCs into OLs is a major pathological characteristic in demyelinating diseases. The development of therapies that promote myelination in neurological conditions, particularly demyelinating diseases, is hampered by the limited translatability of existing preclinical animal models, and the lack of reliable in vitro models. Human induced pluripotent stem cells (hiPSCs) can be used to generate OLs for in vitro applications, however, current differentiation protocols are often lengthy, challenging to reproduce, and are difficult to scale. Our proprietary opti-ox™ (optimised inducible overexpression) technology enables highly controlled expression of transcription factors, deterministically programming hiPSCs into specific cell types of interest, to provide a robust, consistent, and reliable source of human cells for in vitro applications. We have used opti-ox to rapidly program hiPSCs into oligodendrocyte-like cells (ioOligodendrocyte-like cells), a population of oligodendroglial cells resembling a pre-myelinating oligodendrocyte state. By day 1, the cells present an OPC-like morphology and are positive for oligodendroglial lineage markers OLIG2, SOX10 and O4. By day 8, the cells show increased complexity with an OL-like morphology, and increased expression of other mature oligodendrocyte markers such as MBP, MAL, CNP and MYRF, seen by qRT-PCR, bulk RNA and scRNAseq. Furthermore, whole transcriptome analysis demonstrates equivalent expression profiles between three different manufactured lots indicating consistency and experimental reproducibility. ioOligodendrocyte-like cells provide a relevant, consistent, and scalable source of human cells that can be used for investigations into novel therapeutics and molecular mechanisms that regulate this critical glial cell type that is implicated in various human diseases.

W1191

RAPID, EASY, AND EFFICIENT BIALLELIC KNOCK-IN SYSTEM UNIFIED WITH AN INDEPENDENT SECOND SYNTHETIC CISTRON FOR PERSONALIZED TRANSGENE EXPRESSION

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Unspecific, inefficient, lack of control of the copy number and/or small cargo knock-in of transgenic elements confines the capacity of cell and gene therapies. For example, viruses and transposases allow quick integration of considerable cargo sizes into the genome but lack both specificity and controlled copy number. Nucleases like TALENS and CRISPR-associated proteins help integrate large cargo sizes into specific loci, but they rely on homology-directed repair, which is very inefficient. Thus, the selection of the edited cells is laborious and time-consuming. Prime editing is

another system that allows small editions into the targeted loci and combined with recombinases it can be used to insert big fragments, however, the selection of monoallelic or biallelic edited cells is still an arduous task. To address these challenges, we have developed a highly efficient methodology for inserting large cargo into a specific locus while enabling the rapid selection of biallelic knock-in cells. Furthermore, transgenic elements can be expressed under any synthetic promoter, making the system very customizable. In human cells, we achieve this by targeting a constitutive gene with template vectors and ribonucleoprotein complexes to insert the desired landing pad via homology-directed repair. The landing pad enables both the visualization and selection of biallelic-edited cells while also supporting the addition of a secondary cistron, where new transgenic elements can be expressed under any synthetic promoter and inserted via recombinases. As proof of principle, we tested our system using fluorescent gene reporters for easy visualization and selection of cells. However, any transgene can be inserted, making the system adaptable for testing therapeutic genes or mutated genes for disease modeling under the expression of synthetic promoters.

Funding Source: Cedars-Sinai.

W1193

RESTORATION OF CALCIUM HANDLING ABNORMALITIES IN BECKER MUSCULAR DYSTROPHY PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELL DIFFERENTIATED CARDIOMYOCYTES WITH GIVINOSTAT TREATMENT

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Dystrophinopathy, caused by DMD gene mutations, include Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD). DMD presents with severe progressive skeletal muscle weakness, while BMD is milder. Both conditions can lead to early-onset cardiomyopathy and severe heart failure. Increase histone deacetylase (HDAC) activity in dystrophinopathy causes epigenetic changes that inhibit muscle regeneration and promote chronic inflammation, fibrosis and

adipogenesis. The HDAC inhibitor Givinostat, has shown promise as a treatment, with preclinical and clinical studies demonstrating its positive impact on DMD skeletal muscle pathology. A phase 3 clinical trial in ambulatory DMD boys aged 6 years and older showed that Givinostat significantly delayed motor deterioration. It has been approved by the US FDA for treating DMD patients aged 6 years and older. However, data on the cardiac effects of Givinostat are limited. In this study, we established integration-free induced pluripotent stem cell (iPSC) lines from 2 patients with BMD and 2 healthy subjects. Both patients have mild skeletal muscle weakness but experienced early-onset of severe cardiomyopathy and heart failure, necessitating heart transplantation. We differentiated the patient-derived iPSC into cardiomyocytes (iPSC-CM) for pathological and functional characterization, and drug screening with Givinostat. Our patient-derived iPSC-CMs showed reduced dystrophin expression, increase cell membrane fragility with significantly elevated ATP and creatine kinase release after hypotonic challenge, and impaired calcium handling properties. Givinostat treatment restored intracellular calcium transient properties in the patient-derived iPSC-CMs, shortening calcium transient duration and accelerated time to peak and time of decay in a dose-dependent manner. However, higher doses were associated with irregular heartbeats. In summary, we found that the HDAC inhibitor Givinostat, had a potential positive functional impact on cardiac pathology of dystrophinopathy using our patient-derived iPSC-CM model. We will continue to investigate the epigenetic mechanisms using single-cell ATAC-seq and paired single-cell RNA-seq to explore Givinostat's genetic regulation of its cardiac effect in dystrophinopathy.

Funding Source: GRF grant (Ref: 17123122) Dystrophinopathy patient-derived iPSC disease model for novel epigenetic mechanisms evaluation and advancing small molecules therapies development (2022-ongoing).

W1195

AUTOMATION OF FULL WORKFLOW FOR CARDIAC DIFFERENTIATION AND FORMATION OF 3D CARDIAC ORGANOIDS FROM HUMAN IPSC, AND FUNCTIONAL ANALYSIS OF COMPOUND RESPONSES

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3D organoid models are increasingly important for biological research and drug development, however because of complexity of steps those processes are difficult to automate. To enable automated control of cell culture, we developed the automation solution CellXpress.ai. CellXpress.ai contains four essential components for automated organoid culture: liquid handler, automated incubator and imager, plus integrated AI-powered software that that provides automated processing of complex protocols. Automated 3D organoid culture includes processes of plating organoid domes, periodic media exchanges, and periodic monitoring by imaging and analysis. It provides automated passaging of organoids, which can be triggered by AI-based organoid classification based on organoid phenotypes. We developed AI-based protocols and present results from the automation of three different organoid types: mouse intestinal organoids, human intestinal organoids, and patient-derived colorectal tumor organoids. 3D organoid cultures were started from seeding organoids into matrigel domes in 24 well plate format. We automated

organoid culture protocols using media kits for mouse and human organoids recommended by STEMCELL Technologies. Media exchanges were done automatically every 24 hours. Passaging organoids were also performed automatically using liquid handling, but timing for passaging depends on maturation of organoids, which requires decision action done either by scientists of by software. Cultures were monitored by imaging every 12 hours with transmitted light and 4X magnification by integrated imager. Then machine-learning based image analysis allowed to detect organoids and provide analysis of organoid objects to measure variety of phenotypic read-outs including size, density, and texture measurements. Phenotypic classification of organoids for mature and immature phenotypes was done by pre-trained model that combined un-supervised and supervised machine learning. We created models for three tested organoid types. Passaging steps were then triggered automatically by a user-defined percentage and number of mature organoids in the culture, typically >50%. The AI-based classification allowed to fully automate 3D culture and expansion of organoids, also to increase productivity and throughput.

W1197

TRANSLATIONAL RESEARCH IN DRUG DISCOVERY TARGETING BRAIN CELLS USING CYNOMOLGUS MONKEY INDUCED PLURIPOTENT STEM CELL-DERIVED ASTROCYTES

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Translational research with non-human primates (NHPs) is widely accepted in central nervous system disease areas because of their close anatomical and physiological similarities to humans, allowing for accurate disease modeling and evaluation of therapeutic candidates. We previously established a chronic stroke model in cynomolgus monkeys and demonstrated the therapeutic effect of AS9102246-00 (AS), a lipid nanoparticle that delivers a transcription factor-encoding mRNA into astrocytes to induce neurogenesis, on motor dysfunction. While in vitro assays under controlled conditions are desirable to evaluate whether the efficacy observed in NHPs can be extrapolated to humans, the application of NHP in vitro models has been limited. Here, we report the generation of astrocytes derived from cynomolgus monkey induced pluripotent stem cells (monkey iPSCs) and in vitro efficacy evaluations of AS using these cells. Monkey iPSCs purchased from the Japanese Collection of Research Bioresources were differentiated into neural progenitor cells (NPCs) using dual SMAD inhibition. The NPCs were cultured in Astrocyte Medium for approximately 10 weeks with regular passaging to generate astrocytes. The majority of the cell population co-expressed GFAP and S100β and exhibited a star-shaped morphology, indicating typical astrocyte characteristics. Additionally, few doublecortin (DCX)-positive cells were observed, suggesting minimal neuronal cell contamination. In efficacy evaluations of AS, we treated iPSC-derived astrocytes and assessed induced-neurogenic effects 7 days post-treatment. The number of DCX-positive cells increased with rising concentrations of AS compared to the control on the same day. These DCX-positive cells exhibited a round cell body and neurite-like structures with very little GFAP expression, indicating neuronal characteristics. Furthermore, we successfully detected the efficacy of AS in human cells, suggesting its neurogenic potential may extend to humans as well. In conclusion, we conducted in vitro translational research using monkey iPSC-derived astrocytes. Our approach utilizing iPSCs has the potential to facilitate similar studies in other cell types, improving estimation of clinical responses in human and reducing the number of in vivo studies in animals.

Funding Source: Astellas Pharma Inc.

W1199

A BIPOTENTIAL ORGANOID MODEL OF RESPIRATORY EPITHELIUM RECAPITULATES HIGH INFECTIVITY OF SARS-COV-2 OMICRON VARIANT

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The airways and alveoli of the human respiratory tract are lined by two distinct types of epithelium, which are the primary targets of respiratory viruses. We previously established long-term expanding human lung epithelial organoids from lung tissues and developed a ‘proximal’ differentiation protocol to generate mucociliary airway organoids. However, a respiratory organoid system with bipotential of the airway and alveolar differentiation remains elusive. Here we defined a ‘distal’ differentiation approach to generate alveolar organoids from the same source for the derivation of airway organoids. The alveolar organoids consisting of type I and type II alveolar epithelial cells (AT1 and AT2, respectively) functionally simulate the alveolar epithelium. AT2 cells maintained in lung organoids serve as progenitor cells from which alveolar organoids derive. Moreover, alveolar organoids sustain a productive SARS-CoV-2 infection, albeit a lower replicative fitness was observed compared to that in airway organoids. We further optimized 2-dimensional (2D) airway organoids. Upon differentiation under a slightly acidic pH, the 2D airway organoids exhibit enhanced viral replication, representing an optimal in vitro correlate of respiratory epithelium for modeling the high infectivity of SARS-CoV-2. Notably, the higher infectivity and replicative fitness of the Omicron variant than an ancestral strain were accurately recapitulated in these optimized airway organoids. In conclusion, we have established a bipotential organoid culture system able to reproducibly expand the entire human respiratory epithelium in vitro for modeling respiratory diseases, including COVID-19.

W1201

A HUMAN ELONGATING HEART ORGANOID PLATFORM FOR INVESTIGATING TERATOGEN-INDUCED CONGENITAL HEART DEFECTS

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Congenital heart diseases (CHDs), such as ventricular septal defects (VSDs), tetralogy of Fallot (TOF), and transposition of the great arteries (TGA), are among the most common congenital anomalies, often linked to environmental and chemical exposures during early pregnancy. While epidemiological studies have identified teratogens, such as maternal hyperglycemia and certain medications as risk factors for CHDs, their direct impact on human heart development remains poorly understood due to the limitation of animal models. To address this, we utilized a human induced pluripotent stem cell (hiPSC)-derived elongating heart organoid (eHO) model, which closely mimics the early stages of heart tube formation and subsequent elongation and looping, to investigate how teratogens influence the pathogenesis of CHDs. Using high glucose as a

representative teratogen, we demonstrated its detrimental effects on eHO development, including significantly smaller size and reduced heart tube elongation. Furthermore, high glucose-treated eHOs exhibited aberrant looping dynamics, with accelerated and increased curvature, indicating disrupted morphogenetic regulation. Gene expression analysis revealed notable impairment in cardiac differentiation, with reduced levels of key cardiac markers such as NKX2-5 and TNNT2. Additionally, we observed significant DNA damage, indicating disrupted cellular integrity during heart development. These findings provide the first evidence of a direct, causal link between high glucose exposure and defects in early heart morphogenesis, particularly in the timing and degree of heart tube looping, a process essential for the proper alignment of heart chambers. The eHO model offers significant potential as a robust platform for modeling the effects of teratogens on early heart development and screening preventive interventions and therapeutic strategies aimed at teratogen-induced heart defects.

W1203

A NOVEL CELLUAR SYSTEM FOR NORMAL AGEING AND ANTI-AGEING STUDY

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Ageing is a complex biological process with profound health implications. Scientists have relied on model organisms such as fly and mouse and cellular models that lack normal human physiology-specific relevance. The placenta trophoblast development is an accelerated aging process, and provides unique insights into ageing. We have developed a pioneering technology for aging research: an ageing-emulated biosystem based on the development of human trophoblast stem cells to syncytiotrophoblasts. The biosystem displays all major ageing hallmarks, such as cell cycle arrest, genomic instability, epigenetic modifications, telomere attrition, and SASP, thereby establishing an accelerated ageing model in human normal cells. This robust technology enables simple, rapid and quantitative detection of potential anti-aging activities and thus holds promise for advancing ageing study, expediting anti-ageing discovery, and improving human health and longevity.

Funding Source: This research is supported by Health@InnoHK, the Innovation and Technology Commission of the Government of the HKSAR.

W1205

A NOVEL IN VITRO MICROPHYSIOLOGICAL SYSTEM CULTURING DIFFERENT TYPES OF HIPSC-DERIVED PERIPHERAL NEURONS ON MEA FOR NEUROPATHY ASSESSMENT

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Microphysiological system (MPS) is an in vitro culture technology that reproduces the physiological microenvironment and functionality of humans, and is expected to be applied for drug

screening. In this study, a novel MPS device was constructed on MEA chips for compartmentalized co-culture of different types of neurons, and we successfully detected electrophysiological activities in two types of in vitro peripheral model (i.e., neuromuscular junction (NMJ) model, and pain model) before and after drug administration. The MPS device was combined to MEA surface using a directly photobonding method to avoid cell damage during culturing. Human iPSC-derived motor neurons and human primary skeletal muscles were cultured in different chambers linked by microfluidics. Spontaneous activity could be detected from both cells. Notably, after electrical stimulation in motor neurons, a corresponding evoked response could be measured in the neighbor skeletal muscle, which indicated the formation of NMJ in the model. By using motor neurons derived from ALS patients, an increasing in spontaneous activity (hyperexcitation) was measured compared to healthy motor neurons. And such hyperexcitation was reduced after Rapamycin treatment. Next, human iPSC-derived sensory neurons and human iPSC-derived spinal cord dorsal horn (SCDH) were cultured in different chamber to mimic pain circuit. After adding a TRPA1 agonist AITC to sensory neurons, an acute increasing in spontaneous activity could be measured followed by a slowly increasing in SCDH later. Interestingly, the SCDH activity maintained even when the activity in sensory neuron temporary dropout. In contrast, administration of CNQX in SCDH could induce an acute increasing of activity, which might be through GABA receptors in interneurons. And spike disappearance was detected in sensory neurons later, which indicated the signal transduction between different neurons through synaptic networks. Taken together, the present compartmentalized MPS device enables co-culture of different types of peripheral neurons with axonal network linkage, which is essential to reproduce the relevant human anatomical architecture and for drug assessments by MEA measurement.

W1207

A NOVEL, DSMAD-INDEPENDENT INDUCTION PARADIGM FOR RAPID GENERATION OF HIPSC-DERIVED NEURONS AMENABLE TO REGIONAL PATTERNING

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Induced overexpression of Neurogenin 2 (iNGN2) is a valuable strategy to accelerate the conversion of human induced pluripotent stem cells (hiPSCs) into neurons for modeling of neurodegenerative diseases such as Alzheimer's Disease (AD). However, iNGN2 alone generates neurons of a mixed regional identity, potentially limiting their relevance for modeling AD. We developed an alternative neural induction strategy, which prepatterns hiPSCs before NGN2 induction to obtain a stronger cortical identity. This strategy involves inhibiting different pathways critical for self-renewal and meso-endodermal differentiation, independent of commonly applied dual SMAD inhibition (dSMADi). Combined with iNGN2, this induction paradigm quickly leads to homogeneous cultures of excitatory cortical neurons. Furthermore, the cells are responsive to regional patterning cues during the short neural induction pulse. This enables generation of neurons from different regions of both the central and peripheral neuron systems, including midbrain dopaminergic, motoneurons and sensory neurons, improving in vitro models for a range of neurological diseases and neurodegenerative disorders. Cortical neurons generated with this paradigm are suitable for an AD-relevant tau aggregation assay, similar in performance to cortical neurons derived from the dSMADi protocol, highlighting their applicability in disease – relevant in vitro models. Disclaimer: CH, AW, MJH, NN, CS, LR, TL, CU, LNM, JK, DG, DS, HL, LB, BMS, MB, RW, JR, IW, JDM, MC, PR are employees of AbbVie. VH was employee of AbbVie at the time of the study. AK was employee of the Institute of Molecular and Cell Biology, Mannheim University of Applied Sciences at the time of the study and was funded by the Graduiertenkolleg TASCDT of the MWK Baden-Württemberg and the Albert und Annelise Konanz-Stiftung. R.R and M.H. are current employees of Center for Mass Spectrometry and Optical Spectroscopy, Mannheim University of Applied Sciences and Institute of Medical Technology, Heidelberg University and Mannheim University of Applied Sciences and have no funding to disclose. The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

W1209

A PLURIPOTENT STEM CELL-DERIVED HIGH THROUGHPUT MODEL OF THE HUMAN NEUROVASCULAR NICHE IN HEALTH AND DISEASE

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Human pluripotent stem cell-derived neural organoids/assembloids have provided invaluable models in which to study human neural development, disease and advance drug therapies. Yet lacking in these models is the critical importance of the neurovascular niche and blood brain barrier (BBB). Efforts to date have shown primitive incorporation of endothelial cells into assembloids or cultured organoids onto rudimentary blood vessel networks. Here we present a high-throughput platform for generating healthy and patient specific neurovascular assembloids by integrating independently differentiated human pluripotent stem cell-derived endothelial cells, pericytes, astrocytes, and neurons. These assembloids exhibit in vivo-like features, including tight junction integrity, selective permeability and elaborate vasculature and neuronal maturation over time. Functional stress assays, with mitochondrial and calcium imaging readout in defined neural or endothelial populations, confirm the spatially organised neurovascular crosstalk and coordinated cell-cell (neural-vasculature) communication. Extensive transcriptomic sequencing and developmental profiling validate the reproducibility and temporal precision of these assembloids, uncovering mechanisms of cell-autonomous stress responses and dynamic intercellular signalling during maturation. We utilised this novel throughput model to demonstrate a previously uncharacterised role of pericytes in initiating neurovascular dysfunction in amyotrophic lateral sclerosis patients carrying C9orf72 mutations. By enabling the study of cell-specific contributions to BBB dysfunction and neurovascular disease pathophysiology, these assembloids provide a scalable and robust platform for advancing personalised medicine and therapeutic discovery.

W1211

A STEPWISE, MODULAR DESIGN OF BUILDING UNIFORM BRAIN ASSEMBLOIDS REPRESENTING THE DYNAMIC CELLULAR INTERPLAY BETWEEN NEURONS AND GLIAL CELLS

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Current brain organoid technology fails to provide adequate patterning cues to induce a mature structure that represent the complexity of the human brain. Here, we developed a module-based cellular reconstitution technology to sequentially build uniform forebrain assembloids with mature cortical structures and functional connectivity. The uniformity and maturity of the newly-conceived forebrain assembloids were achieved by creating single-rosette-based organoids at the early stage, whose sizes were big and consistent with the treatment of Wnt and Hedgehog agonists, followed by spatial reconstitution with the Reelin-expressing neuronal layer and non-neuronal glial cells. The resulting single-rosette-based forebrain assembloids were highly uniform and reproducible without significant batch effects, solving major heterogeneity issues caused by

difficulties in controlling the number and size of rosettes in conventional multi-rosette organoids. Furthermore, these forebrain assembloids structurally and functionally recapitulated the physiology of the human brain, including the six-layered cortical structure, functional connectivity, and dynamic cellular interplay between neurons and glial cells. Our study thus provided an innovative preclinical model to study a range of neurological disorders, understanding the pathogenesis of which requires an organoid system capable of representing the dynamic cellular interactions and the maturity of the human brain.

Funding Source: This research was supported by grants from NRF Korea (NRF-2022R1A2C3002702, RS-2023-00223277), Samsung Science and Technology Foundation (SSTF-BA2101-12), New Faculty Startup Fund from SNU, and the BK21FOUR Research Fellowship.

W1213

AAV-CRISPR-MEDIATED LIVER-SPECIFIC KNOCK-IN RESTORED HEMOSTASIS IN NEONATAL HEMOPHILIA B MICE

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AAV-delivered CRISPR/Cas9 (AAV-CRISPR) has shown promising potentials in preclinical models to efficiently insert therapeutic gene sequences in somatic tissues. However, the AAV input doses required were prohibitively high and posed serious risk of toxicity. Here, we performed AAV-CRISPR mediated homology-independent knock-in at a new target site in mAlb 3'UTR and demonstrated that single dose of AAVs enabled long-term integration and expression of hF9 transgene in both adult and neonatal hemophilia B mice (mF9 -/-), yielding high levels of circulating human Factor IX (hFIX) and stable hemostasis restoration during entire 48-week observation period. Furthermore, we achieved hemostasis correction with a significantly lower AAV dose through liver-specific gene knock-in using hyperactive hF9R338L variant. The plasma antibodies against Cas9 and AAV in the neonatal mice receiving low-dose AAV-CRISPR were negligible, which lent support to the development of AAV-CRISPR mediated somatic knock-in for treating inherited diseases.

Funding Source: This study was supported by funds provided by the Research Grants Council of Hong Kong [14116719, 14115520, 14106024 to B.F.], and the Health@InnoHK Program launched by Innovation Technology Commission of the Hong Kong SAR, China.

W1215

ADIPONECTIN KNOCKOUT UNVEILED IMPAIRED CELL FATE AND SURVIVAL IN TENDON-DERIVED STEM/PROGENITOR CELLS: INSIGHTS INTO THE PATHOGENESIS OF CHRONIC TENDINOPATHY

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Chronic tendinopathy is characterized by excessive inflammation and erroneous differentiation of tendon-derived stem/progenitor cells (TDSCs), resulting in a reduced pool of TDSCs available for tendon regeneration. It presents a significant challenge in sports medicine, with limited effective treatment options due to its unclear etiopathogenesis. Adiponectin, a hormone-like factor, exhibits anti-inflammatory, anti-apoptotic, and tissue regenerative properties across various cell types, including macrophages and endothelial cells. Our unpublished data revealed an increased adiponectin expression in both clinical samples and animal model of tendinopathy, implying a potential role of adiponectin in the disease pathogenesis. However, whether it exerts protective or detrimental effects in tendons remains unclear. This study aimed to explore the effects of adiponectin knockout on the cell fate and survival of TDSCs. TDSCs were isolated from wild-type or adiponectin knockout mice. Gene expression of tenogenic and non-tenogenic markers was analyzed, while clonogenicity, apoptosis, viability, and migratory properties were assessed to understand the effects of adiponectin deficiency on stem cell characteristics. This study uncovered key changes in adiponectin knockout TDSCs, including a significant decrease in the gene expression of tenogenic markers and an increase in non-teno genic markers, signaling a shift away from the desired tenogenic lineage. Clonogenicity assay revealed highly reduced colony formation, indicating impaired self-renewal potential, while increased apoptosis suggested disrupted survival mechanisms. Viability assessment showed a significant decrease in cell viability in the absence of adiponectin, and functional studies unveiled reduced migration capability, as evidenced in wound healing and transwell assays. These findings highlight the significant negative impacts of adiponectin knockout on the fate, survival, and function of TDSCs. The upregulation of adiponectin in the clinical samples and animal model of tendinopathy may thus be an attempt to enhance TDSC functions and promote tendon repair. Future studies should test the effects of adiponectin supplementation on TDSCs and tendons, as it may serve as a potential target for treating chronic tendinopathy.

Funding Source: This study was supported by the Research Grants Council (Ref. 14111624) and Health@InnoHK (Ref. ITC RC/IHK/4/7).

W1217

ADVANCED MODELLING OF UPPER MOTOR NEURON MND PATHOLOGY USING HUMAN PLURIPOTENT STEM CELLS

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Motor neuron disease (MND) is a progressive neurological disorder, marked by the degeneration of upper motor neurons in the spinal cord as well as the cortex, with no known cure. Recent research has implicated a role for inhibitory neurons within the cortex in modulating disease progression within the excitatory motor neurons. To better understand disease progression and unravel potential disease initiating mechanisms in MND, our lab has developed new methods for differentiating human pluripotent stem cells (PSCs) into specific neural subtypes, including cortical interneurons (inhibitory) and layer V cortical projection neurons (the laminar population

predominantly affected in MND). Here we have established a 2D co-culture system that combines these differentiated neurons using diseased or control iPSCs carrying mutations in the C9orf72 gene, the most common genetic mutation in familial MND. This model allowed the study of both histological, biochemical and functional changes in both neural subtypes and further investigate the role of interneurons in MND pathology. Our findings show that exposure to chemical stressors leads to an increase in reactive oxygen species, axonal fragmentation, stress granule formation and neural activity in both mutant interneurons and projection neurons. Notably, these pathological changes are more pronounced when mutant and isogenic projection neurons are co-cultured with mutant interneurons. Ongoing work is probing transcriptional and electrophysiological changes in these cultures. These results underscore the importance of including specific and disease-relevant neural subtypes to accurately model MND in vitro and highlight a potential regulatory role of cortical interneurons in MND pathology.

W1219

ALLOGENIC MITOCHONDRIA TRANSFER IMPROVES CARDIAC FUNCTION IN IPSC-DIFFERENTIATIED CARDIOMYOCYTES OF A PATIENT WITH BARTH SYNDROME

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Barth syndrome (BTHS) is an ultra-rare, infantile-onset, X-linked recessive mitochondrial disorder that primarily affects males, owing to mutations in TAFAZZIN, which catalyzes the remodeling of cardiolipin, a mitochondrial phospholipid required for oxidative phosphorylation. Mitochondrial transplantation is a novel technique to treat mitochondrial dysfunction by delivering healthy mitochondria to diseased cells or tissues. In this study, we explored the possibility of using stem cell-derived cardiomyocytes as a source of mitochondrial transplantation to treat BTHS. We established induced pluripotent stem cells (iPSC) from normal and BTHS patients and differentiated them into cardiomyocytes. The BTHS patient-derived iPSC-differentiated cardiomyocytes (BTHS CMs) exhibited less expression of cardiomyocytes markers, such as α-SA, cTnT, and cTnI, and smaller cell size than normal iPSC-derived cardiomyocytes (normal CMs). Multi-electrode array analysis revealed that BTHS CMs exhibited shorter beat period and longer field potential duration than normal CMs. In addition, mitochondrial morphology and function were impaired, and mitophagy was decreased in BTHS CMs compared to normal CMs. Transplantation of mitochondria isolated from normal CMs induced mitophagy in BTHS CMs, mitigated mitochondrial dysfunction, and promoted mitochondrial biogenesis. Furthermore, mitochondrial transplantation stimulated cardiac maturation and alleviated cardiac arrhythmia of BTHS CMs. These results suggest that normal CMs are useful for allogeneic transplantation in the treatment of mitochondrial diseases, including BTHS.

Funding Source: National Research Foundation of Korea (NRF-RS-2023-00208466), the Research Institute for Convergence of Biomedical Science and Technology (30-2021-000), Pusan National University Yangsan Hospital, and KHIDI (HI19C1085).

W1221

ALTERED GRANULE DYNAMICS IN CDKL5 DEFICIENCY DURING DEVELOPMENT

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CDKL5 deficiency disorder (CDD) is an X-linked neurodevelopmental disorder causing by loss of function of a serine/threonine kinase, Cyclin-Dependent-Kinase-Like 5 (CDKL5). The CDD patients suffer from severe neurodevelopment defects and the underlying pathological mechanism is not clear. In previous studies we identified nELAVL as novel substrates of CDKL5. Lack of phosphorylation by CDKL5 leads to altered nELAVL phase separation in rodent models. Induced pluripotent stem cells (iPSCs) derived neuron is a promising human-based model to study neurodevelopment disorders. We established CDD patient-derived neuron model and created an isogenic rescue control by Adenine based editing (ABE). We found increased nELAVL granule formation and increased colocalization between nELAVL granules and P-bodies in CDD neurons. Our findings suggested possible altered granule interaction in CDD neurons, which might lead to pathological outcomes. Investigating the mechanism underlying the interaction between nELAVL and P-bodies would be an intriguing focus for future studies.

Funding Source: This work was supported by Lo Kwee-Seong Biomedical Research Fund (J.I.), Faculty Innovation Award (FIA2020/A/04) from the Faculty of Medicine, CUHK (J.I.), Hong Kong RGC ECS 24117220 (J.I.).

W1223

ALZHEIMER'S DISEASE CEREBRAL AND NERUOIMMUNE ORGANOID UNVEILING EARLY AD PATHOLOGIES AND MECHANISMS

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder marked by amyloid-beta (Aβ) plaque accumulation, tau hyperphosphorylation, neuronal loss, and neuroinflammation. Early intervention is critical for slowing disease progression, but identifying reliable early biomarkers poses a significant challenge. In this study, we developed a cerebral organoid model from human induced pluripotent stem cells (hiPSCs) derived from familial AD (FAD) patients, effectively recapitulating key AD features, including Aβ plaques, tau pathology, and neuronal apoptosis, within just 30 days. Our organoid model exhibited significant Aβ accumulation confirmed by immunofluorescent staining techniques. Electrophysiological recordings using high-density microelectrode arrays (HD-MEA) revealed impaired neuronal activity in FAD organoids compared to control (CTRL) organoids, indicating disrupted synaptic integrity and decreased network functionality. Spatial transcriptomics analysis highlighted the upregulation of UNC5D, a gene associated with neuronal survival and microglia-independent neuroinflammation, suggesting its potential as an early biomarker for AD. To examine the interplay between immune responses and neurodegeneration, we co-cultured FAD organoids with iPSC-derived macrophages (iMACs), which differentiated into microglia-like cells and effectively engaged with Aβ plaques. This interaction demonstrated the potential for iMACs to mitigate Aβ burden and influence the inflammatory environment within the organoids. Our findings establish a robust platform for

studying early AD mechanisms and illustrate the complex relationships between neuroinflammation and neurodegeneration. This work provides critical insights for identifying biomarkers and therapeutic targets aimed at enabling early intervention strategies, ultimately contributing to innovative approaches for slowing AD progression and enhancing neuronal resilience.

Funding Source: YY was supported by Guangzhou Postdoctoral Supporting fundings, Guangdong Postdoc researcher fundings, Huangpu Postdoc Researcher fundings and CAS Special Research Assistant fundings.

W1225

AN IPSC-BASED HIGH THROUGHPUT DRUG SCREENING PLATFORM FOR ALZHEIMER'S DISEASE

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Alzheimer’s disease (AD) is a complex neurodegenerative disease. However, lacking suitable cellular models for drug candidate screening and validation, and lacking enough diversity in existing compound libraries, hindered the development of AD drug discovery. To overcome those hurdles, we generated a series of iPSC-based AD disease models using the cutting-edge genome editing technology mediated by CRISPR/Cas9. We selected an iPSC line with BACE1 overexpression (BACE1 iPSC) for further characterization and drug screening. We differentiated BACE1 iPSCs into neuronal cells and tested the secreted A**β**40 and A**β**42 levels in the culture supernatant. In addition, we manufactured neural progenitor cells (NPCs) derived from these genetically modified iPSCs in a large scale, and then further differentiated them into AD-relevant neuronal cells for high throughput drug screening. Our results showed that, BACE1 iPSC retain pluripotency and normal karyotype after genetic modifications. The expression levels of BACE1 were around 80-100 folds higher in BACE1 iPSCs and their derived neural progenitor cells and neurons compared to their relative control. The expression level of BACE1 encoded protein, β-secretase 1, is around 300-600 ng/mg total protein of BACE iPSC derived NSC and generic neurons, which is significantly higher than their control counterpart. And the ectopic expression level of β-secretase 1was stable during neuronal induction from NSCs. Interestingly, the Aβ42 and Aβ40 level were increased significantly in NSCs, or neurons derived from BACE1 iPSCs compared to control cells. Furthermore, we established a workflow for generating NPCs and generic neurons from BACE1 iPSC, and these cells can be used to screen Aβ42 lowering drugs in vitro. With this in vitro platform, we conducted a high-throughput screening of natural chemical compounds and herb extracts derived from traditional Chinese medicines (TCM) and get 6 out of around 100 TCM that can significantly downregulate Aβ42 level. We further confirmed that 2 of these 6 compounds can reduce Phospho-Tau level in BACE1 iPSC-derived generic neurons. Our results indicate that our AD model offers a golden opportunity for development of novel therapeutics for treatment of AD.

W1227

APPLICATION OF NEUROMUSCULAR JUNCTION-CHIPS TO CHARACTERIZE SYNAPTIC RNA DYSREGULATION IN ALS

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by the loss of motor neurons, resulting in muscle paralysis and eventual death. Emerging evidence suggests the importance of local RNA processing and protein homeostasis at neuromuscular junction (NMJ) synapse may play a critical role in disease progression. To explore this, we developed microfluidic "neuromuscular junction-chips," an innovative in vitro model system combining motor neurons and muscle cells derived from patient-induced pluripotent stem cells (iPSCs). This compartmentalized system provides unique opportunities to answer specific questions that are focused on local signaling at the NMJ. Our findings reveal critical components of the pre-mRNA splicing pathway in axons of iPSC-derived motor neurons and NMJ synapses in vivo, providing an intriguing link that splicing defects in ALS may be localized to NMJs. In C9ORF72-ALS, we observed compromised spliceosome pathways due to interactions with toxic C9 repeat RNA, leading to neurite degeneration and TDP-43 mis-localization—a hallmark of ALS pathology. We have recently developed a state-of-the-art single-cell proteomics approach to measure the proteome profile at the true single-cell resolution even in complex cell mixtures. To deepen our understanding of RNA-protein homeostasis, we applied this cutting-edge single-cell proteomics approach to measure proteomic and transcriptomic changes during motor neuron differentiation at single-cell resolution. This innovative strategy paves the way for future studies using NMJ-chips to quantitatively compare synaptic pre-mRNA processing and protein homeostasis at NMJs versus neuronal somas, and to unravel their alterations in C9-ALS.

Funding Source: Postdoctoral Fellowship, California Institute for Regenerative Medicine, EDUC4-12751.

W1229

ASOS TO HERV-K ATTENUATE PRODUCTION OF PHOSPHORYLATED TDP-43 IN IPSC-DERIVED MOTOR NEURONS FROM ALS PATIENTS WITH C9ORF72 EXPANSION

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The C9orf72 repeat expansion is the most common genetic cause of Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia. A hallmark of these diseases is the cytoplasmic aggregation

of TDP-43 in neurons but the underlying mechanisms by which the two are linked is poorly understood. Recent studies have shown that human endogenous retrovirus HERV-K reactivation in ALS and FTD. In vitro and in vivo studies show that expression of HERV-K is toxic to motor neurons. Hence we determined if HERV-K played a role in C9orf72 repeat expansion mediated pathogenesis in motor neurons. We generated iPSCs from peripheral blood hematopoietic progenitor cells of ALS patients with C9orf72 repeat expansions and controls and further differentiated them into motor neurons. We found HERV-K env expression in the motor neurons of the ALS patients using RT-PCR and increased total and phosphorylated TDP-43 production using Western-blot assay. Treatment with antisense oligonucleotides (ASO) targeting C9orf72 mutation decreased the HERV-K env activation in the C9orf72 motor neurons ($P< 0.05$). Further, treatment with ASOs targeting C9orf72 mutation or HERV-K env attenuated phosphorylated TDP-43 production in C9orf72 motor neurons ($P< 0.05$). The results indicate that C9of72 repeat expansion causes at least partially HERV-K activation and the subsequent TDP-43 neuropathology in motor neurons which can be attenuated using ASOs. The C9orf72 ALS-derived motor neurons reproduce TDP-43 neuropathology in ALS and therapeutic strategies targeting HERV-K could be useful in this patient population.

Funding Source: This project is supported by NINDS intramural fund.

W1231

BISPHENOL A PROMOTES NEURODEGENERATION VIA EXOSOME-MEDIATED MITOCHONDRIAL DYSFUNCTION

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Bisphenol A (BPA), an industrial chemical commonly found in food and water containers as well as various consumer products, acts as an endocrine disruptor due to its estrogen-mimicking properties. BPA exposure leads to its accumulation in the brain, resulting in mitochondrial dysfunction, including increased mitochondrial reactive oxygen species (mtROS) production and dysregulated mitophagy. This study investigated how these disruptions alter exosome secretion of fragmented mitochondria and contribute to neurodegenerative phenotypes. Initially, we observed in the human neuroblastoma cell line SH-SY5Y that high-dose BPA exposure (100 μM) augmented mtROS and mitophagy, which could be regulated by the abatement of membrane estrogen receptor (ER) activity. Notably, BPA significantly enhanced exosome secretion containing mitochondrial fragments, evidenced by elevated levels of TOMM20 and mitochondrial RNAs related to oxidative phosphorylation (ND1-ND6, COX1-COX4) in SH-SY5Y cells. However, BPA reduced exosome secretion from the mouse hippocampal neuronal cell line HT-22 and primary microglia, which was increased in mouse primary astrocytes. In a co-culture system between mouse primary astrocytes and HT-22 cells, BPA increased mitochondrial secretion into the media from HT-22 cells, which were subsequently absorbed by astrocytes. Additionally, BPA exposure significantly elevated GFAP protein levels in astrocytes, indicating astrocytic activation. In conclusion, these findings suggest that BPA increases mitochondrial secretion via exosomes, thereby diminishing mitochondrial content in neurons and contributing to neurodegeneration.

W1233

BREATHING LIFE INTO STEM CELLS: GENERATING CENTRAL RESPIRATORY NEURONS TO STUDY INVOLUNTARY BREATHING

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The rhythm of breathing is vital for mammalian life, required for daily activities and during sleep. The central nervous system (CNS) cells responsible for initiating and autonomously maintaining breathing rhythm are the preBotzinger complex (preBotC) neurons, residing in the brainstem. In rodents, preBotC development requires an orchestrated rostro-caudal signalling to achieve the correct rhombomeric regional identity within the hindbrain, as well as the correct dorso-ventral patterning. Although the biology of these neurons has been investigated in rodent studies, very little information exists from investigations in human systems. Here, we aimed to establish a novel, small molecule-based, human pluripotent stem cell (hPSC) protocol to differentiate regionally-specified preBotC neurons for advancing our understanding of human-specific features of the preBotC. Following dual-SMAD inhibition for neural induction in vitro, we found that retinoic acid was necessary to upregulate the expression of HOX4 gene, required for the correct rostro-caudal rhombomeric patterning. Finetuning of dorso-ventral patterning of our cells successfully generated progenitors that expressed DBX1, a key marker of respiratory neural progenitors in the hindbrain. Maturation of these progenitors gave rise to neurons expressing markers consistent with preBotC identity and are electrophysiologically functional as demonstrated by multi-electrode array recordings. This protocol represents one of the first to generate human preBotC neurons by modulating rostro-caudal and dorso-ventral patterning of human PSCs. Establishing this hPSC protocol of preBotC neuron differentiation will facilitate future gene editing and disease modelling studies to further understand breathing and the rhythm of breathing in humans, as well as therapeutically supporting patients living with CNS-related respiratory disorders.

W1235

CATALPOL PROMOTES THE GENERATION OF CEREBRAL ORGANOIDS WITH ORGS THROUGH ACTIVATION OF STAT3 SIGNALING

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The generation of human cortical organoids containing outer radial glia (oRG) cells is crucial for modeling neocortical development. Here we show that Catalpol, an iridoid glucoside derived from Rehmannia glutinosa, significantly enhances the generation of cerebral organoids with expanded oRG populations and increased neurogenic potential. Catalpol-treated organoids exhibited thicker ventricular zone/subventricular zone (VZ/SVZ) and outer subventricular zone (oSVZ) regions, with increased numbers of SOX2 + HOPX+ and SOX2 + TNC+ oRG cells and elevated expression of oRG markers HOPX and FAM107A. We found that Catalpol promoted oRG generation through non-vertical divisions of ventricular radial glia (vRG) cells, indicating enhanced oRG generation via asymmetrical divisions. Furthermore, we demonstrated that Catalpol augmented oRG cell numbers through activation of the STAT3 signaling pathway. These findings highlight Catalpol's potential in promoting the generation of cerebral organoids with expanded oRG populations and increased neurogenic potential through STAT3 activation, offering new insights into neocortical development modeling.

Funding Source: This work was supported by Basic Science Research Program (NRF-

2022R1A6A1A03053343, 2021M3E5E5096464 and RS-2023-00248303) and by the ABC-based Regenerative BioTherapeutics grant (RS-2024-00433755).

W1237

CHANGES IN BEHAVIOR, ORGANIZATION, AND STATE OF HIPSC-DERIVED ENDOTHELIAL CELLS EXPOSED TO SHEAR STRESS REVEALED BY LIVE CELL MICROSCOPY AND QUANTIFICATION

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Endothelial cells (ECs) play critical roles in the vasculature and are subject to shear stress as blood flows through the vessels they line, which influences their structure, function, and morphology. Here, we use the alignment and re-alignment of human induced pluripotent stem cell-derived ECs (hiPSC-ECs) under fluid shear stress as a model system for investigating cell state transitions in a holistic manner. We differentiated endogenously tagged hiPSC lines from the Allen Cell Collection (www.allencell.org) into hiPSC-ECs and performed 3D, live cell imaging as they respond to fluid shear stress to capture changes in their morphology, behavior, and organization. We found that hiPSC-ECs exhibited distinct responses to different magnitudes of applied shear stress. Under low shear stress (0.8-6 dyn/cm2) the hiPSC-ECs elongated, collectively migrated upstream, aligned parallel relative to the direction of fluid flow, and developed VE-cadherin puncta localized to the contacts of lateral cells. When subjected to high shear stress (15-25 dyn/cm2), the cells aligned perpendicular to the direction of flow, migrated in all directions, and rarely developed VE-cadherin puncta. When the magnitude of shear stress is switched from high to low or vice versa, hiPSC-ECs changed their collective migration behavior and organization accordingly. To quantify the transition

between these different cell states that occur in response to the distinct shear stress magnitudes, we developed a segmentation-free machine learning framework to extract single-cell features from 2D time-lapse image data. Using these features, we are implementing recently developed machine learning methods for stochastic dynamics to infer the underlying dynamical rules that govern the observed ensemble of single-cell behaviors. We are initially developing this workflow for images of fluorescent VE-cadherin-tagged hiPSC-ECs and plan to expand to image data of other subcellular structures and image modalities. We expect that these analyses of how the environmental cue of shear stress influences the cell state of hiPSC-ECs will improve our understanding of endothelial and cell biology and demonstrate the utility of studying cell state transitions in a holistic manner.

W1239

CHARACTERISTICS OF ELECTRICAL ACTIVITIES AT SINGLE-CELL RESOLUTION IN HIPSC-DERIVED NEURONS WITH A NOVEL FIELD POTENTIAL IMAGING METHOD ON HD-CMOS-MEA

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The technology for measuring the electrical activity of the nervous system is essential for understanding neurological diseases, drug discovery development, and toxicity evaluation of compounds. Recent development of microelectrode array (MEA) with large amounts of electrodes at a high density provides a high spatio-temporal resolution at the single-cell level, and noninvasive measurements of large areas which increase insights on underlying neuronal function. In the present study, we used a HD-CMOS-MEA with 236,880 electrodes covering a wide sensing area in presenting a detailed and single-cell-level neural activity analysis platform. Samples of human iPSC-derived cortical neurons, sensory neurons, and human brain organoids were prepared on CMOS-MEA and the electrophysiological activity were measured before and after drug administration. A novel field potential imaging analysis was performed with optimization upon different samples. For cultured human iPSC-derived cortical neurons, field potential imaging analysis revealed that the synaptic strength was influenced by compounds based on single-cell time-series patterns. With both network neural analysis parameters and single neuron analysis parameters, several novel information in evaluating the drug responsiveness of neural networks was revealed successfully. For cultured human iPSC-derived sensory neurons, we succeeded in classifying neurons with different responses to TRP channel agonists based on single neuron firing patterns. Furthermore, axonal conduction characteristics in each sensory neuron could be analyzed. After administration of anticancer drugs, a decrease in axonal conduction velocity was detected which indicated peripheral neuropathy. Finally, we successfully detected the network activity of brain organoids and assembloids, and extracted the differences from diseased organoids and transitions to compounds. These above results provide new understanding of the basic mechanisms of brain circuits in vitro and ex vivo on human neurological diseases, and show the possibility of the current field potential imaging technology utilization for drug discovery, and compound toxicity assessment.

Funding Source: The grant of collaborative project with Sony semiconductor solutions Inc. Japan Agency for Medical Research and Development (AMED).

W1241

CHARACTERIZING AND ELUCIDATING THE ROLE OF SLC35A2 IN HUMAN CORTICAL BRAIN DEVELOPMENT

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Focal Cortical Dysplasia Type Ia (FCD 1a) is a category of drug-resistant epilepsy that is difficult to diagnose and frequently requires surgery for treatment. A common hallmark of FCD 1a is hyper-columnar radial organization of neurons. Interestingly, the X-linked gene SLC35A2, which has been implicated in a variety of rare brain disorders, has been found in over 29% of FCD 1a cases. SLC35A2 encodes for a UDP-galactose translocator, but its specific role in cortical development and epilepsy remains unknown. We seek to model malformations in cortical development caused by SLC35A2 in order to understand the role of the gene in cortical development. Given the importance of proteoglycans which play a critical role in neuronal migration during development, we hypothesize that SLC35A2 is crucial to the proper production of proteoglycans and neuronal migration in the developing brain. Furthermore, we hypothesize that somatic mutations in SLC35A2 must occur in neural stem cells whose progenitors will go on to abnormally migrate and generate the disease phenotype. To test this hypothesis, we generated a human induced pluripotent stem cell (iPSC) line with the SLC35A2 locus knocked out and subsequently generated in vitro cortical organoids. We found that SLC35A2 is required for the generation of neural stem cells, and brain organoids generated from the SLC35A2KO iPSC line exhibited reduced ability to form neural rosette structures when examined using immunofluorescence (IF). We then used lentiviral mediated delivery of SLC35A2 short hairpin RNA (shRNA) to the cortical organoids to accurately model the induction somatic mutation during development. This is a crucial first step to model the cortical malformations that arise due to SLC35A2 mutations. These results will enable further investigation into the specific mechanism of SLC35A2 causing FCD 1a. In a broader context, this study can offer insights into human brain development and the function of SLC35A2, potentially guiding new therapeutic approaches for related glycosylation related disorders.

Funding Source: NSF GRFP.

W1243

COMBING SCALABLE ORGAN CHIP WITH DEEP-LEARNING IMAGING ANALYSIS FOR DISEASE MODELING, DRUG TESTING, AND PRECISION MEDICINE

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The traditional drug development process, relying on two-dimensional (2D) screening and animal models before human trials, is time-consuming, costly, and often fails to predict clinical outcomes

accurately. To bridge this translational gap, we have developed OC-Plex, a scalable Organ Chip (OC) platform optimized for automation and imaging-based assays. We demonstrate its applications in modeling cell-cell interaction and mechanobiology in the tumor microenvironment. We also described the establishment of a Liver Chip model for drug-induced liver injury, which achieved over 90% predictive accuracy in a validation study, surpassing the performances with 2D and animal models. Finally, we showed the feasibility of cancer precision medicine by combing a patient-derived Cancer Chip model with deep-learning imaging analysis. The integration of scalable OC technology with artificial intelligence represents a transformative approach to modernizing drug discovery, improving predictive validity, and accelerating clinical translation.

W1245

CONSTRUCTION OF SCA3 CEREBELLAR ORGANOID MODEL AND MECHANISM OF NEURONAL SELECTIVE VULNERABILITY

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Developing effective organoid models for cerebellar diseases is crucial for understanding disease mechanisms and exploring new treatments. Spinocerebellar ataxia type 3 (SCA3) is a genetic neurodegenerative disease affecting the cerebellum with no effective treatment available. In our study, we used control and SCA3 induced-pluripotent stem cells to create human cerebellar organoids, following an updated 2024 protocol that improves cellular purity. These organoids were assessed for their development stages, cellular composition, and SCA3 pathology markers to evaluate their suitability for disease modeling. Through immunofluorescence staining, the organoids displayed markers of cerebellar neuron progenitors (PTF1A, ATOH1, SKOR2) and mature neurons (Calbindin, BARHL1, MAP2, NEUN, SYN1). Electrophysiological activity detected by multi-electrode array (MEA) revealed spikes and burst networks, indicating an integrated functional neuronal network. Crucially, immunofluorescence staining showed the presence of nuclear inclusion bodies, a hallmark of SCA3 pathology. Single-cell RNA sequencing is underway to further characterize the cellular composition and gene expression profiles within these organoids, providing deeper insights into cellular heterogeneity and mechanisms of neuronal vulnerability in early SCA3. Overall, our findings suggest that these organoids successfully replicate key aspects of SCA3 pathology and possess cerebellar characteristics, making them valuable for studying disease mechanisms and testing potential treatments for cerebellar diseases.

W1247

CONVERGENT TRANSCRIPTIONAL DYNAMICS OF MICROGLIA IN THE MOUSE SPINAL CORD DURING AGING AND ALS

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Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disease resulting in paralysis and death three to five years after diagnosis. It is a late-onset disease that typically presents in older populations, usually between the ages of 51 and 66, with aging being its greatest risk factor. However, cell models do not fully recapitulate aged conditions and often require external stressors to display disease phenotype. Thus, it is crucial to understand the transcriptomic events that occur during aging in order to modulate these effects in cell models. We have previously shown that aging pathways are disrupted in ALS spinal cords and spinal motor neurons. However, the anatomical and cell-specific aging within the spinal cord and its contributions to ALS pathogenesis are still unclear. In this study, we have created a single-nuclei transcriptomic atlas of mouse spinal cords from wild type (WT) and ALS mice carrying the SOD1G93A transgene. We profiled WT mice from embryonic day 13.5 up to 800 days old, and we profiled the ALS littermates from the same life stages up until they reached their paralytic endpoint at 160 days old. Our findings allowed us to identify dynamic changes in the transcriptome of each cell type during ALS progression and aging. Construction of gene co-expression networks also allowed us to define cell-specific aging and their contribution to ALS. Further analysis of regulatory networks revealed transcription factors that may drive these aging and ALS processes. These insights provide a framework for further studying factors driving ALS disease progression and offer valuable targets for faithfully modeling aging in in vitro models, developing ALS therapies, and guiding comparative aging studies.

Funding Source: California Institute for Regenerative Medicine.

W1249

CORTICOSPINAL ASSEMBLOID MODELS REVEAL EARLY TDP-43 PATHOLOGY AND NETWORK DYSFUNCTION IN AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by progressive motor neuron degeneration and TDP-43 proteinopathy. While postmortem studies provide insights into end-stage pathology, they fail to capture early disease dynamics and cellular changes that precede neurodegeneration. To address this, we developed corticospinal assembloids by integrating cortical and spinal cord spheroids derived from TDP-43 mutant and isogenic control iPSCs. Although assembloid models remain relatively immature compared to adult human brain, they offer a unique opportunity to investigate early pathological events in a physiologically relevant context. Single-cell RNA sequencing identified a novel mutant-specific cellular cluster, suggesting transcriptional reprogramming associated with TDP-43 pathology. Longitudinal electrophysiological recordings further revealed progressive network dysfunction, synaptic deficits, and altered excitability in mutant assembloids. These findings highlight the potential of assembloids to model early-stage ALS pathology and provide a powerful platform for identifying disease-modifying targets and evaluating therapeutic interventions before irreversible neurodegeneration occurs.

W1251

CRISPR/CAS9 MEDIATED CORRECTION OF GENETIC MUTATIONS IN GBA, VPA35 and LPR10 OF IPSCS FROM PATIENTS WITH PARKINSON'S DISEASE

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Patient-specific induced pluripotent stem cells (iPSCs) have great therapeutic potential for neurodegenerative diseases such as Parkinson' disease (PD). However, dopaminergic neurons (iPSC-DAN) derived from the iPSCs of PD patients with genetic mutations have decreased function and survival after these iPSC-DANs are transplanted to animal models or the PD patients. Therefore, the mutations in the iPSC-DAN have to be corrected before these cells are translated to the clinical application. we have generated three iPSC lines with mutations of GBA, VPA35 and LPR10 by electroporating the transgene-free episomal plasmids expressing OCT3/4, SOX2, KLF4, L-MYC, and LIN28 into the skin fibroblast cells from patients with PD. These three iPSC lines are carrying the mutations of GBA RecNciI mutation (c.1448T > C, c.1483G > C, and c.1497G > C), VPS13A mutation (c. 4282_4289delinsA), and LRP10 mutation (c.688C > T) respectively. We have also modified the protocol to efficiently differentiate iPSCs to iPSC-DANs with more than 60% cells expressing TUJ1/TH by adding the small molecules to different stages of differentiation by regulating the dopaminergic lineage commitment and maturation. To correct mutations of GBA, VPA35 and LPR10 in three iPSC lines, we have used the CRISPR/Cas9 editing to correct these mutations in the iPSC lines. The GBA mutation was corrected and was confirmed by sequencing analysis. We are comparing the phenotypes and mitochondrial functions of the iPSC-DANs carrying GBA mutation to that without GBA mutation-corrected. Next we are going to transplant the iPSC-DANs from normal individuals, patient carrying GBA mutation and corrected GBA mutation to the rat PD model to see whether or not iPSC-DANs with corrected GBA mutation has similar ability as that from normal individuals to improve the motor deficits of PD rats.

Funding Source: The Key Basic Research project (ZR2019ZD39). Natural Science Foundation of Shandong Province, China.

W1253

CT PROMOTES SPHEROID FORMATION AND EXTRACELLULAR VESICLE SECRETION OF HUMAN UMBILICAL CORD MESENCHYMAL STEM CELLS AND ITS ROLE IN SKIN INJURY TREATMENT

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Human umbilical cord mesenchymal stem cells (huc-MSCs) are a promising source for therapeutic applications due to their pluripotency. Extracellular vesicles (hucMSC-EVs) secreted by huc-MSCs contain bioactive molecules and have shown therapeutic potential. However, conventional 2D

cultures face challenges like limited space, inconsistent cell quality, and inefficient use of culture area. Scaffold-free MSC spheroid cultures offer a more in vivo-like environment, preserving cell characteristics and optimizing space. Despite these advantages, spheroid culture faces challenges in spheroid formation and EV yield. This study explores how combining Chroman 1 and Trans-ISRIB (CT) enhances MSC spheroid formation, EV secretion, and their therapeutic potential for skin injury. The CT combination was screened for its ability to promote MSC spheroid formation and EV secretion using CEPT. Transcriptome sequencing and PCR were used to explore the mechanisms behind CT's effects on spheroid formation. MSC EV-related gene expression was analyzed, and EV yield was quantified by nanoparticle tracking analysis (NTA). The therapeutic effects of CT-3D MSC-EVs on skin injury were assessed in vitro using HUVECs and fibroblasts, and in vivo in a mouse skin injury model. CT significantly promoted MSC proliferation and increased paracrine factor expression. In spheroid cultures, CT enhanced spheroid formation and upregulated EV-related (CD9, CD81, CD63, Alix) and stemness (Nanog, Sox2, Oct4) genes. Transcriptome analysis and PCR revealed that CT promoted spheroid formation by upregulating cell adhesion molecules. Scratch assays demonstrated that CT-3D MSC-EVs enhanced migration of HUVECs and fibroblasts, outperforming 2D-MSC-EVs. Tube formation assays confirmed that CT-3D MSC-EVs better promoted HUVEC tube formation. In vivo, CT-3D MSC-EVs accelerated skin wound healing and dermal collagen regeneration in mice. The CT combination enhances MSC spheroid formation and EV secretion by upregulating cell adhesion molecules, improving the therapeutic effects of spheroid-derived MSC-EVs for skin injury. These findings highlight the clinical potential of MSC-EVs in therapeutic applications

W1255

DECIPHERING RETINOBLASTOMA TUMORIGENESIS WITH RB1 KNOCKOUT CONE REPORTER RETINAL ORGANOIDS

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Retinoblastoma, the most prevalent childhood intraocular malignancy, originates from maturing cone photoreceptor precursors with biallelic RB1 inactivation. In explanted fetal retina, pRB-depleted post-mitotic cone precursors proliferate, followed by a 3-5 month premalignant indolence phase before retinoblastoma-like masses emerge at tissue ages mirroring in vivo disease. Research on this transition is limited by fetal tissue availability, but CRISPR engineered retinal organoids (ROs) provide a promising alternative. This study introduces a robust organoid platform to model and investigate the retinoblastoma indolence-malignancy transition. We generated cone-reporter iPSC lines through CRISPR knock-in of EGFP-P2A at the GNAT2 locus. A second round of CRISPR editing produced homozygous RB1 knockout. Chimeric RB1+/+ ROs and RB1-/- RBROs were generated from edited iPSCs mixed with unedited parental iPSCs. ROs and RBROs were embedded in hydrogel and live-imaged episodically to track EGFP+ cone proliferation dynamics. Deep full-length scRNA-seq was carried out on FACS isolated EGFP+ RB1+/+ and RB1-/- cones at various timepoints. In RB1 WT ROs, EGFP specifically, robustly and innocuously labeled immature and mature cones. In RBROs, bi-weekly live confocal imaging revealed initial

EGFP+ RB1-/- cone proliferation followed by a pre-malignant indolence phase starting at ~d150. The majority of the initially proliferating cones were Ki67-negative with some adopting mature cone morphology. Nascent retinoblastoma-like foci co-expressing EGFP, cone markers, and Ki67 formed after ~d280, a tissue age that equates to the first post-natal month when early retinoblastomas typically emerge. Single cell transcriptomics of RB1-/- cones from multiple tumorigenesis stages showed distinct molecular signatures of proliferation, differentiation and stress cell states and suggest a role of p53 pathway in indolence entry and escape. In summary, we established a human retinoblastoma organoid model that faithfully recapitulates the cell-of-origin and timing of multi-step retinoblastomagenesis. This model revealed distinct molecular signatures at each tumorigenesis stage and highlighted altered expression of a p53 pathway regulator during the retinoblastoma indolence-malignancy transition.

Funding Source: 2R01CA137124-12A1.

W1257

DECRYPTING THE IMPACT OF DEFECTIVE PRDM13 DURING CEREBELLAR AND SPINAL DEVELOPMENT IN HUMAN-BASED ORGANOID MODEL

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The functioning of the human nervous system relies on the emergence, during development, of a highly organized cellular diversity. Even slight disruptions in its cellular composition can have major consequences, contributing to neurodevelopmental disorders (NDD). Through whole-genome genomics approaches, we recently discovered recessive mutations in PRDM13 in patients affected by a rare and severe NDD, marked by cerebellar and brainstem hypoplasia. PRDM13 encodes a neural-specific member of the PRDM protein family of epigenetic regulators, known to modulate cell fate choices decision in various developmental contexts. In mouse models, Prdm13 was previously shown to impact cell fate choices in the spinal cord. Our functional analyses in the zebrafish model indicate that loss-of-function of prdm13 also impairs the generation of cerebellar inhibitory neurons, notably Purkinje cells, and induces profound perturbations in neuronal cell fate specification in the brainstem. However, the molecular mechanisms underlying these defects remain to be deciphered, in particular in the human context. Leveraging a cytidine base editing approach, we obtained isogenic clones of human induced pluripotent stem cells carrying a nonsense mutation in PRDM13 and derived cerebellar and spinal organoids, using in-house optimized protocols. We are currently characterizing these mutant organoids, using immunohistochemistry, bulk and single-cell transcriptomics. Our preliminary analyses support a multifaceted role for PRDM13 in orchestrating cell fate specification during human posterior brain development. These models will also provide a valuable opportunity to elucidate, within a human

context, the epigenetic mechanisms involving PRDM13 that regulate posterior nervous system development.

Funding Source: Works supported by funds from INSERM, CNRS, Université Paris Cité, Institut Imagine and Institut Jacques Monod. This project benefits from ANR and the patient association CSC grants.

W1259

DEFINING THE MOLECULAR BASIS OF CARDIOMYOPATHY USING HUMAN PLURIPOTENT STEM CELL MODELS

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Cardiomyopathies are diseases of the heart muscle. The key to developing therapies that directly target the cause of cardiomyopathies is understanding the molecular mechanisms underlying pathology. To do this we have taken a broad approach profiling clinical samples, mouse models and iPSC derived cardiac cells by both proteomics and transcriptomics. Firstly, we performed a large-scale analysis of single nuclei RNA-seq data sets from patients with dilated cardiomyopathy (DCM), combining publicly available datasets with our paediatric samples. Our findings suggest that defined fetal gene re-activation in DCM is not restricted solely to CMs but is broadly re-engaged in non-CM populations, including cardiac fibroblasts. We have evaluated these gene networks in hiPSC-derived cardiac cells, encompassing various genetic forms of DCM. Through this evaluation, we identify a conserved set of fetal genes that may represent the core functional gene network driving DCM. These core genes could provide critical insights into the underlying molecular mechanisms driving DCM from the earliest stages of cardiogenesis and potentially serve as targets for therapeutic interventions. In parallel, we have used proteomics to profile hypertrophic cardiomyopathy (HCM). Misfolded and aggregated sarcomeric proteins in heart muscle cells disrupt cellular functions and contribute to HCM disease progression. Therefore, protein quality control mechanisms, such as the ubiquitin-proteasome system (UPS) and the autophagy-lysosomal pathway, play a crucial role in maintaining sarcomere homeostasis and contraction in cardiac muscle cells. Our clinical and in vitro studies identified a signalling nexus at the M-Band of the sarcomere, containing a number of E3 ubiquitin ligases. Deletion of one of these E3 ubiquitin ligases, TRIM55, compromises contraction demonstrating the UPS is necessary for normal contraction. Mass spectrometry and RNA sequencing analysis revealed sarcomere organisation and muscle contraction was altered in TRIM55 deficient cardiomyocytes. Thus, we have identified a diverse array of molecular networks underlying cardiomyopathies.

Funding Source: Novo Nordisk Foundation Center for Stem Cell Medicine, reNEW (Grant number NNF21CC0073729).

W1261

DERIVATION OF HUMAN POST-MITOTIC CARDIOMYOCYTES FROM TETRAPLOID INDUCED PLURIPOTENT STEM CELLS

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Human induced pluripotent stem cell (iPSC)-derived cardiomyocytes (iPS-CMs) have great promise for disease modeling, drug development, and regenerative medicine. iPS-CMs have already been used for drug development. Transplantation therapies using iPS-CM-engineered heart sheets have also advanced to clinical stages. However, the immaturity of iPS-CMs resembling the human fetal state, remains a significant challenge for broader applications. To address this issue, we focused on the polyploidy of human mature cardiomyocytes. While over 80% of human cardiomyocytes become tetraploid during maturation, only 10% of iPS-CMs achieve polyploid using conventional methods. This ploidy difference could limit the maturation of iPS-CMs. To overcome this limitation, we completely changed the differentiation strategy from the conventional scheme by first establishing tetraploid iPSCs (4N-iPSCs) and then differentiating them into cardiomyocytes to replicate the polyploidy of human mature cardiomyocytes. In this study, we successfully established 4N-iPSCs by Sendai virus-mediated fusion of diploid iPSCs. 4N-iPSCs had doubled DNA content within a single nucleus without any chromosomal abnormality. These 4N-iPSCs were then differentiated into cardiomyocytes using a general protocol. Almost all cardiomyocytes derived from 4N-iPSCs (4N-iPS-CMs) were tetraploid, replicating the polyploidy of adult human cardiomyocytes. Gene expression analysis revealed reduced expression of mitotic genes in 4N-iPS-CMs compared to diploid iPS-derived cardiomyocytes (2N-iPS-CMs), indicating that 4N-iPS-CMs were more mature, post-mitotic cardiomyocyte-like cells. Besides, the mitochondrial content was higher in 4N-iPS-CMs, suggesting an increased capacity for ATP production. Functionally, 4N-iPS-CMs exhibited higher contractile force and faster upstroke velocity than 2N-iPS-CMs based on electric impedance measurement. Furthermore, when tested for sensitivity to Terfenadine, a hERG channel blocker, 4N-iPS-CMs demonstrated greater resistance. These findings indicate that 4N-iPS-CMs replicate the polyploidy of adult human cardiomyocytes, resulting in greater maturity compared to conventional iPS-CMs. We propose that 4N-iPS-CMs are a promising platform for drug discovery and regenerative medicine.

Funding Source: JSPS, Grant-in-Aid for Scientific Research (B) (24K02028), and for JSPS Research Fellows (24KJ0995). JCRF, Bayer Scholarship for Cardiovascular Research. SENSHIN Medical Research Foundation. TERUMO Life Science Foundation.

W1263

DEVELOPMENT AND CHARACTERIZATION OF CHICKEN LUNG ORGANOIDS WITH PROSPECTS OF MODELING HIGHLY PATHOGENIC AVIAN INFLUENZA IN VITRO

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Avian influenza viruses typically reside in wild waterfowl, inducing no clinical disease. When these low pathogenic strains spill over to domestic poultry, they evolve into highly pathogenic strains (HPAIV), with devastating effects on the industry. Spillover events have been increasingly reported in mammals (including humans), causing severe disease with high mortality. There is, therefore, a critical need to understand the viral tropism, host immune responses, and replication dynamics of HPAIVs in various avian species. 2D cell cultures, which have been used to study HPAIVs, have shortcomings, including their lack of cellular heterogeneity. 3D organoids, composed of multiple cell types, may represent a more faithful model to study mechanisms of HPAIV dynamics and pathogenicity. Adult stem cell-derived lung organoids from three-week old SPF White Leghorn chickens were developed and their transcriptome analyzed via bulk and single-nuclei RNA sequencing. Morphology was characterized using brightfield imaging, H&E, immunohistochemical (IHC), immunofluorescent, and special staining in tissues and organoids. Three organoid lines were established, passaged (n >10), and cryopreserved. Histological staining revealed columnar, cuboidal, squamous, and acidic mucin-producing cells, representing regions throughout the lung. Single-nuclei RNA sequencing identified epithelial and fibroblast cell populations in the culture, confirmed with IHC. Epithelial cells were further resolved to be either cycling or non-cycling. Bulk RNA sequencing revealed highly conserved gene expression between organoids and parent tissues. Furthermore, similar expressions (in TPM) of both lung-relevant and virus-related genes were noted. This study established the first chicken lung organoids derived from adult stem cells. RNA and protein expression in the avian lung organoids and parent tissues suggest organoids can be used to model epithelial cell populations in birds for HPAIV studies.

Funding Source: Internal Funding UGA.

W1265

DEVELOPMENT OF A FULLY HUMAN GLIOBLASTOMA-IN-BRAIN-SPHEROID MODEL FOR ACCELERATED TRANSLATIONAL RESEARCH

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In glioblastoma (GBM) the intricate interplay between tumor cells and the surrounding microenvironment plays a crucial role in tumor progression, invasion, and therapeutic resistance. So far, studying these interactions in a controlled and representative model system has been challenging. Here, we present the development of hGliCS, a human glioma-cortical spheroid model that allows the elucidation of the biology of GBM cells and their interactions with a human-specific brain-like microenvironment and neurons. GBM cells efficiently invade the cortical spheroids, forming a well-connected network of communicating cells. The heterogeneous cellular states of the GBM cells within this model closely resembled findings previously observed in glioblastoma patients and in mouse xenografts. In contrast to the substantial changes observed in the tumor cell population, the impact of the GBM cells on the neurons was minimal. Finally, we further demonstrate the suitability of hGliCS to test and validate compounds targeting tumor-specific neurobiological features, which could easily be adapted to high-throughput conditions.

W1267

DEVELOPMENT OF AN HD-CMOS–MEA–BASED METHOD FOR CARDIOTOXICITY DETECTION AND MECHANISM-OF-ACTION PREDICTION IN HUMAN IPSC-DERIVED CARDIOMYOCYTES USING BEAT PROPAGATION PATTERNS

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In new drug development, cardiotoxicity—particularly QT prolongation—often leads to clinical trial termination or market withdrawal. Although the ICH S7B guideline focuses on hERG blockade, it is insufficient for multi-ion channel mechanisms. The CiPA initiative aims for more comprehensive analyses, but challenges remain with complex compounds. In this study, we employed an HD-CMOS-MEA platform equipped with 240,000 densely arranged microelectrodes to measure human iPSC-derived cardiomyocytes. For the pharmacological tests, we selected compounds and concentrations whose toxicity could not be fully captured by conventional MEA, as well as negative compounds—14 in total—and recorded their extracellular field potentials. The high resolution of the HD-CMOS-MEA system allows tens of electrodes to record the activity of a single cell, enabling us to construct 17 parameters from the acquired data, including the number of initiation sites, variability of initiation site positions, conduction velocity, and propagation area. In the case of isoproterenol, a β1-adrenergic receptor agonist, we detected a specific increase in the number of initiation sites. Mexiletine, a Na^+ channel inhibitor, led to a marked decrease in conduction velocity, whereas the hERG channel blocker E4031 caused a reduction in propagation area. Furthermore, we identified distinct conduction velocities and propagation patterns according to the mechanism of action of each compound, suggesting that HD-CMOS-MEA–based cardiotoxicity assessment may sensitively capture channel-specific activities at different concentrations, even in compounds with multiple modes of action. Additionally, when human iPSC-derived cardiomyocytes were exposed to doxorubicin at 0.1 μM—a concentration known to exhibit chronic cardiotoxicity—both propagation area and conduction velocity were found to decrease 24 hours post-exposure. Compared with conventional toxicity tests, the HD-CMOS-MEA system demonstrated the ability to detect cardiotoxicity at lower concentrations and over shorter chronic exposure periods. These findings highlight an enhanced level of precision in cardiotoxicity evaluation for drug development and offer

a new platform to elucidate complex interactions among multiple ion channels.

Funding Source: The grant of collaborative project with Sony semiconductor solutions Inc., Japan Agency for Medical Research and Development (AMED).

W1269

DIFFERENTIAL RESPONSE OF INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES TO CHRONIC BETA-ADRENERGIC STIMULATION AND ELECTRICAL PACING IN A MODEL OF EXERCISE-INDUCED LONG QT SYNDROME

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Intense physical activity, while beneficial for cardiovascular health, has been linked to adverse cardiac outcomes in certain predisposed individuals. Exercise-induced long QT syndrome (exiLQTS) is a life-threatening condition identified by our group, characterized by QT interval prolongation and repolarization abnormalities on the ECG that resolve upon cessation of physical activity. To investigate the mechanisms underlying exiLQTS, we generated six human induced pluripotent stem cell-derived (hiPSC) lines from competitive athletes diagnosed with exiLQTS (cases) and healthy controls. Using perforated patch-clamp techniques, multielectrode arrays (MEA), and optical action potential measurements, we performed detailed electrophysiological characterizations of hiPSCs differentiated towards cardiomyocytes (hiPSC-CMs). To mimic beta-adrenergic stimulation taking place during physical activity, hiPSC-CMs were subjected to chronic treatment with a cell-permeable cAMP analog, dibutyryl cAMP (dbcAMP). Additionally, to simulate the sustained elevated heart rate during training, hiPSC-CMs underwent chronic 2 Hz electrical stimulation for 7 days. At baseline, field potential duration (FPD), single-cell action potential (AP) parameters, and AP rate-dependency were similar between groups, except for the spontaneous beat-to-beat interval, significantly longer in hiPSC-CMs derived from exiLQTS cases (2.4 ± 1.04 s) compared to controls (1.83 ± 0.99 s). Following chronic dbcAMP treatment, hiPSC-CMs from exiLQTS cases exhibited significantly prolonged corrected FPD compared to controls (323 ± 60 ms vs 259 ± 85 ms, $p \leq 0.001$ at day 9). Chronic electrical tachypacing resulted in reduced AP duration (APD) in both groups; however, hiPSC-CMs from exiLQTS cases displayed longer APD values under these conditions (459 ± 147 ms vs 378 ± 125 ms, $p = 0.017$). Together, these findings support the feasibility of modeling exiLQTS using hiPSC-CMs and suggest the presence of molecular differences between the two groups. Additional pacing protocols and transcriptomic analyses will further enhance our understanding of this condition.

Funding Source: EU Horizon Europe (PREPARE, No. 101105561 to AK), PRIN 2022 PNRR

H53D23007640001 to LC, Leducq Foundation grant 18CVD05 to PJS, Next Generation EU project No. H45E22001210006 to LS, EU Horizon 2020 (ERA-CVD - EMPATHY No. 298736 to LC).

W1271

DIVERGING AND CONVERGING MECHANISMS IN POLYQ-RELATED DISEASES

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Polyglutamine (polyQ) diseases are a group of nine neurodegenerative disorders caused by the expansion of CAG trinucleotide repeats in specific genes, leading to toxic polyglutamine tracts in the encoded proteins. While their genetic basis is well-established, the molecular mechanisms driving selective neuronal vulnerability remain elusive. Emerging evidence highlights aging and epigenetic dysregulation as critical factors in polyQ disease progression. Spinocerebellar ataxia types 3 (SCA3) and 6 (SCA6) are two polyQ diseases characterized by spinocerebellar ataxias but differ in the functions and expression patterns of the affected proteins, ATXN3 and α1ACT, respectively. These differences, along with shared pathological features, make them ideal models for comparative analysis. In this study, we use stem cells to generate cerebellar brain organoids and investigate the normal and mutant roles of ATXN3 and α1ACT in disease progression. Specifically, we examine their involvement in epigenetic regulation across developmental stages, from stem cells to mature neurons. By identifying shared and disease-specific mechanisms, our work provides novel insights into the molecular mechanism of polyQ disorders. Together, our findings illuminate key molecular pathways driving neurodegeneration in polyQ diseases, offering potential targets for therapeutic interventions that address these debilitating conditions.

W1273

EFFECT OF EXTRACELLULAR VESICLES DERIVED FROM GLYCOLYTIC MESENCHYMAL STEM/STROMAL CELLS ON THE PHENOTYPIC AND METABOLIC REPROGRAMMING OF SYNOVIAL MACROPHAGES IN PATIENTS WITH OSTEOARTHRITIS

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Osteoarthritis (OA) is a chronic inflammatory disease for which no cure exists; only palliative treatments are available. OA is characterized by an exacerbated inflammatory response primarily driven by the activity of synovial macrophages. Mesenchymal stem/stromal cells (MSCs) are well-known for their therapeutic potential; however, alternative strategies are essential to enhance their immunomodulatory properties. We show that glycolytic MSC (MSCglyco) and their small extracellular vesicles (EVs) holds promise for developing new therapies for OA. MSCs were

isolated from the umbilical cords of healthy donors, with informed consent obtained for the use of these samples. MSCs were treated with oligomycin for 24 hours to induce glycolytic metabolism. EVs were isolated from the MSCglyco (MSCglyco-EVs) using ultracentrifugation, quantified by nanoparticle tracking analysis (NTA), and characterized by transmission electron microscopy (TEM) and flow cytometry. Macrophages were isolated from the synovial membranes of OA patients (OAM), for which informed consent was also obtained. EVs from MSCs or MSCglyco were added to the culture media of the OAM at various doses. After 24 hours, OAM were recovered to evaluate EV internalization using qPCR, surface marker expression through flow cytometry, cytokine secretion via ELISA, and glycolytic flux using SCENITH, a flow cytometry assay. The metabolic reprogramming of MSCs does not alter the phenotype or size of the released EVs, nor their capacity of internalization. OAMs treated with MSCglyco-EVs internalize these EVs, leading to an increased expression of CD206, which indicates an anti-inflammatory phenotype, while decreasing HLA-DR and CD86 associated with a proinflammatory phenotype. Additionally, OAMs treated with MSCglyco-EVs exhibits a reduction in the secretion of inflammatory mediators. EVs from metabolically reprogrammed MSC have improved therapeutic properties. We demonstrate that MSCglyco-EVs dose-dependently reduce inflammatory profiles in OAM and increase anti-inflammatory profiles, providing compelling evidence for their enhanced therapeutic effects. This offers a promising approach for the development of novel acellular therapies for OA, focusing on immunomodulation using EVs from metabolically reprogrammed MSCs.

Funding Source: This research was supported by ANID-Chile through Beca Doctorado Nacional folio 21220015; FONDECYT Regular Nº1211353; FONDEF-ID: 21I10194; IMPACT-FB 210024; and TA24I10054.

W1275

EFFECTS OF BACTERIOPHAGES ON STEM CELLS

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Bacteriophages are increasingly recognized as a viable alternative to antibiotics for combating bacterial infections. However, their biocompatibility with mammalian cells is a critical consideration for therapeutic applications. This study aimed to evaluate the cytotoxic effects of commercially obtained bacteriophages on stem cells from the apical papilla (SCAP), a key cell type in dental tissue regeneration. SCAP cells were exposed to two concentrations of bacteriophage preparations (0.5 mL and 1 mL) in vitro, and cellular viability was assessed using the MTT assay. Results demonstrated that bacteriophage exposure at both concentrations resulted in minimal cytotoxic effects, with cell viability remaining above 90 at the tested doses. The findings suggest that the bacteriophages tested are generally biocompatible with SCAP cells at therapeutic concentrations. This study highlights the importance of evaluating the safety of bacteriophages on mammalian cells and supports their potential use in clinical applications. Further research should explore the long-term effects of phage exposure and their interactions with other cell types in regenerative medicine.

W1277

ELUCIDATION OF THE MECHANISMS OF CARDIOMYOPATHY BY USING HUMAN IPS CELL-DERIVED CARDIOMYOCYTES FROM FUKUYAMA MUSCULAR DYSTROPHY PATIENTS

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Fukuyama congenital muscular dystrophy (FCMD) is a progressive neuromuscular disease caused by mutations in the FKTN gene. The phenotypes of FCMD include developmental retardation, muscle weakness and dilated cardiomyopathy. Particularly, the prognosis is determined by the severity of respiratory failure and cardiomyopathy. Fukutin protein coded by the FKTN gene elongates sugar chains linked with α-dystroglycan, a membrane protein that connects to the extracellular matrix. However, it is unclear how sugar chains on membrane proteins are involved in the onset of cardiomyopathy. This study aims to clarify the mechanism by human iPS cell (hiPSC)-derived cardiomyocytes. HiPSC lines were established from two FCMD patients and two healthy controls, F-hiPSC and C-hiPSC, respectively. Furthermore, two control hiPSC lines were generated from F-hiPSCs by the CRISPR-Cas9 system. First, the elongation of sugar chains linked to α-dystroglycan was evaluated by comparing the fluorescence intensity of sugar chains antibodies in differentiated cardiomyocytes. Furthermore, the size and beat rate were measured at the single-cell level, and the field potential duration (FPD), which is the same meaning as clinical QT duration, was calculated by a multi-electrode array system. For analysis, a two-sample t-test with unequal variance was used, and a p-value of less than 0.05 was used as the significant value. The fluorescence intensity of sugar chains in F-hiPSC-derived cardiomyocytes (F-CM) was reduced compared to that in C-hiPSC-derived cardiomyocytes (C-CM) and gene-corrected F-hiPSC-derived cardiomyocytes (C-F-CM) (C-CM vs. F-CM: p=0.0032, F-CM vs. C-F-CM: p=0.0013). Although the cell size varied, there was no significant difference between C-CM and F-CM, and C-F-CM was significantly larger than F-CM (p=0.030). The beat rate was 62.4±22.4 bpm for C-CM, 50.1±23.3 bpm for F-CM, and 60.7±30.7 bpm for C-F-CM, with no significant difference. The FPDs corrected for beat rate by Fridericia formula was 241.7±29.3 msec for C-CM and 236.3±41.6 msec for C-F-CM, while it was 283.0±46.3 msec for F-CM, which was longer, but no significant difference was detected (C-CM vs. F-CM: p=0.13, F-CM vs. C-F-CM: p=0.11). These results indicate that the F-CM might have cardiac vulnerability.

Funding Source: Kakenhi.

W1279

ENGINEERING ANTIBODY-CAR-MACROPHAGES (AB-CAR-M) FOR TUMOR MICROENVIORNMENT REMODELLING (EATER)

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Chimeric antigen receptor (CAR) immunotherapy in solid tumors remains limited due to the immunosuppressive microenvironment and poor infiltration of immune cells. Overcoming both is essential for CAR immunotherapy in solid tumors. Here, we designed AntiBody-CAR-Macrophage

(AB-CAR-M) to remodel the immunosuppressive microenvironment and improve infiltration for the treatment of Hepatocellular Carcinoma (HCC). We engineering CAR-M to secrete single chain variable fragments (scFv) to block immunosuppressive signals. AB-CAR-M reinvigorated cytotoxic T-cells, macrophages and downregulates bystander CD4+ T-cells Treg programs by counteracting immunosuppressive signals, indicating that immunosuppressive signal blocking converted the immunosuppressive microenvironment into immunogenic. AB-CAR-M promoted in vitro phagocytosis and killing of liver cancer cells. We administered AB-CAR-M to the tumor-bearing immunodeficient NSG mice to examine infiltration. AB-CAR-M formed perivascular immunological hubs with host macrophages in the tumor. Co-administration of AB-CAR-M and T-cells reduced tumor volume on the ipsilateral site, while the contralateral site was unaffected, suggesting the spatial confinement of macrophage immunotherapy which is promising for local delivery of blocking scFv. Moreover, murine AB-CAR-M shrinks orthotopic liver cancer in immunocompetent mice models. Patients' tumor slice cultures are underway to determine the nature of the immunological hubs formed by AB-CAR-M. Overall, these data reinforce AB-CAR-M as a solution for solid tumors. Our ultimate goal is to apply the AB-CAR-M in universal donor human pluripotent stem cells for off-the-shelf generation of cell therapy products.

W1281

ENGINEERING SPLICING-REGULATORY NETWORKS TO PRODUCE MATURE STEM CELL-DERIVED NEURONS

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Incurable aging-related neurodegenerative diseases are a growing public health crisis. The ability to generate substantial quantities of disease-pertinent neuronal types, with and without predisposing mutations, holds great promise for probing disease mechanisms and developing therapies. However, current protocols yield neurons that fail to mature in vitro and stall at an embryonic identity. We understand how to make neurons, but what controls maturation? Could it be RNA metabolism? The mammalian nervous system employs alternative splicing (AS) at distinct developmental stages to massively expand transcriptomic diversity and protein function. The function of these programs is poorly understood, but presumably of importance since the content and timing of splicing switches are deeply conserved. Here, we show that a major bottleneck towards maturation of mouse and human stem cell-derived neurons is the inability to activate sequential programs of alternative splicing switches, affecting hundreds of genes. Through systematic characterization, we identify master splicing factors that control distinct AS programs during different phases of maturation. Critically, we manipulate developmental AS master regulators in mouse and human neurons to demonstrate that maturation can be distinctly advanced and accelerated through activation of stalled alternative splicing programs. Of note, our strategy yields cells with mature electrophysiology profiles on a shorter timescales, and generates neurons of mouse and human origin that express endogenous levels of mature tau isoforms within seven days in culture. Overall, this undertaking explores a novel function for alternative splicing during neurodevelopment, improves understanding of mechanisms that control maturation of neurons, and provides a refined stem cell-based model for studying disease, particularly tauopathies. Moreover, this technology has important applications for the exploration of aging biology and will be broadly useful to the scientific community for modeling neurodegenerative diseases and drug discovery.

Funding Source: New York Stem Cell Foundation, NIH.

W1283

EPIGENETIC MEMORY AND INFLAMMATORY PROFILING OF ADIPOCYTES DIFFERENTIATED FROM UMBILICAL CORD MSCS IN OBESITY

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Adipose tissue plays a critical role in regulating metabolism and inflammation. In obesity, adipose tissue adopts a pro-inflammatory profile that contributes to metabolic diseases. Obese adipose tissue derived mesenchymal stromal cells (MSCs) significantly contribute to inflammation. Notably, adipose tissue retains an “obesogenic memory” even after weight loss, predisposing to an accelerated response to future obesogenic stimuli. MSCs are known as modulators of the immune response and secrete a range of cytokines and chemokines, influencing both local and systemic inflammation. Umbilical cord (UC) MSCs are of fetal origin but highly influenced by the intrauterine environment. These unique MSCs retain inflammatory signatures and can inform on the transgenerational impact on the differentiation and function of offspring adipocytes. These investigations are crucial to understand the intergenerational transfer of metabolic risk from the inflammatory state of the mother resulting from obesity. We have developed methods to extract UC MSCs from Wharton Jelly mechanically as described previously. We aim to extract and investigate MSCs from 10 mothers with pre pregnancy obesity compared to 10 with normal BMI (< 25 kg/m2). This study suggests that MSCs from obese donors will exhibit an inflammatory signature resembling the donor's adipose tissue, supporting the concept of "obesogenic memory." We hypothesize that RNA, protein, and DNA analyses (RNA-seq, Western blot, ATAC-seq, single cell RNA-seq) will reveal donor-dependent differences in inflammatory (IL-6, IL-10, TNF-α) and adipogenic markers (PPARγ, Pref-1). Furthermore, multiplex cytokine assays (ELISA) are expected to demonstrate distinct cytokine secretion profiles in media collected at Days 0 and 21 of differentiation. By exploring these results, this study aims to provide novel insights into obesity-associated inflammation, the intergenerational impact of maternal obesity, and the development of therapeutic strategies to treat metabolic disorders.

W1285

ESTABLISHING AN INDUCED PLURIPOTENT STEM CELL BANK FROM URINE CELLS OF PEDIATRIC NEUROGENETIC DISEASE PATIENTS

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Inadequate knowledge of the fundamental mechanisms underlying pediatric neurological disorders impedes their effective treatment. Induced pluripotent stem cells (iPSCs) are essential to explore the course of neurological diseases because they enable the modeling of these diseases at the cellular level. This study aimed to generate an iPSC bank from urine cells (UCs) for clinical applications, especially for the study of pediatric neurogenetic diseases. Urine sample collection can benefit the large donor population because it is a noninvasive, painless, and simple technique, providing a plentiful cell source for iPSC generation. UCs were isolated from the urine of donors with specific diseases (n = 12; 7 males and 5 females). UCs were reprogrammed into iPSCs using episomal plasmid vectors and key transcription factors (OCT3/4, SOX2, KLF4, L-MYC, and LIN28). qPCR and immunocytochemistry confirmed the expression of pluripotent genes (OCT3/4, SOX2, NANOG, and LIN28) and pluripotent proteins (OCT4, NANOG, SSEA-4, and TRA-1-60). Trilineage differentiation potential was demonstrated by immunostaining embryonic-body-derived iPSCs for β-tubulin III, smooth muscle actin (SMA), and alpha-fetoprotein (AFP). Chromosomal microarray (CMA) was used to assess genomic stability, revealing pathogenic chromosomal deletions or duplications in four out of 12 lines. Notably, repeated CMA testing on earlier-passage lines showed normal genomic profiles in one of the affected lines, emphasizing the importance of genetic screening at multiple stages during iPSC culturing. This study successfully generated an iPSC bank derived from UCs of patients with early-onset neurogenetic diseases. The bank provides a robust, efficient protocol to expand access to patient-specific iPSCs, facilitating pediatric neurogenetic research and enabling disease modeling for the development of targeted therapies.

Funding Source: This work was funded by the New Faculty Startup Fund from Seoul National University and SNUH Lee Kun-Hee Child Cancer and Rare Disease Project, Republic of Korea (grant number: 24C017-0100).

W1287

ESTABLISHMENT OF AN NMOSD RODENT MODEL WITH DISTINCT PATHOLOGICAL CHARACTERISTICS FROM EAE MODEL

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Multiple sclerosis (MS) and neuromyelitis optica spectrum disorder (NMOSD) are inflammatory autoimmune diseases of the central nervous system (CNS) characterized by demyelination, leading to neurological dysfunction. Due to their overlapping clinical manifestations and affected regions, MS and NMOSD were historically considered a single disease entity. However, the identification of anti-aquaporin-4 (AQP4) antibodies (NMO-IgG) in the serum of NMOSD patients has established NMOSD as a distinct disease. Despite ongoing efforts to differentiate these diseases, their underlying pathogenic mechanisms remain elusive. Here, we established an NMOSD rodent model by intrathecally administering patient-derived NMO-IgG and human complement. Using this model, we performed comparative histological and molecular analyses with the experimental autoimmune encephalomyelitis (EAE) model, a representative MS model. GFAP expression showed opposing patterns, with significant upregulation in EAE but acute loss in the NMOSD model, reflecting astrocyte gliosis and destruction, respectively. Similarly, AQP4 expression was markedly reduced in the NMOSD model compared to EAE, consistent with the pathogenic mechanism of AQP4 antibody-mediated astrocyte damage. Moreover, the NMOSD model exhibited more severe demyelination and inflammation, as demonstrated by Luxol Fast Blue (LFB) and H&E staining. These pathological differences were observed across the brain, spinal cord, and optic nerve, with regional variations in their manifestation. Our findings highlight distinct pathological differences between MS and NMOSD, underscoring the utility of our NMOSD model in

advancing the understanding of NMOSD pathogenesis and facilitating therapeutic development.

Funding Source: Korean Fund for Regenerative Medicine grant funded by the Korea government (the Ministry of Science and ICT, the Ministry of Health and Welfare) (22C0628L1-11) and BK21 Four Institute of Precision Public Health.

W1289

ESTABLISHMENT OF IMPLANTATION MODEL WITH HUMAN EXPANDED POTENTIAL STEM CELL DERIVED EMBRYO SURROGATE AND OPEN-FACED ENDOMETRIAL LAYER FOR STUDYING IMPLANTATION IN IVF PATIENTS

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Successful pregnancy requires the implantation of a competent blastocyst in receptive endometrium. Despite the use of in vitro fertilization (IVF), many women still fail to conceive due to endometrial or embryo problems. Studying embryo implantation in vivo is limited by ethical concerns. This study aimed to develop an implantation model with embryo surrogate and an expandable endometrial cell culture system for the study on implantation processes. Our team has generated a trophoblastic spheroid (BAP-EB) derived from human expanded potential stem cells (hEPSC-em) as human embryo surrogate. hEPSC-em-BAP-EB differentiated for 48h (hEPSC-em-BAP-EB-48h) are early trophectoderm (TE) like and less adhesive on the receptive Ishikawa cell, but after they are differentiated for 72h (hEPSC-em-BAP-EB-72h), they are polar TE like and can strongly attached onto receptive endometrial cell line. Endometrial organoids followed by open-faced endometrial layers (OFELs) culture were established using samples from IVF patients. It was found that endometrial organoids preserved the phenotypical and functional properties of endometrial glands. OFELs cultured in three different conditions were found to be hormone responsive. While hormone treatment had no effect on endometrial receptivity markers, it induced early epithelial to mesenchymal transition in OFEL. An attachment assay by coculturing OFEL with hEPSC-em-BAP-EB-72h was established. Critically, the attachment rate of hEPSC-em-BAP-EB on hormone treated OFEL was significantly higher than the non-treated OFEL. In conclusion, receptive OFELs were successfully established from endometrial organoids derived from IVF patients, and the coculture model with hEPSC-em-BAP-EB represented the first step toward the establishment of a 3D endometrium model that better mimics the implantation process.

Funding Source: This work was partly supported by Health and Medical Research Fund (grant numbers: HMRF #11222296) from the Food and Health Bureau, Government of the Hong Kong Special Administrative Region.

W1291

EVALUATION OF BOTULISM NEUROTOXIN EXPOSURE IN A HUMAN HPSC-DERIVED NEUROMUSCULAR JUNCTION PLATFORM

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The human neuromuscular junction (NMJ) is a specialized synapse that modulates neurological control over muscle contraction, facilitating precise communication between motor neurons, skeletal muscle cells, and terminal Schwann cells. While the neurophysiological properties of the NMJ have been extensively characterized in animal models, our knowledge of human NMJ neurophysiology remains limited. Compared to animal models, human NMJs are smaller, less complex, and more fragmented, with divergent molecular and cellular characteristics. Furthermore, NMJs are vulnerable entry points for pathogens and toxins that can impair motor function and propagate central nervous system (CNS) infections. To address this, we optimized a protocol for generating functional human NMJs through directed differentiation of human pluripotent stem cells (hPSCs), producing cultures that recapitulate key features of native human NMJs. These cultures form self-organizing bundles of aligned muscle fibers encircled by innervating motor neurons. Detailed imaging demonstrates the presence of skeletal muscles, satellite cells, glia cells, terminal Schwann cells, interneurons, and spinal cord motor neurons. In contrast to monocultures or co-cultures of only two distinct cell types, these cultures reflect the varied composition of cell types seen in vivo. To validate the utility of this platform for investigating neurotoxin-induced pathologies, we characterized the differential paralytic effects of various Botulinum Neurotoxins on synaptic transmission using electrophysiological methods and in vitro imaging. This model system offers a comprehensive framework for creating targeted therapies to treat neuromuscular dysfunction and advances our knowledge of human NMJ pathophysiology, in addition to evaluating the mechanisms by which infections and toxins impair NMJ function.

Funding Source: This work was supported by the Defense Threat Reduction Agency—Joint Science and Technology Office (Project MCDC2201-002).

W1293

EXPANDED POTENTIAL STEM CELL-DERIVED SKIN ORGANOIDS FOR STAT1-GOF DISEASE MODELING

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STAT1 gain-of-function (STAT1-GOF) mutations are associated with immune dysregulation and increased susceptibility to the skin infections, particularly Chronic Mucocutaneous Candidiasis (CMC). These infections are reported to be linked to abnormalities in skin structure and development. To better understand the pathophysiology of CMC in STAT1-GOF patient and evaluate potential therapeutic interventions, we aim to develop a skin infection model using skin organoids derived from expanded potential stem cells (EPSCs) of STAT1-GOF patients. The patient-derived skin organoids recapitulate key structural in human skin, providing a physiologically relevant platform for studying infection dynamics. By infecting the organoids with pathogens, we intend to investigate the pathophysiology of CMC and identify underlying mechanisms driving susceptibility. Furthermore, the model will allow for testing and evaluation of candidate therapeutic compounds and drugs, enabling the identification of effective treatments to mitigate infection and restore skin integrity. The development of this model has the potential to facilitate critical insights into disease mechanisms and accelerate the discovery of targeted therapies for patients with

STAT1-GOF mutations.

W1295

EXPLORING HORMONAL AND INTESTINAL ADAPTATIONS DURING PREGNANCY USING INTESTINAL ORGANOID MODELS

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Pregnancy and lactation demand significant energetic and metabolic resources, requiring maternal systems to undergo rapid adaptations comparable to those of high-endurance athletes. The small intestine plays a pivotal role in this process by increasing its absorptive capacity to support maternal metabolism and fetal development with an additional 500–800 kcal daily. These dynamic changes are influenced by key pregnancy hormones—human chorionic gonadotropin (hCG), progesterone, and estradiol (E2)—which drive essential biological functions. Intestinal growth factors, such as Epidermal Growth Factor (EGF), R-Spondin, WNT-3a, and Noggin, regulate the proliferation and health of intestinal epithelial stem cells, providing optimal conditions for the development of enteroids or “miniguts.” EGF is critical for supporting organoid cell proliferation and growth. However, pregnancy hormones interact with overlapping pathways that modulate cellular behaviors in the presence or absence of EGF. For instance, progesterone promotes cell proliferation through the WNT pathway in certain mammalian cells while inhibiting the WNT/β-Catenin pathway in others. Insufficient levels of hCG are associated with maternal and fetal complications, underscoring the importance of understanding these hormonal dynamics. Notably, preliminary data demonstrate that hCG can substitute for EGF in murine and human enteroid cultures, suggesting an early adaptive mechanism by which the maternal gut anticipates the metabolic demands of pregnancy. Despite these insights, interactions between pregnancy-related hormones and intestinal adaptations remain poorly understood. Organoids derived from the intestinal stem cell niche offer a promising platform to replicate gut physiology during pregnancy. These models can elucidate mechanisms underlying intestinal adaptation, predict treatment outcomes, and assess the safety of interventions. Furthermore, they hold potential for developing countermeasures against enteropathies, such as celiac disease and inflammatory bowel disease, that negatively impact pregnancy outcomes. Thus, my continuous experiments will explore the integration of organoid technology to enhance our understanding of maternal-fetal health and address unmet needs in pregnancy research.

Funding Source: Bill and Melinda Gates Foundation.

W1297

EXPLORING MYELINATION DYNAMICS IN 2D AND 3D MODELS USING HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED OLIGODENDROCYTE PROGENITORS

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Myelin production by oligodendrocytes is essential for efficient neuronal communication, and disruptions in myelination are linked to various central nervous system disorders. Oligodendrocyte progenitor cells (OPCs) derived from human induced pluripotent stem cells (hiPSCs) offer a valuable platform for studying myelination in both 2D and 3D models. These systems enable controlled investigations of oligodendrocyte differentiation, myelination, and OPC-neuron interactions, supporting high-throughput drug screening for myelin repair and disease therapies. To accelerate therapeutic discovery for demyelinating diseases, BrainXell developed an optimized protocol that reduces OPC differentiation time from three months to under one month, yielding highly pure OPC populations. Quantitative analysis showed significant MBP expression, with 60-80% of cells positive for MBP, and flow cytometry confirmed ~95% O4+ cells. qPCR demonstrated upregulation of oligodendrocyte-related genes (CNP, OLIG2, CSPG4) and myelin markers (PLP1, MBP). In 2D co-culture with hiPSC-derived cortical glutamatergic neurons, MBP+ oligodendrocyte projections wrapped around MAP2+ neurites, indicating functional OPC-neuron interactions. In 3D nanofiber scaffolds, OPCs exhibited similar differentiation and maturation, demonstrating their myelination potential in a scaffold-based system. Both 2D and 3D cultures showed complex, arborized morphologies, confirming OPC maturation. Notably, in 3D cultures and co-cultures, OPCs displayed enhanced structural complexity with extended processes, resembling the in vivo environment more closely than monocultures. This underscores the value of 3D and co-culture systems for studying oligodendrocyte development and myelination, as they better mimic natural OPC-neuron interactions. This optimized protocol facilitates the rapid generation of hiPSC-derived oligodendrocytes, accelerating myelin repair and regeneration therapeutic discovery. The platform supports functional OPC-neuron interactions in both 2D and 3D models, providing a robust system for drug screening and advancing therapies for myelin loss-related disorders.

W1299

EXPLORING THE IMPACT OF HEPATIC FIBROGENESIS ON GAP JUNCTIONS: A MULTICELLULAR SPHEROID MODEL APPROACH

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Connexin (Cx)-formed gap junctions mediates important physiological and pathological events in the liver. However, little is known about the impact of liver fibrogenesis on gap junctions. To address this issue, a TGF-β induced in vitro fibrogenesis model was applied on multicellular spheroids (MS) obtained from HepG2, GRX (liver cell lines) and menstrual blood-derived mesenchymal cells CeSaM (n=10, Clementino Fraga Filho University Hospital Ethics Committee approval 056/09). Before, and after 7 and 14 days in culture, control and fibrotic (treated with 10 ng/mL of TGF-β) MS were submitted to volumetric (V=4/3πR3) and phase contrast analyses. The presence of Cx26, Cx32, Cx43, albumin, αSMA, COL I and III, Ki67 and Cyp3a4 was evaluated by immunofluorescence analyses. Confocal and transmission electron microscopy were performed to detect structural alterations on MS. To evaluate functionality, MS (n=5) were treated with different concentrations of acetaminophen (1, 5, 25, 50, 60, 70, 80 mM) for 2, 5, and 24h. MS viability was accessed by PrestoBlue assay. Intracellular Ca2+ signalization assay was performed to investigated whether fibrogenesis impacts on MS physiology. Volumetric analysis revealed significant changes when comparing MS obtained from different cell densities (2 and 4x10^4 cells, $p< 0.01$). Fibrotic MS differed from control in size (smaller at 7 and 14d; $p< 0.05$). Histological analysis revealed a spherical and uniform MS. Sirius red staining highlighted collagen distribution on fibrotic MS. Immunofluorescence analyses showed differences between Cx26, Cx32, Cx43 and COL I & III distribution between control and fibrotic spheroids. TEM showed morphological changes between control and fibrotic MS and revealed that fibrogenesis impairs architecture of liver organelles. Dose-response assay shows that acetaminophen was metabolized and contributed to a significant decrease in viability at 2 and 24h. Sequential confocal images obtained in Fluo-4/AM loaded SM showed that fibrogenesis promoted a significant impact on Ca2+ signaling amplitude and ATP responsiveness when compared to control MS. Taken together, these data suggest that liver fibrogenesis can impact on gap junctions promoting structural alterations that impairs on liver physiology.

Funding Source: Capes, FAPERJ, CNPq, INCT Regenera, INCT Nanobiofar, Ministério da Saúde.

W1301

EXPLORING THE ROLE OF NOTCH SIGNALING IN PATHOLOGICAL OSTEOGENIC DIFFERENTIATION DURING CALCIFIC AORTIC VALVE DISEASE

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The Notch signaling pathway is an evolutionarily conservative mechanism of cellular communication, regulating the processes of differentiation. Notch is essential for bone regeneration through the regulation of osteoblasts (OBs) and osteoclasts production as well as arteriovenous differentiation of endothelial cells (ECs). Calcific aortic valve disease (CAVD) is characterized by pathological osteogenic differentiation of aortic valve interstitial cells. The Notch pathway is an essential signaling pathway of aortic valve development and the NOTCH1 mutations are the first

genetic variants identified for CAVD in human. However, the role of Notch in the pathologic osteogenic differentiation in CAVD remains unclear. The aim of this work was to analyze how the activation/suppression of Notch pathway components in endothelial cells influences osteogenic differentiation in endothelial-mesenchymal cocultures in normal and pathological osteogenic differentiation. Primary human OBs and HUVECs cocultures were used as normal osteogenic differentiation; aortic valve endothelial cells (VECs) and valve interstitial cell (VICs) were used for the analysis of pathological osteogenic differentiation. ECs were modified with lentiviral human intracellular domains of Notch1 or Notch3 receptors for activation, or by short hairpin RNA to a NOTCH1 or NOTCH3 genes for knockdown. Transduced ECs were co-cultured with intact OBs/VICs in osteogenic conditions. NOTCH1 activation increased osteogenic differentiation only in HUVECs-OBs co-cultures, while activation of NOTCH3 in ECs enhanced calcium deposition in both VECs-VICs and HUVECs-OBs cocultures. Suppression of the NOTCH1 in ECs had an inhibitory effect on osteogenic differentiation of both cocultures. Suppression of the NOTCH3 inhibited osteogenic differentiation in HUVECs-OBs, but had the opposite effect in VECs-VICs cocultures. Thus, modification of the Notch pathway in aortic valve endothelial cells could be a promising tool for developing a therapy to prevent CAVD progression.

Funding Source: Supported by RSF grant 23-15-00320.

W1303

FUNCTIONAL MATURATION OF HUMAN IPSC-DERIVED MODELS OF THE ENTERIC NERVOUS SYSTEM

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The enteric nervous system (ENS) is essential for many key functions of the gastrointestinal tract, including water and nutrient absorption, hormone secretion and motility. Perturbations in the ENS can result in debilitating disease. Understanding the development, function, and communication of this complex neural network in humans will be essential to developing new therapies to treat enteric neuropathies. The development of enteric neurons and glia can now be mimicked in vitro, by the sequential and guided differentiation of human induced pluripotent stem cells (hiPSC) towards an enteric neuronal and glial fate. However greater insight into the developmental and functional properties of hiPSC-derived ENS cells is critically needed to validate their potential as disease models and for cell therapy. In this study, we investigated the functional activity of hiPSC-derived enteric neurons and glia during early time points post-differentiation. Using an established method, hiPSCs were differentiated into enteric neurons and glia. Live calcium imaging was performed to examine neuronal and glial activity at differentiation day (D) 22, D29, D36, D43, D50 and D57. Additionally, we reconstructed a neural network using ‘graph theory’, to investigate crosstalk and network communication dynamics among enteric neurons. Ca2+ transients were observed in response to high K+ application, as well as the neurotransmitter receptor agonists DMPP (10uM) and ATP (10uM). Further, spontaneous Ca2+ transients were observed. Maturation

of spontaneous and evoked responses were identified along the cells' developmental trajectory. Further, network analysis demonstrated that around 15% of neurons play a critical role in network communication, and that modules with stronger connectivity are not organised based on spatial proximity but are dispersed across the neural network. This is the first study to characterise the functional maturation of neuronal and glial hiPSC-derived ENS cells.

W1305

FUNCTIONALLY RELEVANT PSC-DERIVED HEPATOCYTES AND LIVER ORGANOIDS FOR HEPATOTOXICITY AND LIVER BIOLOGY MODELING

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Functionally relevant human hepatocyte models are critical for drug safety and efficacy screening, cell therapy, and disease modeling. However, currently available in vitro models, such as primary human hepatocytes and immortalized cell lines, often rapidly de-differentiate or lack metabolic maturity. Human pluripotent stem cell (hPSC)-derived hepatocytes and liver organoids represent convenient, scalable, and patient-representative alternatives to conventional models. STEMdiff™ Hepatocyte Kit supports efficient and reproducible generation of hPSC-derived hepatocyte-like cells (HLCs) over 21 days. hPSCs are patterned to definitive endoderm cells, then differentiated to hepatic progenitors, and finally matured to HLCs. The resulting HLCs exhibited hepatic marker expression (66 ± 8% ALB+A1AT+; mean ± SD) and mature functionality, including CYP3A4 enzymatic activity and albumin secretion (n = 2 - 15). HLCs were also replated in Matrigel® domes to establish passageable liver organoids amenable to cryopreservation using STEMdiff™ Hepatic Organoid Growth Medium (n = 11). These organoids were further matured in 3D culture using STEMdiff™ Hepatic Organoid Differentiation Medium (ODM), resulting in significant downregulation of fetal hepatocyte gene AFP (5.7-fold decrease; $p < 0.0001$), and increased expression of several mature hepatic genes, including ALB, ASGR1, CYP3A4, CYP2C9, UGT1A1, and OTC (n = 2 - 20). ODM-differentiated cultures also exhibited increased albumin secretion (2.9-fold increase; $p = 0.0199$) as well as CYP3A4 activity that was modulated using ketoconazole (average 2.0-fold decrease) and calcitriol (average 2.5-fold increase). HLC-derived organoids were sensitive to ketoconazole-, rifampicin-, and acetaminophen-induced hepatotoxicity (average ODM-differentiated organoid IC50 = 58 µM, 527 µM, and 1.8x104 µM, respectively; n = 2), and could be used to generate viability data that successfully distinguished between low and high liver injury-causing compounds that are structurally similar. These results demonstrate the utility of STEMdiff™ Hepatocyte Kit and STEMdiff™ Hepatic Organoid Media in hPSC-derived liver modeling and drug screening applications.

W1307

GEL-FREE ENGINEERED HEART TISSUE FOR DUAL EXCITATION-CONTRACTION RECORDING AND DISEASE MODELLING

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Human iPSC-derived cardiomyocytes are increasingly used to assess drug toxicity through effects on ion channels and electrophysiology, yet contractility is not always evaluated. There is growing interest in including both electrophysiology and contractility data from engineered heart tissue to more comprehensively assess human cardiac responses. In this study, we developed a scalable platform for generating engineered heart tissue without the use of collagen or fibrin gels. The iPSC-CMs self-organized into 3D heart tissue with a thickness of over 100 μm within 10 days. This device is compatible with commercial multi-electrode array systems, enabling the simultaneous recording of electrophysiology and contractility data. A range of drugs with varying torsade de pointes risks were used to validate the system, underscoring the advantage of comprehensive, multi-parameter readouts over single-parameter analyses. We found that drug-induced arrhythmias not only affect heart rhythm but also significantly reduce force output. Additionally, we used this device to model myosin-binding protein C deficiency, successfully replicating contractile deficits in engineered heart tissues. Collectively, this platform demonstrates that simultaneously measuring electrophysiological and mechanical properties is crucial for evaluating drug-induced cardiotoxicity, holding promise for regulatory authorities, pharmaceutical companies, and research institutions in the comprehensive assessment of drug efficacy and toxicity in humans.

W1309

GENERATION AND CHARACTERISATION OF A HUMAN IPSC LINE LACKING NEUREGULIN-1 TO INVESTIGATE MACROPHAGE GROWTH FACTOR BIOLOGY

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We have recently discovered that myeloid cells produce Neuregulin-1 Class VII, but its role in tissue homeostasis is not known. Neuregulin-1 (NRG1) is a pleiotropic growth factor and a key regulator of the stem cell niche, influencing cellular survival, proliferation and differentiation via the NRG1/ErbB signalling complex. The seven isoform classes of NRG1 exhibit distinct, tissue-dependent expression patterns. Unique N-terminal sequences characterise each class of NRG1, and direct alternate patterns of expression, trafficking, and receptor affinity in the resultant protein. However, the functional implications of NRG1 isoform diversity are poorly understood. To address this gap we developed a novel, human iPSC model with complete knockout of functional NRG1 – a previously unfeasible approach in rodent studies due to embryonic lethality associated with many NRG1 mutations. The NRG1KO line was generated on the MCRI-PB001.1 cell line utilising CRISPR/Cas9 technology and sgRNAs to achieve targeted deletion of all known transcriptional start sites and the signalling domain from the NRG1 locus. Supplementation of the NRG-1 KO line with exogenous NRG1 ensured viability during targeting and clonal expansion. PCR analysis and sanger sequencing were used to confirm deletion and expected DNA breakpoints. Immunofluorescence was used to confirm loss of protein expression, and flow-cytometric analysis demonstrated robust expression of key pluripotency markers. We herein demonstrate successful

differentiation of the line to hemogenic endothelium and to myeloid cells of comparable marker expression to wild-type controls. In generating this novel NRG1KO HiPSC line, we hope to facilitate future investigations as to the specialised roles played by NRG1 isoforms in health and disease, thereby addressing a longstanding gap in scientific understanding of the NRG1 signalling network. This cell line will serve as an important toolkit in our efforts to discover the functional role played by myeloid NRG1.

Funding Source: NHMRC APP1186371 to CW The FEIT-MDHS PhD stipend to BS.

W1311

GENERATION OF IPSC LINES FROM PATIENTS WITH BARDET BIEDL SYNDROME AND STARGARDT DISEASE AS IN VITRO HUMAN MODELS OF INHERITED RETINAL DYSTROPHY

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Inherited Retinal dystrophies (IRDs) are a group of disorders involving the degeneration of photoreceptor cells or retinal pigment epithelia leading to vision loss or legal blindness. IRDs are clinically and genetically heterogeneous, with 271 associated genes reported. Therefore, the recapitulation of IRDs has been challenging especially in light of complexities associated with the translation of findings from animal models to human context. Retinal organoid (RO) technology derived from induced pluripotent stem cells (iPSCs) has been an indispensable alternative to closely mimic in vivo systems in order to study the disease pathophysiology. However, many IRDs are underexplored due to the diseases´ genetic heterogeneity and the limited availability of iPSC lines. In this study, we reprogrammed peripheral blood mononuclear cells from two patients suffering from Bardet Biedel Syndrome (BBS-POMGNT1, c.1539+1G>A) and Stargardt disease (STGD1, ABCA4, c.5917del.), respectively using Sendai viruses. Two stem cell lines harboring the patients´ mutations were generated from this approach. Alkaline phosphatase treatment and immunostainings using OCT-4, TRA1-60 and SOX2 were utilized to confirm the pluripotency of the generated cell lines. Germ layer differentiation was performed and characterization was done using endodermal, mesodermal, and ectodermal markers. We confirmed the lines' genome integrity using karyotyping and the presence of the mutations by genetic sequencing. We subsequently generated ROs using our already established RO protocol confirming the ability of the lines to generate retinal tissue. We aim next to generate isogenic control lines and then retino-cortical assembloid models to better recapitulate and understand the disease pathophysiology.

W1313

GENERATION OF IPSCS LINE FROM PATIENT WITH SINGLETON-MERTEN SYNDROME

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Singleton-Merten syndrome (SMS) is a dominant autosomal orphan disease classified as an interferonopathy. Patients with SMS experience severe pathologies, including dental dysplasia, early-onset osteoporosis, psoriasis, and calcification of heart valves, vessels, and organs. The syndrome is linked to mutations in DDX58 and IFIH1. Despite its genetic basis, the molecular mechanisms of calcification and the role of endothelial cells remain unclear. We derived and characterized the first induced pluripotent stem cell (iPSC) line, SMS-DDX58, from a patient with SMS. Reprogrammed from peripheral blood mononuclear cells (PBMs) using viral vectors, SMS-DDX58 retains the patient-specific c.902C>G mutation in DDX58, confirmed by Sanger sequencing, while STR analysis verified its identity with the original PBMCs. SMS-DDX58 displays stem cell characteristics, expressing pluripotency markers OCT4, NANOG, and SSEA4. mRNA analysis confirmed POU5F1, SOX2, and NANOG expression. The cells differentiate into derivatives of all three germ layers under spontaneous and directed conditions, expressing lineage-specific markers. To investigate endothelial involvement in calcification, SMS-DDX58 cells were directed towards the endothelial lineage, expressing endothelial markers CD31 and vWF. Analysis of these derivatives demonstrated their potential to serve as a cellular model for studying the mechanisms of endothelial calcification in SMS. This iPSC line represents a promising model to study SMS pathogenesis and the role of endothelial cells in calcification. Beyond SMS, it offers a valuable tool for exploring mechanisms underlying interferonopathies, providing insights that could inform therapeutic strategies for related conditions.

Funding Source: This study was supported by the Ministry of Science and Higher Education of the Russian Federation (Agreement No. 075-15-2022-301, dated 04/20/2022).

W1317

HAPLOINSUFFICIENCY IN HUMAN NEURAL PROGENITOR CELLS: IMPLICATION FOR AUTISM SYNDROME DISORDERS

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Haploinsufficiency describes a phenomenon where only one functioning allele of a gene is insufficient for a normal phenotype of a diploid cell/organism. Although haploinsufficiency underlies several human diseases, the effect of haploinsufficiency on human embryogenesis is largely unknown. Here, we aimed to identify genes affecting the normal growth of human embryonic stem cells (hESCs) when one of their two alleles is lost. To establish a genome-wide loss-of-function screening for heterozygous mutations, we fused normal haploid cells with a library of mutant haploid hESCs. We have identified over 600 genes with a negative effect on hESC growth in a haploinsufficient manner and characterized them as genes depleted from telomeres and X chromosome. Interestingly, a large fraction of these haploinsufficiency genes is associated with the extra-cellular matrix and the plasma membrane. We have revealed an enrichment of genes causing haploinsufficiency disorders within WNT and TGF-b signal transduction pathways. We

could thus identify haploinsufficiency-related genes and pathways that show growth retardation in early embryonic cells, suggesting dosage-dependent phenotypes in hESCs. There are several neurodegenerative disorders affected by haploinsufficiency phenomenon, and many of them are related to autism. We differentiated the library of heterozygote mutations into neural progenitor cells (NPCs) and identified about 250 genes essential for their differentiation in a haploinsufficient manner. We were able to identify neuronal-related dosage-sensitive pathways and detected several autism-related genes with a haploinsufficiency effect, suggesting that they can be modelled at early stages of differentiation. By using small molecule activators of haploinsufficient pathways in the heterozygous mutated cells, we were able to see improvements in some of the pathological phenotypes, thus affecting the molecular damages caused by haploinsufficiency in autism-related genes, and creating opportunities for future therapeutic research. Overall, we have constructed a novel model system for studying haploinsufficiency in both hESCs and NPCs, and thus were able to characterize important dosage-dependent genes and pathways involved in their normal growth and pathology.

W1319

HCMV INFECTION OF MACROPHAGES INDUCES INFLAMMATION BY UPREGULATING IL-6/IL-6ST SIGNALING IN CHOLANGIOCYTES IN BILIARY ATRESIA

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Biliary Atresia (BA) is a rare childhood disease in which bile is accumulated in the liver due to a complete or partial blockage of the biliary network. The pathogenesis of BA remains unclear. It is suggested that infection with human cytomegalovirus (HCMV) and activation of pro-inflammatory macrophages in the newborn's liver contribute to the disease initiation and progression of BA. To test this hypothesis, we developed a human isogenic pluripotent stem cell (hiPSC)-derived cholangiocyte and macrophage co-culture to investigate how HCMV-infected macrophages interact with cholangiocytes and contribute to BA pathogenesis. hiPSC-derived macrophages were infected with HCMV (multiplicity of infection = 1.0), and the samples were divided into mock and HCMV-infected groups. Macrophages were co-cultured with hiPSC-derived cholangiocytes in a 1:1 ratio. HCMV-mediated immune responses and cholangiocyte organoid development were evaluated. HCMV infected co-culture formed smaller, multi-cystic and deformed organoids. sc-RNA-seq analysis from post-infection day 1 (PD1) showed that HCMV-infected macrophages expressed pro-inflammatory mediators (IL6 and other chemokines) and acted as pro-inflammatory macrophages. Moreover, ligand and receptor interaction revealed that macrophages derived IL-6 interacts with IL6ST receptors on cholangiocytes, and induced robust inflammatory responses in cholangiocytes, upregulated downstream target genes (SPP1, IL-8, IL-1B) and pro-inflammatory pathways. Immunohistochemistry staining revealed that IL-6, SPP1 and IL-8 expressions were significantly upregulated in HCMV+ BA liver biopsies. Our findings were further validated in the RRV (rhesus rotavirus) experimental BA mice model, in that IL6 and SPP1 expressions were significantly upregulated in experimental BA liver tissues. Our data suggest that macrophage induced

inflammation of cholangiocytes via IL-6/IL6ST signaling contribute to the disease initiation and progression of BA.

Funding Source: Theme-based Research Scheme (T12-712/21-R) RGC Hong Kong SAR Government, Hong Kong SAR, China.

W1321

HEMATOPOIETIC STEM CELL-DERIVED CAR MACROPHAGES REMODEL TUMOR NICHE

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Immunotherapy has shown promising efficacy in lymphoma and leukemia, but clinical trials for solid tumors have yielded disappointing results. Chimeric antigen receptor (CAR) macrophage therapy holds potential for solid tumors due to its superior infiltration capabilities compared to CAR-T cells. In this study, we leveraged the robustness of cord blood hematopoietic stem cells (CB-HSCs) as an ideal source of CAR-macrophages. We validated the large-scale expansion of macrophages, their in vitro sustainability over two weeks, and their robust phagocytosis capacity from HSCs. Furthermore, we demonstrated that DNA-PK inhibition enhanced AAV-CRISPR mediated knock-in at the AAVS1 loci in CB-HSCs by a factor of 1.5, and the knock-in efficiency could reach 80% by droplet digital PCR detection. Additionally, aGPC3 CAR macrophages successfully engrafted into Huh-7 luciferase-bearing mice and demonstrated significant tumor burden reduction. Subsequently, we observed sustained macrophage presence in the liver post-injection into tumor-bearing mice for four weeks, as confirmed by immunohistochemistry staining. Notably, no cytotoxicity was observed in the mouse organs. Finally, we identified IL-12 and IL-18 primed NK cells, which secrete IFNg, as potential to reinvigorate bystander cells. Our findings demonstrate the robustness of CB-HSCs as a reliable source for immunotherapy and the potential of aGPC3 CAR macrophages to effectively eliminate liver cancer-bearing mice with satisfactory safety. This study presents CB-HSC as an off-the-shelf source of CAR macrophages and tumor microenvironment remodeling in solid cancer.

W1323

HIPSCS-DERIVED IN VITRO MODELS OF THE BLOOD-BRAIN BARRIER FOR STUDYING NANOPARTICLE TRANSPORT

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The primary barrier restricting access to the central nervous system (CNS) is the blood-brain barrier (BBB). Its properties differ in the presence of neurological diseases. This restriction and diversity of features remain a significant challenge in most CNS-related studies investigating nanoparticle (NP) delivery to the brain for a particular purpose, like glioblastoma multiforme (GBM) therapy. For these studies, maintaining BBB's integrity and studying its characterization is crucial at first glance. Although decades have been devoted to better understanding and mimicking BBB via several in vitro BBB models, the variability of BBB data and the lack of personalized in vitro BBB modeling for NP transport have been presented. Herein, our study approach is to exploit human induced pluripotent stem cells (hiPSCs) to create a perfect human BBB modeling in vitro. In our study, hiPSCs were transduced from peripheral blood mononuclear cells of one healthy human and successfully differentiated into brain microvascular endothelial cells (BMECs). More importantly, for the first time, a relatively novel NP, titanium carbide/oxide (Ti3C2Tx) MXene quantum dots (MQDs), was tested for its delivery across the hiPSCs-derived BBB model and its uptake by brain tumors needed for treating GBM. Our experimental results found that the hiPSCs-derived model resembled in vivo BBB more than the model with hCMEC/D3 cell lines, and it expressed several BBB genes essential for the brain endothelium. Accordingly, 10-fold higher occludin gene expression in the model with hiPSCs-derived BMECs than that of immortalized BMEC lines was found, indicating better BBB integrity. Moreover, in our transport-related studies, Ti3C2Tx MQDs were tracked by their auto-fluorescence, and the percentage of fluorescence by tumor cells in our MQD-treated model was 2-fold higher than in the untreated model, showing MQD transport across the BBB, and detection inside the brain tumor cells. Therefore, our study demonstrates the creation of well-developed BMECs, forming a protective membrane with the help of hiPSCs. It also emphasizes important points researchers consider in the BBB modeling for in vitro studies in neuro-nanotechnology. Finally, it offers a pivotal in vitro BBB model for personalized GBM therapy with MQDs and guides future studies in vivo.

Funding Source: Tubitak 1004, Regenerative and Restorative Medicine Research and Applications, 20AG003-P8. Project, Obtaining a human induced pluripotent stem cell-based blood brain barrier model, TSG-2024-3419.

W1325

HUMAN AIRWAY AND NASAL ORGANOIDS REVEAL ESCALATING REPLICATIVE FITNESS OF SARS-COV-2 EMERGING VARIANTS

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The high transmissibility of SARS-CoV-2 Omicron subvariants is generally attributed to immune escape. However, it remains unclear whether these emerging variants have gradually acquired replicative fitness in human respiratory epithelial cells. We sought to evaluate the replicative fitness of BA.5 and earlier variants in physiologically active respiratory organoids. BA.5 exhibited a dramatically increased replicative capacity and infectivity compared to B.1.1.529 and the ancestral

wildtype (WT) strain in human nasal and airway organoids. The BA.5 spike pseudovirus demonstrated significantly higher entry efficiency than those carrying the WT or B.1.1.529 spike. Notably, we observed prominent syncytium formation in BA.5-infected nasal and airway organoids, which was elusive in WT- and B.1.1.529-infected organoids. BA.5 spike-triggered syncytium formation was verified through lentiviral overexpression of the spike in nasal organoids. Moreover, BA.5 replicated modestly in alveolar organoids, with a significantly lower titer than B.1.1.529 and WT. Collectively, the higher entry efficiency and fusogenic activity of the BA.5 spike facilitated viral spread through syncytium formation in the human airway epithelium, leading to enhanced replicative fitness and immune evasion. In contrast, the attenuated replicative capacity of BA.5 in alveolar organoids may account for its benign clinical manifestation.

W1327

A NOVEL STEM CELL PLATFORM FOR ANTI-MONKEYPOX VIRUS STRATEGIES
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The recent widespread of Monkeypox Virus (MPXV) infection worldwide has caught global attention on the impact of this viral pandemic. Although MPXV infection was long identified decades ago, its re-emergence in 2022 has caused the declaration of Public health emergency of international concern. Unfortunately, the current available treatment strategies are not effective in treating MPXV infections. Here, we identified a new cell model for dissecting monkeypox infections, which has a high virus susceptibility and cell survivability after infection. This novel platform could bring new and invaluable insights into the current drug development for MPXV infections, as well as other viral infections.

Funding Source: This project is supported by the National Key Research and Development Program of China (nos. 2022YFA1105401); Health@InnoHK, Innovation Technology Commission; HKSAR.

W1329

HUMAN TROPHOBLAST CELLULAR MODEL IS HIGHLY PERMISSIVE TO SARS-COV-2 INFECTION AND ENABLES THE DISCOVERY OF ANTIVIRAL NATURAL COMPOUNDS POTENTLY AGAINST SARS-COV-2
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Non-human and/or abnormal human cell lines have long been adopted for virology studies, particularly for COVID-19. However, they may not fully mimic human physiological contexts particularly those together with pregnancy and alternative models are needed. Here we utilized our previously established normal human early syncytiotrophoblast (eSTB) model for infecting with SARS-CoV-2 of different variants and assessing antivirals. The eSTBs were highly susceptible to SARS-CoV-2 infection while being more sensitive to antivirals but resistant to post-infection cell

death than other conventionally used cell models. We further validated several clinically administered antivirals and revealed that Nirmatrelvir is effective, while Molnupiravir is not, which aligns with clinical manifestations. Using SARS-CoV-2 infected eSTBs, natural compound libraries were screened and potential candidates with high anti-SARS-CoV-2 functions were identified. Selective candidate nature compound effectively reduced the infection of primary human airway cells and organoids in vitro and in both mice and hamsters in vivo by SARS-CoV-2. The eSTB cellular model is an ideal platform for virology study and antiviral discovery.

Funding Source: This work was financially supported by Health@InnoHK, Innovation Technology Commission (ITC), HKSAR, China.

W1331

HYPOXIC CONDITIONING RESCUES RETINAL PRECURSOR CELLS FROM COCL2-INDUCED FERROPTOSIS VIA DRP1 AND VDAC1

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Traumatic optic neuropathy leads to irreversible vision loss due to ischemia and inflammation-induced apoptosis of optic-nerve axons. Models of retinal ganglion cell and optic nerve degeneration involve induction by chemicals (cobalt chloride, LPS, and N-methyl-D-aspartate), mechanical stress (optic nerve compression, light), and ischemia (transient retinal ischemia). Although exposure to severe hypoxia in R28 cells, and retinal precursor cells (RPCs) with retinal phenotypes, such as glial or retinal ganglion cells, can cause cell damage and alter mitochondrial metabolism, the effects and mechanisms of hypoxic conditions on these cells are unclear. Cobalt chloride (CoCl2) is used to mimic hypoxia in the retina and in in vitro models of optic-nerve injury. In this study, CoCl2 was used to cause hypoxic damage to R28 cells, which were subsequently transferred to a hypoxic chamber. The levels of proteins related to hypoxia, mitochondrial homeostasis, neuro-regeneration, and retinal ganglion-like cell markers were investigated by immunoblotting. Additionally, the expression levels of genes associated with ferroptosis were evaluated. Mitochondrial respiration, glycolysis, and ATP synthesis were analyzed using the Seahorse XF. After transfer to a 0.3% hypoxic chamber following CoCl2-induced hypoxic damage, the viability of RPCs was increased compared to the control. Mitochondrial ATP production, which was reduced by CoCl2, was restored in the 0.3% hypoxic chamber. Additionally, the expression levels of ferroptosis-related SUMO specific peptidase 1 (Senp1), glutathione peroxidase 4 (Gpx4), and transferrin receptor (Tfrc) were increased. Furthermore, the reductions in the levels of the neuro-regeneration markers Nf, Gap43, Stx12, and Brn3a were reversed. The 0.3% O2 hypoxic chamber rescued RPC mitochondrial homeostasis from CoCl2-induced hypoxic damage using a hypoxia mimic, thereby reducing ROS production and inhibiting ferroptosis. Thus, hypoxic conditions are implicated in the induction of, and recovery from, apoptosis or ferroptosis through mitochondrial regulation in RPCs. Therefore, we suggested that 0.3% O2 hypoxic chamber has significant neuroprotective potential.

Funding Source: This research was supported by grants from the National Research Foundation of Korea (NRF): 2021R1A2C2010523.

W1333

IDENTIFICATION OF A LONG-TERM SURVIVING HUMAN MESENCHYMAL STROMAL CELL SUBPOPULATION UPON INTRADERMAL INJECTION IN IMMUNODEFICIENT MICE

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Complications due to mutations in COL7A1 encoding type VII collagen (C7). Mesenchymal Stromal Cells (MSC) show promise in improving wound healing and reducing skin inflammation in RDEB patients owing to their ability to express C7 as well as their anti-inflammatory properties. Our objective was to refine in vitro conditioning of human bone marrow-derived MSC (hBM-MSC) and evaluate their fate upon local injections in murine models. hBM-MSC were tagged with a bioluminescent lentiviral vector and subjected to various culture conditions before being administered intradermally (ID) in immunodeficient mice. The survival of these cells was assessed using the bioluminescent reporter by in vivo imaging. Previous results have shown 4 months of survival of ID injected BM-MSCs cultured under standard in vitro culture conditions. Surprisingly, although most injected cells died within the first two months in all tested conditions, a small population (10%) of live bioluminescent cells persisted for at least one year-post-injection. We sampled murine skins injected with hBM-MSC 2 months post ID injection and analyzed the surviving subpopulation by immunostaining and spatial transcriptomic. In parallel, we analyzed the hBM-MSC populations cultured under the different conditions prior to injection through single-cell RNA sequencing (scRNAseq). Spatial transcriptomics data indicated that the surviving cells maintained the expression of THY1, ENG and NT5E in vivo and shared several characteristics with cutaneous fibroblasts. They expressed COL7A1 and showed enriched expression of genes involved in extracellular matrix and collagen fibril organization which have substantial therapeutic value for wound healing. Moreover, integration of spatial transcriptomic and scRNAseq data indicated that the surviving cells were initially present in the injected cell population and originated from the same cluster regardless of their culture condition. The identification and characterization of this subpopulation of hBM-MSC capable of long-term survival following ID injection could lead to improved cell therapy protocols for RDEB with long-lasting effects.

Funding Source: EB Research Partnership.

W1335

IDENTIFICATION OF NEURON-GLIA SIGNALING FEEDBACK IN HUMAN SCHIZOPHRENIA USING PATIENT-DERIVED, MIX-AND-MATCH FOREBRAIN ASSEMBLOIDS

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Although abnormal activities across multiple cell types are believed to contribute to the development of various neurodevelopmental disorders, current brain organoid technologies fall short in accurately modeling the dynamic cellular interactions in the human brain. Recently, we developed a cellular reconstitution technology to create human forebrain assembloids with enhanced cellular diversity, representing dynamic interactions between neurons and glial cells. Here, we created patient-derived, mix-and-match forebrain assembloids, in which neurons, astrocytes, and microglia from both healthy individuals and schizophrenia patients were reconstituted in a combinatorial manner, and identified aberrant cellular interactions between neurons and glial cells in human schizophrenia. At the early stage, schizophrenia forebrain assembloids showed premature neurogenesis induced by the abnormal proliferation and differentiation of neural progenitor cells. Integrated modular analysis of gene expression in post-mortem schizophrenia brain tissue and brain assembloids found increased expression of tumor protein p53 (TP53) and nuclear factor of activated T-cells 4 (NFATC4), which functioned as master transcriptional regulators to epigenetically reprogram the transcriptome involved in the cellular dynamics of neuronal progenitor cells, leading to premature neurogenesis. At the later stage, we observed weakened structures of laminar organization of the cortical layers in forebrain assembloids and identified the neuron-dependent transcriptional plasticity of glial cells and their altered signaling feedback with neurons, in which neuronal urocortin (UCN) and protein tyrosine phosphatase receptor type F (PTPRF) elicited the expression of Wnt family member 11 (WNT11) and thrombospondin 4 (THBS4) in astrocytes and microglia, respectively. These aberrant signaling axes altered the neuronal transcriptome associated with neuronal response to various stimuli and synthetic processes of biomolecules, resulting in reduced synapse connectivity. Thus, we elucidated developmental stage-specific, multifactorial mechanisms by which dynamic cellular interplay among neural progenitor cells, neurons, and glial cells contribute to the development of the human schizophrenia brain.

Funding Source: This research was supported by grants from NRF Korea (NRF-2022R1A2C3002702, RS-2023-00223277), Samsung Science and Technology Foundation (SSTF-BA2101-12), New Faculty Startup Fund from SNU, and the BK21FOUR Research Fellowship.

W1337

IMMUNE PHENOTYPE OF IPSC-DERIVED ASTROCYTES FROM PATIENTS WITH SPINOCEREBELLAR ATAXIA TYPE 17

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Spinocerebellar ataxia type 17 (SCA17) is an orphan neurodegenerative disease caused by trinucleotide repeat expansion in the TBP gene. SCA17 is characterized by severe symptoms and irreversible progression, leading to disabilities and death. The mechanisms of the disease pathogenesis are unclear. It is thought that neuroinflammation, driven primarily by proinflammatory reactive astrocytes, may contribute to SCA17 pathogenesis. To explore this hypothesis, we generated astrocytes from induced pluripotent stem cells (iPSCs) derived from TBP mutation carriers. This included two lines from SCA17 patients and one from an asymptomatic carrier. SCA17 astrocytes showed identical morphology and astroglial markers expression to the healthy controls. However, SCA17 astrocytes demonstrated elevated expression of the proinflammatory cytokine TNF. Analyses of transcriptome and secretome revealed significant upregulation of immune pathways in SCA17 astrocytes, with a stronger proinflammatory response observed in astrocytes from symptomatic patients than in those from the asymptomatic carrier. Our findings indicate that the TBP mutation induces a proinflammatory phenotype in astrocytes, suggesting a potential role of astrocytic immune dysregulation in the pathogenesis of SCA17.

Funding Source: This study was supported by a grant 075-15-2019-1669 from the Ministry of Science and Higher Education of the Russian Federation.

W1339

IN VITRO DISEASE MODELING OF FTLD USING PATIENT-DERIVED IPSCS IDENTIFIED POTENTIAL THERAPEUTIC AGENTS AND CELLULAR/MOLECULAR MECHANISMS.

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Frontotemporal Lobar Degeneration (FTLD) is a neurodegenerative disorder that primarily affects the frontal and temporal lobes. Clinically, FTLD is characterized by behavioral abnormalities, language deficits, and/or movement impairments, with no approved therapeutic drugs currently available. While most cases of the FTLD cases are sporadic, several gene mutations including GRN, MAPT, TARDBP and C9ORF72 are associated with its pathogenesis. Pathologically, FTLD is classified into subtypes such as FTLD-TDP and FTLD-Tau, based on the type of protein aggregation observed in the patients' brains. The purpose of this study is to identify potential therapeutic agents from existing drugs using patient-derived induced pluripotent cells (iPSCs). A

phenotypic screening was conducted using cortical neurons differentiated from FTLD patient-derived iPSCs harboring the GRN mutation. These patient-derived neurons exhibited accelerated cell death and lysosomal enlargement compared to neurons derived from healthy donors. From a library of small compounds, three drugs were identified as effective in suppressing cell death and lysosomal enlargement: Sulfisoxazole, Telmisartan, and Ropinirole (ROPI). In this study, we highlight ROPI as an example of these candidate compounds, presenting its potential efficacy across in vitro FTLD models. Subsequent investigations examined its effects on other FTLD cases, including five familial FTLD cases such as GRN mutant iPSCs (FTLD-TDP) and MAPT mutant iPSCs (FTLD-Tau) as well as four sporadic cases. ROPI consistently suppressed neuronal cell death in all familial cases and three out of four sporadic cases, suggesting that ROPI is effective in the majority of FTLD cases. Further analysis revealed that ROPI also improves several cellular and molecular phenotypes, including lysosomal abnormalities. These findings highlight the therapeutic potential of Sulfisoxasole, Telmisartan, and ROPI as promising candidates for FTLD treatment. Further investigations into their mechanisms offer new hope for developing effective treatments for FTLD.

W1341

INDUCED PLURIPOTENT STEM CELL-DERIVED MESENCHYMAL STEM CELLS IMPROVE CARDIAC FUNCTION THROUGH EXTRACELLULAR VESICLE-MEDIATED TISSUE REPAIR IN A RAT ISCHEMIC CARDIOMYOPATHY MODEL

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Induced pluripotent stem cell-derived mesenchymal stem cells (iPS-MSCs) are a novel type of stem cell which have a therapeutic effect on various diseases. Allogeneic iPS-MSCs are expected to be ideal cell sources because of their consistent quality and ability to avoid immune rejection. However, the therapeutic mechanism underlying systemic iPS-MSC-based therapy for ischemic cardiomyopathy (ICM) remains unclear. We investigated the therapeutic effects of iPS-MSCs through extracellular vesicle (EV)-mediated tissue repair in a rat model of ICM. We developed a rat ICM model by left anterior descending coronary artery ligation. iPS-MSCs were administered intravenously every week for four weeks in the iPS-MSC group, and Alix-knockdown iPS-MSCs in which Alix, a protein involved in the biogenesis of EVs, was knocked down by siRNA, were administered to the siAlix group. Left ventricular ejection fraction (LVEF) was significantly improved in the iPS-MSC group compared with that in the control of sham operation group. In the siAlix group, LVEF was significantly lower than that in the iPS-MSC group. Histological analysis showed a significant decrease in fibrosis area and a significant increase in microvascular density in the iPS-MSC group. A cell-tracking assay revealed iPS-MSC accumulation in the border zone of the myocardium during the acute phase. Comprehensive microRNA sequencing analysis revealed that EVs from iPS- MSCs contained miRNAs associated with anti-fibrosis and angiogenesis. Gene ontology analysis of differentially expressed genes in myocardial tissue also showed upregulation

of pathways related to antifibrosis and neovascularization and downregulation of pathways linked to inflammation and T-cell differentiation. Our study revealed that systemic administration of iPS-MSCs improved cardiac function through EV-mediated angiogenetic and antifibrotic effects in an ICM, suggesting the clinical possibility of treating chronic heart failure.

W1343

INHIBITION OF SENESCENCE IN PROGERIA SKIN FIBROBLASTS CULTURED IN XENO-FREE MEDIA

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Hutchinson-Gilford Progeria Syndrome (HGPS) is a rare genetic disorder characterized by the accumulation of the abnormal progerin protein, which leads to restricted cell proliferation and premature aging. This study investigated the effects of xeno-free media on the proliferative capacity of HGPS fibroblasts. Commercially available HGPS skin fibroblasts were cultured in conventional Eagle's Minimum Essential Medium (EMEM) and xeno-free media, and the resulting changes were analyzed. HGPS cells cultured in xeno-free media exhibited significant alterations, including decreased cell size, absence of cell stacking, increased cell number, and reduced population doubling time. Additionally, there was an increase in BrdU-positive cells, elevated levels of phosphorylated retinoblastoma protein (Rb), decreased expression of p21 and progerin, enhanced mitochondrial health, and reduced β-galactosidase-positive cells. In conclusion, xeno-free media demonstrates the potential to inhibit the senescence of HGPS fibroblasts. Further research is warranted to elucidate the specific mechanisms underlying these effects.

Funding Source: RS-2022-NR-069586, KFRM-24B0101L1.

W1345

INTERACTION BETWEEN SYMPATHETIC NEURONS AND CARDIOMYOCYTES IN A PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELL TAKOTSUBO-MODEL

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Takotsubo syndrome (TTS), also known as stress-induced cardiomyopathy, is characterized by acute left ventricular dysfunction, typically occurring in the absence of stenosis. Since a dysregulated central autonomic nervous system leading to excessive sympathetic stimulation is

believed to play a role in the condition, TTS can be described as brain-heart syndrome. In previous studies, significantly elevated catecholamine levels in patients experiencing acute TTS were reported. Further, our previous research identified increased sensitivity to catecholamine-induced stress toxicity and a genetic predisposition in TTS using a TTS induced pluripotent stem cell (iPSC) cardiomyocytes (CM) model. This study aims to investigate whether iPSC-derived TTS sympathetic neurons (SN) influence iPSC-CM and whether they contribute to the development of TTS. First, we established a 3-step protocol, including neuronal progenitor aggregates and 2D monolayer stages, based on Kirino et al. 2018. SN were generated from TTS-iPSC-lines harboring different genetic variants (e.g. AHNAK mutations). Immunohistochemical and transcript analyses proved the commitment to SN by increasing levels of PHOX2B, TH, and DBH while flow cytometry of 25-day-old cultures indicated ~80% of the population as autonomic progenitors. Finally, spontaneous and KCl-inducible noradrenaline release proved SN functionality. TTS-lines secrete more noradrenaline than wild-type or AHNAK-rescue lines. However, SN differentiation capacity needs to be further quantified. In the next step, we co-cultured SN and CM and observed higher beating frequency compared to CM monoculture, indicating basal stimulation of CMs by functional SN. Functional connectivity of SN and CM was further validated by an increased beating rate after nicotine stimulation of SN. Overall, we developed an efficient protocol for differentiating functional iPSC-derived sympathetic neurons, enabling their co-culture with TTS-iPSC-cardiomyocytes to validate their functional interactions. Investigating the influence of TTS genetic predisposition on SN alongside CM is essential for identifying potential therapeutic targets for TTS. In the future, using a mix-and-match strategy, the impact of TTS-related neuronal mutations on the cardiac phenotype will be investigated.

W1347

INVESTIGATING FAN1 AS A GENETIC MODIFIER OF RETT SYNDROME IN HUMAN STEM CELL-DERIVED NEURAL PRECURSOR CELLS AND DORSAL FOREBRAIN ORGANOIDS

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Rett syndrome (RTT) is a rare neurodevelopmental disorder with limited treatment options. RTT is caused by mutations in the MECP2 gene, a key regulator of brain development. Individuals affected by RTT exhibit variable severity in clinical presentation, which cannot be completely explained by mutation type alone. Therefore, we consider genetic modifiers, as they can alter disease severity and may inform therapeutic targets. A previous RTT modifier screen in Mecp2/Y mice found that deleterious mutations in the DNA repair gene Fan1 increased longevity and improved health in the animal model. To determine the translational relevance to the primarily female RTT population, we aim to examine if FAN1 ablation alters 2D and 3D RTT human induced pluripotent stem cell (hiPSCs) derived models for molecular phenotypes relevant to RTT disease biology. An isogenic RTT hiPSC line was edited to insert a stop codon in exon 1 of FAN1, resulting in an RTT FAN1KO line. The RTT FAN1KO, RTT and isogenic control hiPSCs were differentiated

into neural precursor cells (NPCs) and bulk RNA sequencing was performed. Ablation of FAN1 resulted in over 50 DEGs between RTT and RTT FAN1KO NPCs. Gene set enrichment analysis found that ablation of FAN1 in RTT NPCs resulted in the upregulation of neuronal processes such as “regulation of neurotransmitter levels”, “positive regulation of synaptic transmission,” and “regulation of dendritic extension,” suggesting that FAN1 ablation may alter aberrant neuronal morphology and activity in RTT. At the single-cell transcriptional level, RTT dorsal forebrain organoids (DFOs) indicate aberrant neuronal processes. Initial single-nuclei RNA sequencing (snRNA-seq) data of RTT DFOs show downregulation of neuronal processes such as “regulation of post-synapse organization and regulation of neuron migration” in RTT inhibitory neurons compared to control. Further analysis with the deep learning model NEUROeSTIMater found that RTT inhibitory and corticofugal projection neurons showed increased neural activity compared to control. Further functional experiments will be performed to investigate the impact of FAN1 ablation on neuronal activity and morphology in RTT DFOs. These transcriptional level analyses have revealed aberrant neuronal processes in RTT that may be ameliorated via FAN1 ablation.

Funding Source: This project was supported by Brain Canada, CIHR, NSERC, Children's Hospital Research Institute of Manitoba, Research Manitoba, the Ontario Rett Syndrome Association and NSERC Canada Research Chair funding.

W1349

INVESTIGATING SARS-COV-2 SPIKE VARIANTS IN PSC-DERIVED CARDIOMYOCYTES FOR EARLY CARDIAC DEVELOPMENT

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Recent studies suggest that maternal SARS-CoV-2 infection may influence the cardiovascular health of infants, potentially contributing to heart disease after infection as well as impacting early cardiac development. Human pluripotent stem cell–derived cardiomyocytes (PSC-CMs), which retain an immature phenotype resembling fetal/neonatal heart cells, offer a valuable model to investigate how the spike protein of SARS-CoV-2 might alter cardiac function. Here, we employed lentiviral pseudoviruses encoding spike proteins from the Wuhan, Delta, and Omicron strains of SARS-CoV-2 to elucidate spike-mediated effects in human embryonic stem cell–derived cardiomyocytes (hESC-CMs) and human induced pluripotent stem cell–derived cardiomyocytes (hiPSC-CMs). After a 24-hour exposure, transcriptomic profiling revealed dysregulation of calcium-handling and ion channel genes, hinting at compromised electrophysiological stability. Functional assays demonstrated irregular contractions and enhanced beat-to-beat oscillations, indicating a proarrhythmic risk. Fluorescence microscopy further showed sarcomeric disarray and syncytia formation, consistent with structural remodeling. Notably, each spike variant triggered distinct disruptions in calcium homeostasis, underscoring the variant-specific nature of spike protein pathogenicity. By using lentiviral pseudoviruses to isolate the effects of the spike protein, our findings offer insights into how SARS-CoV-2 variants can directly affect fetal/neonatal-like cardiomyocytes and potentially contribute to both post-infection heart disease and developmental cardiac abnormalities. These results underscore the utility of PSC-CMs in modeling virus-induced cardiac dysfunction within a developmentally relevant context, ultimately informing strategies to mitigate cardiovascular risks in young populations, including those born to SARS-CoV-2–infected mothers.

Funding Source: NSTC112-2636-M-006-010, NSTC112-2321-B-006-012, NSTC113-2320-B-006-047, NSTC113-2321-B-006-012.

W1351

INVESTIGATING THE EARLY CELLULAR CHANGES ASSOCIATED WITH LRRK2 G2019S MUTATION

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The G2019S mutation in LRRK2 kinase is the most common genetic mutation among patients with Parkinson's disease (PD). Although the G2019S mutation is known to increase the kinase activity of LRRK2, more work is needed to determine which LRRK2-regulated biological processes are primarily responsible for the pathogenesis of PD. Using CRISPR/Cas9, we generated isogenic induced pluripotent stem cells (iPSCs) containing different combinations of LRRK2 alleles – G2019S/G2019S, G2019S/wt, wt/wt, knockout (KO)/wt, KO/KO. This set of iPSC lines represents the entire spectrum of LRRK2 kinase activity from maximal (G2019S/G2019S) to null (KO/KO) and can be a helpful tool to clarify abnormalities caused by increased LRRK2 activity. We differentiated these iPSCs into dopaminergic neurons (DAns), which are lost in PD progression, and performed omics analyses. Both transcriptomic and proteomic analyses detected upregulation of nucleic acid metabolic pathways in mutant DAns. Phosphorylation of some RNA-binding proteins correlated with LRRK2 kinase activity, suggesting their role as physiological substrates of LRRK2. Transcriptomic and proteomic data also revealed altered composition of extracellular matrix which was subsequently validated throughout the stages of neuronal differentiation. Furthermore, multi-omics results indicated upregulation of innate immune response correlated with LRRK2 kinase activity. In addition, we observed an aberrant store-operated calcium entry (SOCE) in DAns with

the G2019S mutation. High SOCE is known to increase mitochondrial calcium level, so we hypothesized that immune response in mutant DAns might be caused by mitochondrial DNA (mtDNA) leakage. While exploring immune signaling in DAns, we showed that LRRK2 knockout DAns have higher basal expression of type-I interferon (IFN-I) and interferon-stimulated genes, whereas all isogenic DAns have impairments in IFN-I response after extracellular mtDNA treatment. We are now investigating the role of such impairments that may elucidate possible dysregulation of neuronal immune signalling in PD. Taken together, the diverse set of isogenic genome-edited iPSCs in combination with omics approaches helps identify early pathogenic consequences of the G2019S mutation associated with increased LRRK2 kinase activity.

Funding Source: This work was supported by grant 075-15-2019-1669 from the Ministry of Science and Higher Education of the Russian Federation.

TRACK: ETHICS, POLICY AND STANDARDS

W1353

CURRENT REGULATIONS ON HUMAN EMBRYO RESEARCH AND THE ORIGINS OF THE 14-DAY RULE IN JAPAN

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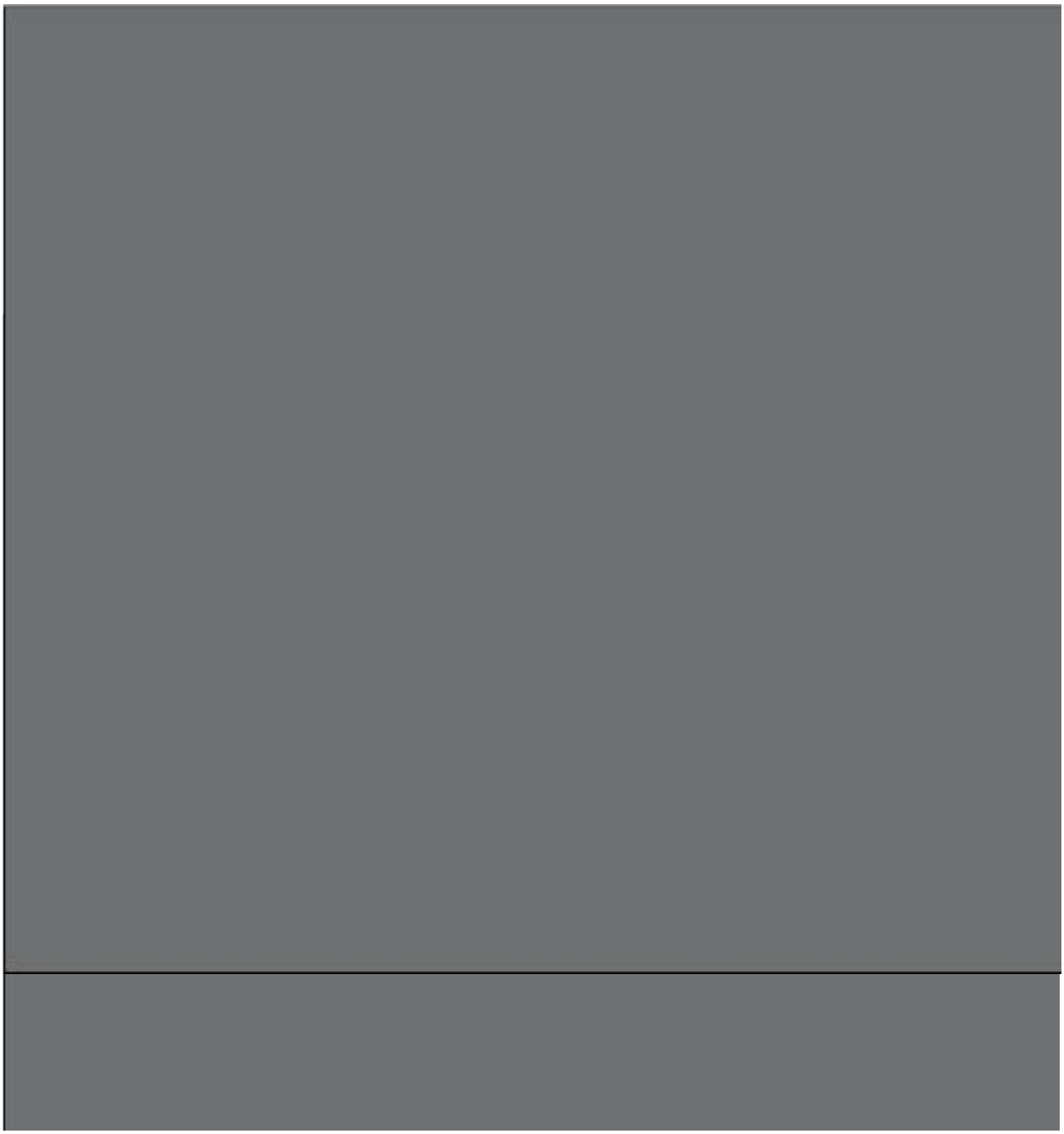
The 2021 ISSCR guidelines did not prohibit the cultivation of human embryos beyond 14 days, prompting discussions in various countries, including Japan, about revising the so-called 14-day rule. The rule's acceptance has been shaped by unique national contexts, and without understanding them, discussions on revision may fail to create culturally and socially appropriate regulations. However, in Japan, little attention has been paid to how this rule was historically adopted. This study reviews Japan's current regulatory framework for human embryo research and examines how the 14-day rule was incorporated. This study examines current government guidelines and analyzes meeting records from their establishment. Japan's human embryo research guidelines are complex. Different guidelines exist for research such as new embryo creation, ES cell derivation from surplus embryos, and genome editing of surplus embryos. Meanwhile, creation of clone embryos and specific chimera embryos follow the same guidelines. These regulations commonly include the 14-day rule and require dual ethical review—by an institutional and a government ethics committee—justified by the notion that embryos are the "germ of life" and require careful handling. In contrast, research using surplus embryos outside these categories follows general human research guidelines without requiring dual review, revealing a double standard. Moreover, while decisions on extending embryo culture beyond 14 days depend on individual ethical reviews, the general guidelines do not explicitly regulate the 14-day rule. The rule's origin in Japan traces back to the 1985 Japan Society of Obstetrics and Gynecology guidelines, though related discussions remain unclear due to a lack of records. Government ethical guidelines emerged in the 2000s, and in 2001, separate guidelines were

introduced—one for ES cell derivation and another for chimera embryo creation—both incorporating the 14-day rule. However, meeting records from that time show little in-depth discussion of the rule itself. In Japan, the 14-day rule was treated as a given in government guidelines. To reconsider its application, it is necessary to reassess its significance within Japan's cultural and societal context from the ground up.



W1357

ETHICALLY SAFE HUMAN-MOUSE CHIMERISM FOR HUMAN ORGANOGENESIS WITH NEURAL AND GERMLINE EXEMPTIONS

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The potential of human pluripotent stem cells to differentiate into all the cell types from the three germ layers forms the basis for their utilisation in developmental study and tissue regeneration. Blastocyst complementation with such cells has been attempted to generate human organs in animals for patients in need, however it causes ethical concerns that human cells may contribute to the central nervous system (CNS) and germlines in the resultant chimera after injection into an animal blastocyst. Here, we knocked out BLIMP1 and PAX6, essential for germline and neural development, respectively, in human embryonic stem cells (hESCs) to prevent human cell contribution into the CNS and germlines in human-mouse chimeras. Indeed, hESCs with the double knockout (DKO) failed to differentiate into germ cells and neural cells in vitro and in vivo. To enhance the chimeric efficiency, we engineered inducible BCL2 (iBCL2) in the wild-type and DKO hESCs. DKO/iBCL2 hESCs injected into the mouse blastocyst formed chimeric fetuses at a high success rate with human cells present throughout the body except neurons in the CNS and germ cells in the gonads. Igf1r knockout in the mouse blastocyst increased human cell contributions to the chimeric embryo with the exceptions as above. Further, mesenchymal stem cells differentiated from DKO/iBCL2 hESCs remarkably rescued chondrogenesis in chimeric fetuses formed with Sox9+/- mouse and contributed to mesenchymal tissues in neonates. Thus, this study demonstrates that deletion of BLIMP1 and PAX6 prevents hESCs from germline and neural contributions in human-mouse chimeras while sustaining possibly most other developmental potency. This paves a way to generating human organs in animals with much reduced ethical concerns.

Funding Source: Ministry of Science and Technology of China National Key Research and Development grant, National Science Foundation China general grant, Macau Science and Technology Development Fund (FDCT) grants, and University of Macau grants to R.X.

TRACK: SOMATIC STEM CELLS AND CANCER (SSCC)

Poster Session 1 (EVEN)

W1002

AUTOMATING RESIDUAL BEAD QUANTIFICATION IN CAR-T THERAPY USING IMAGING FLOW CYTOMETRY

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Gene-modified cell therapies, such as Chimeric Antigen Receptor T-cell (CAR-T) therapy, have revolutionized cancer treatment and show potential for treating genetic disorders and autoimmune diseases. The manufacturing process involves isolating, activating, and expanding human T cells, for which CD3/CD28 Dynabeads offer a streamlined solution. These beads have been used in over 200 clinical trials; however, since they are non-biodegradable, their removal from the final product is crucial. Newer systems with detachable beads still consider residual beads as contaminants, with the FDA requiring fewer than 100 beads per 3 million cells in the final product. Consequently, a precise and accurate assay for detecting and quantifying residual beads is essential for product release testing. Current methods are either labor-intensive, have low throughput, or require specialized equipment. This study introduces a novel imaging flow cytometry-based assay designed to improve quality control and reliability in product release testing. The study employed the Attune CytPix flow cytometer, which combines traditional cytometry with high-content imaging, making it ideal for detecting rare events like residual beads in cell products. Over 300,000 images per sample were captured and analyzed using the instrument's software to assess object and pixel attributes. Twenty-six image analysis parameters were explored in various combinations to determine the optimal gating and analysis strategy for distinguishing cells and beads. The analytical procedure was validated according to ICHQ2R2 guidelines to ensure consistency, accuracy, and reproducibility. The method proved reproducible across different users, instruments, and labs, with a lower detection limit of 20 beads and a lower quantification limit of 20 beads per million cells. This novel assay offers a reliable alternative to existing residual bead detection methods and can be implemented on a platform commonly available in cell therapy manufacturing facilities.

W1004

NEURONAL DIFFERENTIATION ON HUMAN STEM CELLS FROM APICAL PAPILLA INDUCED BY QUERCETIN TREATMENT

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Neurodegenerative diseases are caused by dysfunction of neuronal cells, determining clinical presentation. Exploring alternative cell sources that have the neuronal differentiation’s potential should be investigated for further transplantation. Human stem cells from apical papilla (hSCAPs) are derived from ectomesenchyme origin that exhibited the potential for neuronal differentiation. Quercetin is defined as a natural flavonoid compound that can promote neuronal differentiation. Therefore, this study aims to demonstrate the potential effect of Quercetin on neuronal differentiation of hSCAPs. The hSCAPs were isolated from extracted human-impact third molars of Thai patients and characterized as mesenchymal stem cells (MSCs). Consequently, the cell viability of Quercetin pre-treatment (0-80 μM) on hSCAPs was performed by using a colorimetric (MTT) assay. The non-cytotoxic concentrations were selected to investigate neuronal gene

expression (NES, TUBB3) by using qRT-PCR and determined the highest potential concentrations. Neurogenic induction was sequentially performed to completely induce neuronal differentiation. The characterization of neuronal-like cells was evaluated by cell morphology, β-III tubulin immunofluorescence staining, and Cresyl violet staining. The results demonstrated that the pre-treatment of Quercetin at 0-40 μM showed non-cytotoxicity for 24 hours and cell morphology presented a fibroblasts-like shape to indicate the undifferentiated cells. The highest expression of NES and TUBB3 was observed at 2.5 μM of Quercetin, which was determined as the optimal concentration (Quercetin-hSCAPs). Under neurogenic induction, hSCAPs and Quercetin-hSCAPs differentiated into neuronal-like cells, which positively expressed β-III tubulin by immunofluorescence staining. Cresyl violet staining demonstrated that neuronal-like cells revealed round cell bodies with neurite outgrowth and intensely expressed Nissl substance (a typical neuronal hallmark). Whereas, the undifferentiated cells still presented fibroblast-like morphology. Interestingly, the highest percentage of Cresyl violet positive cells was detected at optimal concentration of Quercetin with neurogenic induction that indicated the potential of Quercetin on neuronal differentiation.

W1006

TRANSCRIPTION ELONGATION FACTOR SPT6 MAINTAINS EPIDERMAL HOMEOSTASIS AND SUPPRESSES SKIN INFLAMMATION IN MICE

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Epidermal stem/progenitor cells (EPSCs) are vital for skin homeostasis, protecting against infections and preventing water loss. Disruption of EPSC development can lead to severe skin disorders. We have shown previously that SPT6, a histone chaperone, promotes EPSC differentiation by facilitating transcriptional elongation. However, its role in skin homeostasis in vivo is unclear. Since systemic SPT6 deletion causes embryonic lethality, studies on SPT6 knockout (KO) mice are rare. We are the first to generate an EPSC-specific SPT6 knockout mouse model. These mice exhibited severe scaly crusting, neutrophilic microabscesses, delayed hair follicle development, and impaired wound healing. Transmission electron microscopy revealed increased keratohyalin granules in the granualar layer, decreased cell-cell junction, relaxed euchromatic nucleus, and disrupted basement membrane in the SPT6 KO epidermis. RNA-Seq data showed strong correlations with psoriasis and atopic dermatitis gene signatures. Using single-cell RNA sequencing and ChIP-seq, we uncovered the molecular mechanisms by which SPT6 regulates skin homeostasis. This study investigated the role of SPT6 in adult stem cells in vivo for the first time and demonstrated that SPT6 regulates skin inflammation, hair follicle development, and wound healing, offering new therapeutic targets for skin diseases like psoriasis.

Funding Source: This work was supported by the National Natural Science Foundation of China (82273561,82073469 and 82473557 to J.L), Natural Science Foundation of Guangdong Province (2023A1515010146 and 2024A1515013194).

W1008

DEVELOPMENT OF A DIGITAL ANALYSIS SYSTEM FOR AN IN VITRO CELLULAR TRANSFORMATION ASSAY USING A NOVEL 3-DIMENSIONAL CULTURE METHOD

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In the manufacture of human cell-based therapeutic products (hCTPs), the presence of malignantly transformed cells represents a significant safety concern. Although such cellular impurities can be assessed by detecting anchorage-independent growth in the conventional soft agar colony formation (SACF) assay, its sensitivity is often insufficient. To overcome this limitation, we previously developed a novel tumorigenicity-associated testing method called the digital SACF assay (D-SACF), which combines partitioned culture of test cells to concentrate target cells with colony detection through image analysis. Recently, we reconfirmed its effectiveness after verifying its feasibility at multiple facilities. However, conventional soft agar culture involves complicated operations, such as preparing multi-layered culture media and controlling temperature, and further technical optimization is necessary for the widespread use of the D-SACF assay. In this study, we developed a new assay that incorporates a three-dimensional culture method using a culture medium with a commercially available low-molecular-weight agar polymer (LA717, developed by Nissan Chemical Corporation, Japan) in low-adhesion 96-well plates. This approach enables control of cell migration and uniform dispersion while facilitating the detection of colonies derived from transformed cells through image analysis. We evaluated the performance of the test system using mesenchymal stromal/stem cells as the product model and HeLa-GFP cells as the transformed cell model. The results indicated that the new liquid/low-molecular-weight agar colony formation (LACF) assay is easier to operate than conventional methods and can detect transformed cells quickly and accurately. Based on these findings, we have established a digital analysis system for the LACF assay (D-LACF assay), which streamlines the overall workflow from performance evaluation of the test method to product testing and result interpretation. This evaluation system is expected to serve as a promising approach for enhancing the quality and safety of hCTPs.

Funding Source: Japan Society for the Promotion of Science Grant-in-Aid for Scientific Research (C) (#18K12137).

W1010

EPIGENETIC CONTROL OF ADULT STEM CELLS AND ITS IMPLICATION IN AGING: EXPLORING THE MULTIFACETED REGULATORY ROLE OF KDM3A

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Adult stem cells, such as mesenchymal stem cells (MSCs) and neural stem cells (NSCs), are essential for maintaining tissue homeostasis and promoting regeneration. However, aging significantly impairs the function of adult stem cells, characterized by reduced responsiveness to tissue injury, disrupted proliferative potential, and diminished functional capacity. These age-associated changes ultimately compromise cell replacement and tissue regeneration in older organisms. In this study, we identify two H3K9 demethylases, KDM3A and KDM4C, as key regulators of heterochromatin reorganization during MSC senescence. Our findings reveal that KDM3A and KDM4C transcriptionally activate condensin components NCAPD2 and NCAPG2, which are critical for maintaining proper chromosome organization. MSCs derived from Kdm3a−/− mice exhibit defective chromosome architecture, heightened DNA damage responses, and accelerated bone aging. Furthermore, our recent work highlights the critical role of KDM3A in NSCs. Loss of Kdm3a, either globally or specifically in NSCs, impairs hippocampal neurogenesis and results in persistent deficits in learning and memory throughout adulthood in mice. In vitro, Kdm3a deficiency reduces proliferation and neuronal differentiation while promoting glial differentiation in NSCs. Mechanistically, we show that KDM3A localizes to both the nucleus and cytoplasm of NSCs, where it regulates the Wnt/β-Catenin signaling pathway via dual mechanisms. These findings underscore the pivotal roles of KDM3A and KDM4C in maintaining adult stem cell function during aging and provide new insights into their contributions to tissue regeneration and age-related decline.

Funding Source: The work is supported by NSFC (82472409), Guangdong Province Basic and Applied Basic Research Fund (2024A1515012929). This work is also supported by Hong Kong University Grants Committee (GRF 14111519, GRF14116622, GRF14112223).

W1012

STEM CELL MODEL TO IDENTIFY GENETIC INTERACTIONS WITH BRCA2

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BRCA1 and BRCA2 are tumor-suppressor genes that play critical roles in DNA homologous recombination and repair. Deficiency in BRCA1 and BRCA2, caused by mutations in these genes, are frequently associated with breast, ovarian, and other cancers. While homozygous BRCA1/2 mutations are known to promote genomic instability and tumorigenesis by compromising the HR pathway, heterozygous mutations do not impair HR, and their contribution to cancer development remains unclear. Since BRCA1 and BRCA2 proteins are involved in DNA replication, we hypothesize that heterozygous mutations in their genes may increase replication stress. Using the DNA fiber assay, we examined replication dynamics in cells expressing either wild-type BRCA1/2 or heterozygous mutations in one of these genes. We found that the heterozygous mutant strains produced shorter DNA fibers, indicating that the mutations impaired DNA replication. Moreover, treatment with nuclease S1 during the assay further decreased fiber lengths in mutant cells, suggesting an accumulation of single-stranded DNA (ssDNA) gaps. Together, our study shows that heterozygous BRCA1/2 mutations indeed increase replication stress, providing new insights into their molecular function in DNA replication and suggesting a potential mechanism by which they promote cancer development. In addition to studying the BRCA1/2 heterozygous mutations, I plan to use a stem cell model to identify genetic interactors of BRCA2 to better understand its function. BRCA2 deletion in embryonic stem cells (ESCs) causes lethality due to impaired DNA homologous recombination. It was recently reported that the deletion of another gene, PTIP, rescues this

lethality by protecting BRCA2-deficient cells from DNA damage. Based on these findings, I propose to carry out a CRISPR knockout-based genetic suppressor screen in BRCA2 conditional deletion ESCs in order to find additional factors that are involved in BRCA2-mediated DNA damage repair pathways. Together, we hope our research will help to better understand the underlying mechanisms behind BRCA1/2 mutation-associated cancers and identify potential future therapeutic targets.

Funding Source: This research project is supported by a grant from the National Cancer Institute to Dr. Lee Zou (CA263934) at Duke University.

W1014

UTILIZING TETRASPANIN-BASED ISOLATION AND MULTIPLEXING ASSAY FOR CHARACTERIZING MESENCHYMAL STEM CELLS-DERIVED EXOSOME

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Mesenchymal stem cells (MSCs) are highly recognized in regenerative medicine due to their multipotent differentiation and immunomodulatory properties. Recent studies suggest that the immunomodulatory effect of MSCs is mainly mediated through paracrine factors, with exosomes being critical players. This study focuses on the immunomodulatory effects of MSCs-derived exosomes. MSCs were isolated from bone marrow and cultured in Cell Vive™ MSC serum-free, xeno-free media, GMP. Exosomes were isolated from the conditioned medium using a tetraspanin-based MojoSort ™ human microbead exosome isolation kit, with purity confirmed via flow cytometry, western blot, and nanoparticle tracking analysis. The cargo of MSCs-derived exosomes was characterized using LEGENDplexTM assay, a multiplex flow cytometry-based immunoassay system, revealing a diverse array of bioactive molecules crucial for tissue repair and immunomodulation. We investigated the immunomodulatory effects of these exosomes on CD3/CD28 activated T cells. Exosomes were depleted from the MSCs conditioned media, and T cells were cultured in conditioned media (CM) with and without exosome-depletion. Data demonstrates that exosomes-depleted MSCs CM exhibits reduced modulation potency in suppressing activated T cell proliferation, and cytokine profiles from MSCs-derived exosomes were analyzed to highlight potential cytokine candidates contribute the MSCs immunomodulation capabilities. These findings highlight the potential of MSCs-derived exosomes as a cell-free therapeutic option in regenerative medicine.

Funding Source: BioLegend.

W1016

DEEP LEARNING-BASED PREDICTIVE CLASSIFICATION OF FUNCTIONAL SUBPOPULATIONS OF HEMATOPOIETIC STEM CELLS AND MULTIPOTENT PROGENITORS

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Hematopoietic stem cells (HSCs) and multipotent progenitors (MPPs) play a pivotal role in maintaining lifelong hematopoiesis. The distinction between stem cells and other progenitors, as well as the assessment of their functions, has long been a central focus in stem cell research. In recent years, deep learning has emerged as a powerful tool for cell image analysis and classification/prediction. In this study, we explored the feasibility of employing deep learning techniques to differentiate murine HSCs and MPPs based solely on their morphology, as observed through light microscopy (DIC) images. After rigorous training and validation using extensive image datasets, we successfully developed a three-class classifier, referred to as the LSM model, capable of reliably distinguishing long-term HSCs (LT-HSCs), short-term HSCs (ST-HSCs), and MPPs. The LSM model extracts intrinsic morphological features unique to different cell types, irrespective of the methods used for cell identification and isolation, such as surface markers or intracellular GFP markers. Furthermore, employing the same deep learning framework, we created a two-class classifier that effectively discriminates between aged HSCs and young HSCs. This discovery is particularly significant as both cell types share identical surface markers yet serve distinct functions. This classifier holds the potential to offer a novel, rapid, and efficient means of assessing the functional states of HSCs, thus obviating the need for time-consuming transplantation experiments. Our study represents the pioneering use of deep learning to differentiate HSCs and MPPs under steady-state conditions. This novel and robust deep learning-based platform will provide a basis for the future development of a new generation stem cell identification and separation system. It may also provide new insight into the molecular mechanisms underlying stem cell self-renewal.

Funding Source: NHLBI.

W1018

INTRANASAL DELIVERY OF DENTAL PULP STEM CELL-DERIVED EXOSOME-ENCASED PHLOROGLUCINOL MITIGATES DEFICITS AND PROMOTES NEUROGENESIS IN IN VIVO CHRONIC MODEL OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is characterized by dopaminergic (DA) neuron degeneration in the substantia nigra pars compacta (SNpc) driven by oxidative stress, inflammation, and impaired neurogenesis. Dopamine analogues lack antioxidative, anti-inflammatory, and regenerative effects, highlighting the need for non-invasive supportive therapies in PD. Phloroglucinol, a polyphenolic antioxidant, has demonstrated neuroprotective effects in PD models but suffers from limited clinical applicability due to poor blood-brain barrier (BBB) permeability. These compounds need a delivery system that bypasses the gut, crosses the BBB, targets injured regions via inflammatory cues, and ideally offers immunomodulatory benefits. Exosomes derived from dental pulp stem cells (DPSCs) exhibit neuroprotective and immunomodulatory properties and serve as promising vehicles for targeted drug delivery across the BBB. This study aimed to evaluate the therapeutic efficacy of intranasally administered exosome-encased phloroglucinol (Exo-Phl) in a chronic MPTP rat model

of PD. Exosomes displayed high purity and homogeneity. Exo-Phl significantly reduced oxidative stress both in vitro and in vivo, as indicated by decreased ROS and lipid peroxidation levels. Exo-Phl treated MPTP rats demonstrated marked improvement in motor and non-motor behaviours compared to MPTP rats. Immunohistochemical analysis revealed increased TH-positive neurons and enhanced neurogenesis in the SNpc of Exo-Phl-treated animals. Biodistribution studies confirmed efficient midbrain targeting of exosomes, which were localized to dopaminergic-neurons, astrocytes and microglia. Exo-Phl also significantly reduced TNF-α expression, indicating decreased neuroinflammation. This study provides the first instance of using DPSC-derived exosomes as a delivery vehicle for phloroglucinol in a PD model. Exo-Phl demonstrated significant neuroprotective-effects, enhanced DA-neuron survival and neurogenesis, and reduced neuroinflammation. Intranasal delivery of Exo-Phl represents a promising non-invasive therapeutic strategy for PD, offering a dual benefit of antioxidative and neurogenic support.

Funding Source: Indian Council for Medical Research (ICMR).

W1020

TARGETING C5AR1 SIGNALING TO OVERCOME IMMUNE EXCLUSION AND ENHANCE IMMUNOTHERAPY IN COLORECTAL CANCER

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Immunotherapy has shown limited efficacy in colorectal cancer (CRC) primarily due to the immune-excluded tumor phenotype. In this context, immune cells, particularly CD8+ T cells, tend to accumulate at the tumor periphery rather than infiltrating the tumor core. Our studies focus on cancer stem cells (CSCs) and their tumor microenvironment. We revealed that myeloid-derived suppressor cells (MDSCs) play a key role in immunosuppressive niche, which is in part mediated by the C5AR1 signaling module. Using the MC38 mouse colon cancer model, we treated mice with an C5AR1 inhibitor and resulted in about 40% reduction in tumor mass. To investigate tumor and the associate microenvironment, we take the following approaches including imaging, single-cell secretome analysis, scRNA-sequencing, and spatial transcriptomics. We found that inhibition of C5AR1 increased infiltration of immune cells including macrophages, CD8+ T cells, and NK cells to the core of tumors from the tumor periphery. Additionally, Single-cell secretome analysis using Isoplexis confirmed the downregulation of immunosuppressive signals (TGFβ, IL-10) and upregulation of inflammatory signals (TNFα, IFNγ). We further validated our finding using the organoid-transplanted colorectal cancer (CRC) model carrying Apc, Ras, and P53 mutations with Xenium spatial transcriptomics. This technology utilizes a probe-based hybridization method for precise spatial profiling. Intriguingly, Inhibition of C5AR1 converted the CRC from immune-excluded into immune-inflamed, thus enhancing CRC immune responses by disrupting the CSC-MDSC interaction

W1022

THE THERAPEUTIC POTENTIAL OF ADSC-SECRETED LEFTY2 IN TREATING ALZHEIMER'S DISEASE

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Adipose-derived mesenchymal stem cells (ADSCs) have exhibited promising therapeutic potential in Alzheimer's disease (AD), although the underlying mechanisms remain poorly understood. Previously established Alzheimer's disease neuron model derived from Ts21-induced pluripotent stem cells (Ts21-iPSCs) has been shown to exhibit progressive β-amyloid accumulation during neuronal differentiation. In this study, we employed a Transwell co-culture system to investigate the interaction between neurons derived from Ts21-iPSCs and ADSCs. Our findings revealed that co-culture with ADSCs significantly enhanced the survival rate of AD neurons. Proteomics analysis identified a significant upregulation of LEFTY2 protein in the co-culture medium. Supplementation with 2 nM LEFTY2 markedly improved the survival and growth of AD neurons. Western blot analysis confirmed that LEFTY2 increased the expression of key synaptic proteins, including postsynaptic density protein 95 (PSD-95) and synaptophysin (SYN). Additionally, immunofluorescence staining and Western blot analysis demonstrated that LEFTY2 effectively reduced the expression of β-amyloid 1-42 in AD neurons, potentially through downregulation of apolipoprotein E4 (APOE4). Furthermore, LEFTY2 attenuates phosphorylated tau231 levels and regulates Trem2 and MIF in AD neurons. These results collectively suggest that LEFTY2 not only promotes neuronal growth but also effectively reduces β-amyloid production in AD-iPSC-derived neurons, highlighting its potential as a promising therapeutic candidate for Alzheimer's disease.

W1026

ENGINEERING CAR MACROPHAGES: ESTABLISHING A REPRODUCIBLE BIOPROCESS USING HSC AND IPSC-DERIVED CELLS

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Chimeric antigen receptor macrophages (CAR-M) represent a promising immunotherapy candidate for solid tumours, leveraging macrophages' ability to infiltrate and remodel the tumour microenvironment. Yet CAR-M manufacturing is hindered by donor variability, limited scalability, and low transduction efficiency, posing challenges for clinical translation. We have established a standardized bioprocess to generate CAR-M from induced pluripotent stem cells (iPSCs) and umbilical cord blood-derived CD34+ hematopoietic stem cells (CB-HSCs), providing a reproducible alternative cell source to monocyte-derived macrophages. CB-HSCs were expanded 51-fold over 7 days with 97.47% viability using a defined cytokine cocktail (hSCF, hFLT3L, hTPO, IL-3), followed by differentiation into macrophages over 10 days, yielding cells with characteristic morphology and ~90% expression of CD45, CD14, and CD11b. Flow cytometry analysis of transduced HSCs demonstrated a transduction efficiency of up to 50% via GFP expression. The sorted GFP^+ population maintained high cell viability and 95% of CD34 expression. Genetic modification with a lentiviral vector encoding an anti-CEA CAR with dual signalling domains successfully generated functional CAR-Ms. Live-cell imaging confirmed progressive, antigen-dependent phagocytosis of

MC-38-CEA cells, increasing over time and peaking at 48 hours, accompanied by rising pro-inflammatory cytokine secretion. The dual-signalling CAR conferred M1 polarization and superior tumour cell engulfment compared to non-transduced HSCs-derived macrophages. This process establishes a reproducible framework for CAR-M production, addressing key challenges in standardisation and manufacturing for next-generation macrophage-based immunotherapies.

Funding Source: Government of the Kingdom of Saudi Arabia.

W1028

GLUTATHIONE REGULATION IN STEMNESS AS A PREDICTIVE AND THERAPEUTIC TRAIT FOR NEOADJUVANT CHEMOTHERAPY RESPONSE IN BLADDER CANCER

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Radical cystectomy with preoperative cisplatin-based neoadjuvant chemotherapy (NAC) is the standard care for muscle-invasive bladder cancers (MIBCs). However, the complete response rate to this modality remains relatively low, and current clinicopathologic and molecular classifications are inadequate to predict NAC response in patients with MIBC. In previously study, we found that CDK1 phosphorylation of TFCP2L1, a pluripotency-associated transcription factor, orchestrated pluripotency and cell cycling in embryonic stem cells and was aberrantly activated in aggressive bladder cancers (Heo at al., 2019). Importantly, molecular programs involved in embryogenesis are frequently upregulated in oncogenic dedifferentiation and metastasis. In this study, we demonstrate that dysregulation of glutathione (GSH) pathway is fundamental for MIBC NAC resistance. Comprehensive analysis of the multicohort transcriptomes reveals that GSH metabolism and immune-response genes are enriched in NAC-resistant and NAC-sensitive MIBCs, respectively. A machine learning-based tumor/stroma classifier is applied for high-throughput digitalized immunohistochemistry analysis, identifying that GSH dynamics proteins, including glutaminase-1 (GLS), are associated with NAC resistance. GSH dynamics is activated in cisplatin-resistant MIBC cells and combination treatment with GSH dynamics modulator and cisplatin significantly suppresses tumor growth in an orthotopic xenograft animal model. Collectively, these findings demonstrate the predictive and therapeutic values of GSH dynamics in determining the NAC response in MIBCs.

Funding Source: This research was supported by the National Research Foundation of Korea

(NRF-2021R1A2C2005790, NRF-2019R1A2C1088246), the Ministry of Education (RS-2024-00448676, 2022R1I1A1A01066044).

W1030

REGULATION OF HEMATOPOIETIC CELL FATE IN ACTIVATED MESENCHYMAL STEM/STROMAL CELLS

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Hematopoietic stem/progenitor cells (HSPCs) maintain the homeostasis of the hematopoietic system in the bone marrow (BM). The ability of HSPCs to engraft and sustain long-term hematopoiesis is fundamental to the stem cell system in the bone marrow. BM transplantation therapy promotes platelet generation and early neutrophil differentiation, thereby reducing the risk of infection. Furthermore, the administration of stimulating factors during HSPC transplantation can induce the differentiation of myeloid cells. However, the factors that contribute to lineage determination in BM or transplanted cells remain unclear. In this study, we aimed to elucidate the effects of Mesenchymal stem/stromal cells (MSCs) on HSPC differentiation. To achieve this, HSPCs and MSCs were purified using a flow cytometer, and in vitro co-culture experiments were performed. The results showed that activated MSCs secrete the chemokine CCL2, which regulates the differentiation ability of HSPCs and promotes the production of myeloid cells. When CCL2 was knocked out in MSCs, the ability of HSPCs to differentiate into myeloid cells was significantly reduced. The main cell cluster secreting CCL2 upon lipopolysaccharide (LPS) stimulation was MSCs, which were found to regulate differentiation induction by targeting granulocyte/macrophage progenitors. Single-cell RNA sequencing analysis revealed that the stimulation of CCL2 significantly enhances the expression of RAS, highlighting its critical role. MSCs function as sensors for inflammation and infection and are thought to transmit inflammatory signals to hematopoietic cells in vivo. These findings support a model in which MSCs promote the recovery of essential immune cell compartments through CCL2 signaling.

Funding Source: This work was financially supported by the AMED (grant numbers 23bm1223011h0001); the JST, CREST Japan (grant number JPMJCR2124).

W1032

THE HIPPO/YAP PATHWAY IS INVOLVED IN HUMAN EPIDERMAL REGENERATION AND KERATINOCYTE DIFFERENTIATION

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The transcription cofactor YES-associated protein (YAP) regulates migration, proliferation, and the reaction to injury in a variety of tissues and organs. The Hippo pathway inhibits YAP activity and preserves it in the cytoplasm. The importance of the Hippo/YAP pathway for maintaining epidermal homeostasis was demonstrated by altering YAP activity in mouse skin through genetic modifications and by analyzing human skin samples from donors with different diseases. Active nuclear YAP was found in individual cells in the basal layer of the epidermis in healthy human skin. We verified that an abnormal active YAP distribution was present in pathology: most epidermal nuclei were YAP-positive in fibrotic circumstances, but YAP expression declined in chronic wounds. We recently demonstrated, using a human skin xenograft model, that YAP activity decreased during human skin regeneration, which is associated with a decline in keratinocyte proliferation and epidermal rete ridge repair. We applied primary epidermal keratinocytes, HaCaT and A431 cell lines, to describe the cell phenotype under YAP activation or suppression by western blot, immunohistochemistry, and qPCR. We activated YAP signaling using Hippo inhibitor Truli. It dramatically decreased the expression of KLF4 and keratin 1/10, which are linked to epidermal differentiation. Similarly, the expression of basal markers keratin 15 and collagen 17a was downregulated. This was accompanied by an increase in the rate of proliferation. We did not identify any significant alterations in basal keratin 5/14 or wound-response keratin 6 expression. We used shRNA to inhibit YAP expression. It increased expression of differentiation markers and suppressed proliferation. Both YAP activation and inhibition did not influence keratinocyte migration in the wound-scratch test. Summing up, active YAP correlates with low-differentiated and highly proliferative conditions of epidermal keratinocytes that correspond to transit-amplifying cell state rather than basal stem cell state or wound-response phenotype.

Funding Source: This research was funded by the Russian Science Foundation (project no. 21-74-30015-π, prolongation of rscf.ru/en/project/21-74-30015/ (accessed on 4 March 2025).

W1034

ANGIOGENIC PROFILING OF LONG-TERM BIOBANKED DENTAL PULP STEM CELLS

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Dental pulp stem cells (DPSCs) are considered prominent in regenerative dentistry for their multipotency, with biobanks established to preserve these cells for therapeutic purposes. However, the extent to which their angiogenic potential and ability to undergo endothelial differentiation are retained after long-term biobanking remains underexplored. Using high-throughput analyses, including RT-qPCR arrays targeting 42 angiogenesis-related genes, reverse-phase protein arrays profiling 141 CD markers, flow cytometry, and digital western blotting, we evaluated DPSCs stored

in a biobank for over five years. Pro-angiogenic functionality was validated using a microfluidic organ-on-chip system, a chicken chorioallantoic membrane (CAM) assay, and 3D culture in clinical-grade GelMA hydrogels. Endothelial induction resulted in the upregulation of key endothelial genes, including PECAM1 (CD31) and KDR (VEGFR2) at the mRNA level, though protein-level analyses revealed limited expression of endothelial markers, with cells retaining a predominantly pericyte-like expression profile. The organ-on-chip system confirmed that DPSCs, despite low CD31-positive cell counts, acted primarily as pericytes and myofibroblasts to generate perfusable, water-tight vascularised tissue when co-cultured with Human umbilical vein endothelial cells (HUVECs). However, when implanted into the CAM assay (i.e., chicken tissue), human-derived vasculature expressing CD31 was sporadically observed. Furthermore, 3D culture in GelMA hydrogel under endothelial induction for 45 days enabled the biofabrication of vascularised tissue solely by DPSCs. These findings highlight the strong pro-angiogenic potential of DPSCs, largely mediated through their pericyte-like functions, maintained after long-term biobanking. Additionally, under specific conditions, DPSCs may be capable of vasculogenesis solely through either enhanced endothelial differentiation or the selective expansion of endothelial progenitors within their population.

Funding Source: Olav Thon Foundation.

W1036

ATRA-BASED COMBINATORIAL APPROACHES TO BREAK EPIGENETIC BARRIERS IN ACUTE MYELOID LEUKEMIA

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Leukemia-initiating cells (LICs) are central to the pathogenesis and persistence of acute myeloid leukemia (AML) and are heavily influenced by epigenetic dysregulation. While ATRA has revolutionized treatment for APL-AML, its efficacy in non-APL AML remains limited due to epigenetic silencing of genes essential for ATRA signaling. This study aims to overcome these epigenetic barriers in AML by exploring ATRA-based combinatorial strategies with epigenetic modulators to restore pathway activity and target LIC populations. The RA-pathway activation was evaluated using HEK-293 cells transduced with the p-GreenFire-RARE-Tk-Luc construct, exposed to ATRA and a library of 650 epigenetic compounds. Selected compounds were assessed for their impact on cell viability, differentiation, and key signaling pathways (ERK1/2, AKT1, MAPK9, p38) in HL-60, NB4, KG1a, BMNC, and primary AML cells. RNAseq and CUT&Tag analyses were performed on ex vivo LIC-enriched AML samples and HL60 cells treated with the most effective combinations. Eleven compounds demonstrated potential, with a dual PI3K-HDAC inhibitor and a pan-PKC inhibitor showing the most synergy with ATRA, enhancing RARE activity 4-5 fold. Both

compounds were noncytotoxic to BMNC and primary MSC. Notably, the dual PI3K-HDAC inhibitor combined with ATRA achieved over 95% reduction in KG1α cell viability, significantly decreased the LIC population, and enhanced differentiation in AML samples (M1, M2, M4) by 2–4 fold compared to TCP (LSD1 inhibitor) or untreated controls. This combination also reduced the phosphorylation of LIC-associated survival pathways (p-MAPK and p-p38) while enhancing p53 activation. RNA sequencing revealed a distinct gene expression signature induced by the ATRA and drug combination treatment, highlighting its potential as a targeted therapeutic approach. Therefore, combining ATRA with dual PI3K-HDAC and pan-PKC inhibitors offers a promising strategy to target LICs and increase differentiation in AML by overcoming epigenetic resistance mechanisms. However, further validation using patient-derived xenograft models is crucial to confirm the potential of this approach in eradicating LIC populations and improving AML treatment outcomes.

Funding Source: The study was supported by the Polish National Science Centre grant no. 2019/33/B/NZ5/02399.

W1038

BIOREACTOR-BASED ORGANOID CULTURE: BOOSTING EFFICIENCY AND REDUCING COST

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Human organoid 3D cultures are models representing a precious asset for biomedical research, from fundamental discovery to precision medicine. Beyond access to the primary tissue and line establishment, the main limitations to the use of human-derived organoid models are the amount of time and skills required to maintain these cultures and also the costs associated with their production/maintenance (staff time and reagents). We describe a successful way to increase our organoid culture capacities while significantly decreasing the associated costs using a bioreactor. We measured the growth rate (i.e cell counting), compared the morphology (i.e H&E staining) and quantified the culture costs (staff time hours and reagents) in the bioreactor against our standard culture method (100% Matrigel embedded organoids overlayed with complete media in 24 well-plates). We tested different conditions in the bioreactor (various inoculation cell numbers and addition of 0%, 2% or 4% Matrigel) and compared with matching plates (standard culture condition). Our results generated using patient-derived colon and breast cancer organoids demonstrated that producing 10 million of single cell organoids was 4 times faster and 5 times cheaper in the bioreactor than in standard culture. Other non-quantitative metrics include the ease of use, the flexibility of the system allowing to successfully culture organoids from 6ml to 30ml volume of media and starting with a number of single cells as low as 150K. This is particularly valuable when the access to the primary tissue and/or the established organoid line is very limited.

W1040

CHALLENGES IN CULTURING EMBRYONIC MUSCLE PROGENITOR CELLS FOR WHITE MEAT PRODUCTION

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Chicken embryonic muscle progenitor cells (MPCs) are emerging as a promising alternative protein source, particularly appealing to health-conscious individuals due to their potential as a healthy, protein-rich cellular material. These cells offer advantages such as easy procurement, flexible collection timing, and enhanced proliferative capacity compared to adult cells, making them valuable for cell-cultured agriculture. This study evaluated the proliferative capacity, genetic stability, and safety of embryonic MPCs to assess their suitability for cultured meat production. MPCs were isolated from the thigh muscle of 12-day-old chicken embryos (three male and three female) and cultured in vitro to monitor proliferation and genetic stability. Comprehensive analyses, including transcriptomics, whole-genome sequencing, cell lifespan-related gene expression, and cell cycle dynamics, were performed. Notably, significant SNP and INDEL accumulation on chromosome 3 was detected in male MPCs at passage 7 compared to passage 3. Transcriptomic analysis revealed a decline in muscle-specific traits, particularly in pathways related to myofibrils and calcium ion-binding, beyond passage 5. Additionally, real-time PCR showed increased p21 expression and reduced TERT expression at passage 10, alongside a decreased S-phase proportion in the cell cycle. These findings underscore the need for optimized culture systems and safe gene-editing technologies to enhance MPC proliferation and muscle functionality for cultured meat applications.

Funding Source: This work was supported by the grant number PJ016711.

W1042

COREGULATION OF PI3K PATHWAY IN MESENCHYMAL STROMAL CELLS AND CANCER CELLS MODULATES THE SURVIVAL OF TRIPLE-NEGATIVE BREAST CANCER CELLS

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The secretome of mesenchymal stromal cells (MSCs) is reported to have tissue repair potential and anti-inflammatory effects. However, in the cancer microenvironment, the MSCs secretome was

reported to enhance the proliferation and migration of triple-negative breast cancer cells (TNBC). The PI3K pathway is activated in MSCs to secrete the procarcinogen factors CCL5, CCL2, and SDF1. In this work, we investigated the hypothesis that inhibition of the PI3K pathway in MSCs will downregulate the expression of pro-tumorigenic factors, enhance tissue repair, and promote the anti-carcinogenic effects of MSCs secretome. The invasive MDA-MB-231 breast cancer cell line was cultured alone or indirectly with MSCs. Cancer cells and MSCs were treated with PI3K inhibitor and evaluated for regenerative capacities and carcinogenic properties. Our data show that CM of PI3K-inhibited MSCs significantly decreased the proliferation of PI3K-inhibited MDA-MB-231cells (iCM-iMDA). The G2/M phase demonstrated a significant increase to 25.1% and a significant upregulation in the percentage of the polyploidy population to 9.51%. The Ki67 gene expression and protein levels were significantly downregulated. The proportion of viable cells significantly decreased to 74.7%. The proportion of the apoptotic population showed highly significant upregulation to 17.9%. Additionally, the percentage of necrosis demonstrates a significant upregulation to 7.42%. Down-regulation of Bcl2, upregulation of P53 and TNF alpha protein level were detected. There was no significance in the migration of iCM-iMDA group and no change in E-Cadherine. Dual inhibition of PI3K in MSCs and MDA-MB-231 cells diminished the proliferation of MDA-MB-231 cells. Ki67 proliferation marker was downregulated, and apoptosis and necrosis were increased as shown by down-regulation of Bcl2, upregulation of P53 and TNF alpha. The migration ability of MDA-MB-231 cells was not affected. Our data demonstrate that signaling between MSCs and MDA-MB-231 cells through the PI3k pathway supports MDA-MB-231 cells progression, and inhibition of PI3K in the tumor microenvironment may be used as adjuvant therapy for TNBC.

Funding Source: Grant #46721 from the Egyptian Science and Technology Development Fund, Cairo, Egypt Sawiris Foundation scholarship in Stem Cell Research 2024.

W1044

DELINEATING THE PROTEOME DYNAMICS OF ADULT MUSCLE STEM CELLS USING METABOLIC LABELING MODELS

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Quiescent adult stem cells are a reserve pool of stem cells that activate when tissue integrity is severely compromised. Being quiescent, they are known to have a reduced proteome size and metabolic activity. However, recent studies indicate that the quiescence of adult skeletal muscle stem cells (also known as satellite cells, SCs) is under active regulation through several mechanisms. In this study, we investigated the proteome dynamics of SCs by generating a SC-specific metabolic protein labeling mouse line. SCs expressing the mutant methionine-tRNA synthetase MetRS* can incorporate click-able methionine analogs into nascent proteins for detection by fluorescence imaging (FUNCAT) or mass spectrometry-based proteomics (BONCAT). Through pulse-chase labeling, we profiled the nascent and turned-over proteomes of quiescent SCs, revealing an extensive protein turnover during tissue homeostasis. Interestingly, we observed a remarkable heterogeneity of protein metabolic activity of quiescent SCs in vivo. By isolating the metabolically active and inactive SC subsets, we demonstrated that the metabolic activity can define their cell fates and molecular signatures.

Funding Source: Work was supported by the Hong Kong Research Grant Council

(GRF16100322, GRF16102021, GRF16101524), the University Grants Committee, and the Hong Kong Center for Neurodegenerative Diseases, InnoHK, HKSAR.

W1046

DNA SELEX AGAINST PANCREATIC CANCER BIOMARKER

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Pancreatic cancer (PC) has a high mortality rate with poor prognosis. The poor prognosis is partially due to the late detection of PC. At present, tissue biopsy, imaging, and blood testing are three major diagnostic methods for PC. Among these, blood testing is cheaper and more convenient for patients, but the sensitivity of existing blood testing tools is insufficient to screen early-stage PC patients. Now in the market, blood testing for the pancreatic cancer glycan biomarker CA 19-9 uses 1116NS19.9 antibody, but the sensitivity is challenging. Developing a high-sensitivity blood testing tool to detect early-stage PC is urgently needed. Aptamers hold promise as an alternative to antibodies and have the potential to be applied in biosensors along with the antibodies against multiple biomarkers. Here, we report an aptamer SELEX to select out aptamers which could be used in a PC aptasensor. We firstly expressed asprosin, one of the early-stage pancreatic cancer biomarkers. Then, a natural DNA aptamer SELEX was performed against asprosin. We discovered two classes of asprosin aptamer sequence. Class I was A rich sequences, which contains over 50% of A in random regions. And Class II is C rich sequences. Ten asprosin aptamer candidates showed a nanomolar level of binding to our expressed asprosin, and three candidates have high specificity and binding affinity in flow cytometry and ELONA data. Other characterization assays including electrochemical assays are ongoing. In the longer term we aim to develop these asprosin aptamers into aptasensors with potential combination with CA19-9 detection for pancreatic cancer.

W1048

EFFECT OF IPSC-DERIVED NK CELLS WITH SITE-SPECIFIC INTERGRATION OF CAR19 AND IL24 AT THE RDNA LOCUS ON ANTI-TUMOR ACTIVITY AND PROLIFERATION.

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The generation of CAR-NK cells using induced pluripotent stem cells (iPSCs) has emerged as a paradigm for manufacturing off-the-shelf cell products for universal immuno- therapy. However, enhancing the potency, safety and multi-actions of CAR-NK cells is still full of challenges. Interleukin 24 (IL24) and CD19-specific chimeric antigen receptor (CAR19) were site-specifically integrated at the ribosomal DNA (rDNA) locus in human iPSCs by using TALENickases. The engineered iPSCs were differentiated into NK (CAR-iNK) cells by adopting a 38-day differentiation protocol followed by expansion using magnetic beads in vitro. Compared with the CAR-iNK cells,

IL24 armored CAR-iNK (CAR19-IL24-iNK) cells showed higher cytotoxic capacity and amplification ability in vitro. Meanwhile, CAR19-IL24- iNK cells inhibited tumor progression more effectively and exhibited better survival without significant side effects in the B-ALL (Nalm-6(Luc1))-bearing mouse model. Interestingly, RNA-sequencing analysis suggested that IL24 may enhance iNK cell function by attracting neutrophils and upregulating FAS and TNFSF10-related genes while exerting a direct effect on tumor cells. This study encourages the exploration of IL24 and other molecules to enhance antitumor properties of CAR-NK cells, suggesting a novel strategy to modulate tumor microenvironment while attacking tumor cells with the potential of promising off-the-shelf immunotherapy.

W1050

ELUCIDATING THE ROLES OF RETINOIC ACID SIGNALING IN THE REGULATION OF EPIDERMAL STEM CELL POPULATION BALANCE IN THE SKIN

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All-trans retinoic acid (ATRA), the active form of retinoids, is an effective anti-aging molecule with pleiotropic effects on skin pathologies. The interfollicular epidermis is composed of anatomically distinct structures, known as the interscale and scale, which are replenished by slow- and fast-cycling epidermal stem cell populations, respectively. In hematopoietic stem cells, the level of retinoic acid signaling controls the balance between dormancy and activation; however, the effect of ATRA on epidermal stem cell heterogeneity and behavior remains unknown. Here, we show that ATRA treatment in the mouse tail skin reduces the scale compartment while the interscale compartment expands. Notably, we discovered ATRA triggered both epidermal stem cell populations in differentiation, and the remaining Slc1a3+ clones in the basal layers switched their fate to the slow-cycling lineage. Similar changes in epidermal stem cell balance are also observed in human primary culture in vitro. Transcriptome analysis shows that the ERK/MAPK signaling pathway is down-regulated by ATRA treatment, and forskolin, an activator of cAMP/PKA, partially rescues the loss of the fast-cycling compartment. These findings provide insight into the existence of a universal mechanism by which RA signaling regulates the proper balance of tissue stem cell heterogeneity.

Funding Source: Grant-in-Aid for Scientific Research (B) (24K02035, 20H03266) Grant-in-Aid for Challenging Research (Exploratory) (24K21973) Takeda Science Foundation Scholarship supported by Rajamangala University of Technology Thanyaburi.

W1054

ESTABLISHING A MODEL OF A WHOLE TESTICULAR SPERMATOGONIA POOL

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Surviving childhood cancer treatment often results in long-term health complications. Male survivors suffer a significantly higher risk of infertility, due to the gonadotoxic side effects of these cancer regimens. Although in vitro cultivation of prepubertal testis tissues has been previously proposed as an option to preserve fertility, clinical application of this method remains challenging, primarily because producing large amounts of sperm in vitro remains unfeasible. To overcome these limitations, we developed a hydrogel microneedle-based culture system. We then used this system to culture mouse testes from 5 days postpartum (dpp) in vitro, and establish a model of 'whole testicular spermatogonia pool' (WTSP). We found that undifferentiated spermatogonia in WTSP proliferated more than fourfold compared to 5 dpp mouse testis. In contrast, we found that testes showed a declining trend in the number of undifferentiated spermatogonia during in vivo development. Transplantation of WTSP into nude mice resulted in a twofold increase in spermatids count per tubule compared to transplantation of conventional whole testes. Furthermore, in vitro meiosis induction of WTSP significantly enhanced spermatid proportion, thus generating fertile offspring. Lastly, we showed that the cellular states of our WTSP closely resemble those of 5 dpp mouse testes in vivo, and the role of X in promoting spermatogonia proliferation by activating the

PI3K-AKT-mTOR pathway. In conclusion, our WTSP offers a promising method for preserving fertility in prepubertal male cancer patients by maintaining and expanding spermatogonia extracted before treatment.

W1056

EXPLORING THE IMPACT OF CELL DERIVED EXTRACELLULAR MATRICES ON ENDOMETRIAL MESENCHYMAL STROMAL/STEM CELLS IN VITRO

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Endometrial mesenchymal stromal/stem cells (eMSCs) contribute toward the maintenance and repair of uterine tissue after each menstrual cycle. These eMSCs (CD140b+CD146+ cells) have the ability to self-renewal and undergo multi-directional differentiation. Characteristics of endometrial stem cells has opened new avenues for therapeutic applications in regenerative medicine. However, the limited availability of eMSCs and the challenges in maintaining their stemness in vitro have restricted its therapeutic potentials. In this study, the extracellular matrix communication on eMSCs' response was investigated. Cell-derived extracellular matrices (CD-ECMs) are formed through the secretion and assembly of extracellular matrix (ECM) components by cells, which is vital in controlling the survival, growth and differentiation of the cells. Here, eMSCs were cultured on CD-ECM derived from endometrial stromal cell line (T-HESCs) or eMSCs for 7 days. The proliferation and phenotypic expression of cell surface markers were assessed. Both CD-ECM derived from T-HESCs and eMSCs did not alter the proliferation activity of eMSCs when compared to the control group (n=6). While, a decrease in stem cell markers was observed when the eMSCs were cultured on T-HESCs CD-ECM ($P < 0.05$ to control group) but no changes were detected when cultured on eMSC CD-ECM (n=5). These preliminary findings suggest the different components in CD-ECMs provide certain bioactivities for specific applications. The exploration of an in vitro method offers valuable insights to select appropriate ECM-producing cell types for proliferation and differentiation of eMSCs.

W1060

HUMAN SKIN STEM CELLS USE M5C DERIVED SMALL VAULT RNA TO BALANCE SELF-RENEWAL AND DIFFERENTIATION

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Vault RNAs (vtRNAs) are small non-coding RNAs implicated in cellular homeostasis, stem cell regulation, and gene expression. Previous studies have shown that vtRNA1-1 is methylated by NSUN2, regulating its cleavage into small vault RNAs (svRNAs), which function as microRNA-like regulatory RNAs. Here, we demonstrate that Drosha, a key enzyme in miRNA biogenesis, processes unmethylated vtRNA1-1 into svRNA1, while Dicer is dispensable for this process. Using pull-down assays and small RNA sequencing, we show that Drosha preferentially binds unmethylated vtRNA1-1, facilitating svRNA1 production. Functional studies reveal that svRNA1 promotes epidermal stem cell differentiation by targeting epithelial-mesenchymal transition (EMT) pathways, as evidenced by reduced cell migration and upregulation of differentiation markers. Gene ontology analysis identifies svRNA1 targets involved in chromatin remodeling, epigenetic regulation, and neural and germ cell differentiation, linking svRNA1 to NSUN2-mediated pathways. These findings uncover a novel role for Drosha in vtRNA1-1 processing and highlight svRNA1 as a key regulator of stem cell differentiation, offering insights into the interplay between RNA modifications, non-coding RNAs, and cellular plasticity.

Funding Source: Khalifa University of Science and Technology Competitive Internal Research Award RIG-2023-116 Khalifa University of Science and Technology Competitive Internal Research Award CIRA-ADEK 8474000304.

W1062

IDENTIFYING AND PREDICTING GENETIC ANTICANCER DRUG RESISTANCE VIA GENOME-WIDE SCREENING IN HAPLOID HUMAN EMBRYONIC STEM CELLS

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Anticancer drugs are at the frontline of cancer therapy. However, innate resistance to these drugs occurs in up to 50% of patients, exposing them to the side effects of these drugs with no meaningful benefit. To identify the genes and pathways that confer resistance to such therapies, we performed a genome-wide screen in haploid human embryonic stem cells (hESCs). These cells possess the advantages of having only one copy of each gene, a normal karyotype, and lack any underlying point mutations. Initially, we show a close correlation between the potency of anticancer drugs in cancer cell lines to those in hESCs. We then exposed a genome-wide loss-of-function DNA library of mutations in all protein-coding genes to a dozen selected anticancer drugs, which represent six different mechanisms of drug therapies. The genetic screening enabled us to identify genes and pathways which can confer resistance to these drugs, demonstrating several common pathways. We validated several of the resistance-conferring genes, showing a significant shift in the effective drug concentrations to indicate a drug-specific effect to these genes. To highlight the clinical relevance of our findings, we focused on the screen results for paclitaxel and carboplatin, two of the most used anticancer drugs in treatment. After confirming the hESC results for these two drugs, further validation in cancer cell lines was performed. Finally, an algorithm for predicting resistance to paclitaxel or carboplatin was developed. Applying the algorithm to DNA mutation profiles of patient tumors enabled the separation of sensitive from resistant patients, thereby providing a prediction tool. As the anticancer drugs arsenal can offer alternatives in case of drug resistance, early prediction can provide a significant advantage and improvement to treatment. Our results show a capacity to identify relevant genetic mutations for cancer in haploid hESCs, demonstrating the applicability of these findings in generating a predictive algorithm to assist the unmet need for preemptive identification of tumor resistance to drug treatments. These findings may have clinically actionable application, improving both efficacy and quality of cancer treatment.

W1064

INVESTIGATING NEUROINFLAMMATION AND NEURAL NETWORK ALTERED BY GLIOMA-DRIVEN ASTROCYTIC ACTIVITY FOR UNDERSTANDING BRAIN TUMOR MICROENVIRONMENT

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Gliomas are tumors that arise from glial cells in the brain and spinal cord and are the most common brain tumors, accounting for 50% of primary intracranial tumors. Despite significant advances in glioma research, treatment options remain limited, and survival rates have yet to improve significantly. This is due to the lack of appropriate preclinical models that accurately reflect the structure and microenvironment of brain tumors. In this study, we established a three-dimensional assembloid model that mimics the microenvironment of actual brain tumor patients, which has yet to be developed worldwide, based on our experience in culturing brain organoids and brain tumor spheroids in three dimensions. Based on this, we aimed to identify the state of glioma-induced astrocyte activation and control the microenvironment by analyzing specific

neuroimmune factors. In our previous study, we found that adding astrocytic growth factor (AGF) to the medium promoted the development of astrocytes and neurons, confirming that neurons and astrocytes are closely related. Based on this, we analyzed the activation of astrocytes by direct or indirect contact with glioma cells using a co-culture method of glioma cells and neurons to determine the structure and function of tumor-affected neurons. We found that glioma cells inhibited the structure and activity of neurons and astrocytes and increased the secretion of neuroimmune factors. These findings suggest that neuroimmune factors secreted by glioma cells significantly impact the structure and function of neurons and astrocytes. Neuroinflammatory factors play an important role in the survival of neurons and astrocytes. Further research is needed to elucidate the mechanisms of the neuroimmune factors and astrocyte changes activated by glioma cells.

Funding Source: This work was supported by the Korea Foundation for Women in Science, Engineering, and Technology (WISET; 2024-625) Grant funded by the Ministry of Science and ICT(MSIT) under the Program for Returners into Research and Development.

W1066

INVESTIGATION OF AGING IN DENTAL STEM CELLS: DPSC, PDL, AND SCAP

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Stem cells derived from the apical papilla (SCAP), dental pulp (DPSC), and periodontal ligament (PDL) have demonstrated remarkable potential for proliferation and differentiation when cultured in controlled environments. These human stem cells are recognized as reliable sources for diverse regenerative therapies. However, the impact of prolonged cultivation on their characteristics remains poorly understood. This study aims to bridge this knowledge gap by investigating the effects of extended in vitro cultivation on SCAP, DPSC, and PDL stem cells, with a particular emphasis on identifying markers of cellular senescence. Stem cells were isolated from healthy third molars, including SCAP, DPSC, and PDL tissues, and cultured under standard conditions (DMEM with 10% fetal bovine serum) over an extended period. Cultivation spanned nearly four months, encompassing early (1st–3rd), intermediate (10th–12th), and late passages (18th–20th). Cells from these passages were assessed for proliferation, apoptosis, telomerase activity, and beta-galactosidase activity as markers of cellular senescence. Additionally, MTT assays were performed, and HLA expression levels were analyzed. SCAP, DPSC, and PDL cells were successfully isolated and expanded in vitro. No statistically significant reduction in proliferative capacity was observed between early and late passages ($p>0.05$). A slight increase in apoptotic cells was detected in late passages, but telomerase and beta-galactosidase activity remained consistent across passages. Similarly, no significant changes in HLA expression were observed among the cell populations. This study underscores the robust potential of SCAP, DPSC, and PDL stem cells for regenerative medicine. Despite extended cultivation, these cells maintain their proliferative capacity and show minimal signs of senescence, confirming their reliability as candidates for regenerative therapies and tissue engineering applications. These findings provide a foundation for further research and clinical applications involving dental-derived stem cells in regenerative medicine.

Funding Source: Bulgarian National Science Fund МП21/3.4/BG-RRP-2.004-0004-C01.

W1068

MANNOSE BIOISOSTERE ENHANCED PROLIFERATION, SELF-RENEWAL AND ANTI-SENESCENCE OF MESENCHYMAL STEM CELLS

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Mesenchymal Stem Cells (MSCs) are accepted as great cell source for regenerative medicine. Companies move to commercialize MSCs in clinical application. However, their efforts may be limited by the ability to expand their cell numbers in vitro with maintaining good quality of differential potentials and stemness. Previous studies from our group have shown reprogramming induction ability of shikimic acid from plant stem cell extracts through mannose receptor mediated mechanism. In this study, mannose bioisostere of the mannose receptor, enhance the rate of proliferation, self-renewal and anti-senescence of MSCs without loss of differentiation potential. Proliferation enhancement by quinic acid was mediated by Cyclin E. Mannose bioisostere induced Sox2, Nanog, Oct4 and Tert expression by binding to the mannose receptor and leading to MKK1/2/3/6, ERK1/2, P38 and CREB phosphorylation. We confirmed that mannose bioisostere inhibited ageing and enhanced regeneration of mouse heart in myocardial infarction model. These results indicate that quinic acid is an effective agent for expanding MSCs with delayed senescence.

Funding Source: National Research Foundation of Korea(NRF) (RS-2024-00359519).

W1070

MESENCHYMAL STROMAL CELLS WITHIN HEPATOCELLULAR CARCINOMA MICROENVIRONMENT MODULATES CANCER PROGRESSION VIA METABOLIC REPROGRAMMING

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Hepatocellular carcinoma (HCC) is a leading cause of cancer death. Mesenchymal stromal cells (MSCs) hold promise for HCC therapy. However, controversial reports emerged on MSCs role in cancer. MSCs recruited to hepatic site of injury may undergo metabolic reprogramming and acquire cancer associated fibroblast (CAF)-like characteristics, promoting HCC progression. We investigated MSCs metabolic reprogramming within HCC microenvironment (ME). We studied MSCs role in promoting HCC progression via cellular metabolites exchange between indirectly cocultured bone marrow MSCs (BMMSCs) and Huh7 HCC cell line (Huh7). We evaluated cellular bioenergetics with a focus on lactate monocarboxylate transporters (MCT1 and 4). Genotypic analysis of cocultured BMMSCs showed upregulation in CCL2, cMYC, FGFR1, IL8, αSMA, MMP2,

SDF1, Vimentin, VEGFα CAF markers, MCT4 and MCT1 downregulation. Concomitant with increase in cellular proliferation as assessed by MTT assay, cell cycle analysis by flowcytometry and genotypic analysis by qRTPCR. AnnexinV/propidium iodide staining in cocultured Huh7 showed downregulation in apoptosis markers, Bcl2 antiapoptotic gene and upregulation of HTERT, Bax and Caspase3 apoptotic genes. Increase in cell migration was observed in cocultured Huh7 using scratch assay, upregulation of epithelial mesenchymal transition genes, Snail, Slug, Vimentin and downregulation of Ncadherin. Genotypic analysis of cocultured Huh showed downregulation of ALDOC, Glut1, ERRγ and PKM2, upregulation of ENO1, TP1, PGK1, IDH1 glycolytic genes and upregulation of ATP6v, Cox11, NDUF5 and 10 oxidative phosphorylation genes. Along with MCT4 downregulation and MCT1 upregulation, which was confirmed by western blot. Biochemical analysis of glucose consumption, lactate, pyruvate, hydrogen peroxide, lactate dehydrogenase, reactive oxygen species and urea showed downregulation in cocultured Huh7 and upregulation in cocultured BMMSCs. Our data show that HCCME may induce BMMSCs metabolic reprograming into CAF-like cells promoting HCC progression, which is mediated via dysregulation of intracellular gene expression of metabolites and their associated metabolic pathways, mainly achieved via lactate transporters. MSCs fate in HCCME thus needs further evaluation and their putative HCC therapeutic use.

Funding Source: Grant #46721 from the Egyptian Science and Technology Development Fund, Cairo, Egypt Grant#5275 from Academy of Scientific Research and Technology, Cairo, Egypt.

W1072

MITOCHONDRIAL TRANSFER FROM STRESSED ADIPOSE-DERIVED MSC EXTRACELLULAR VESICLES INDUCES FERROPTOSIS IN GLIOBLASTOMA

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Glioblastoma (GBM) is an aggressive brain tumor with poor prognosis, primarily due to its resistance to oxidative stress, which inhibits various forms of cell death, particularly ferroptosis. Recent studies have identified extracellular vesicles (EVs) as important mediators of intercellular communication, capable of transferring mitochondria between cells. This study explores whether EVs derived from adipose-derived mesenchymal stem cells (Ad-MSCs) under oxidative stress can transfer dysfunctional mitochondria to GBM cells, thereby overcoming their resistance to oxidative stress and specifically inducing ferroptosis. EVs were characterized and introduced to GBM cell lines, where ferroptosis was assessed by monitoring reactive oxygen species (ROS), lipid peroxidation, and mitochondrial membrane potential. Our findings demonstrate that EVs from stressed Ad-MSCs significantly increase ROS levels and lipid peroxidation in GBM cells, directly inducing ferroptosis. Downregulation of key ferroptosis markers, including GPX4, and the observation of mitochondrial dysfunction and iron dysregulation further confirm the induction of ferroptosis. These results provide new insights into overcoming GBM's resistance to ferroptosis and suggest that Ad-MSC-derived EVs could be a promising strategy for targeted cancer therapy.

W1076

NANOTOPOGRAPHIES ENHANCE NON-VIRAL TRANSFECTION AND NON-VIRAL NEURONAL TRANSDIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS

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Transfection and transdifferentiation strategies capable of producing clinically applicable products could have a significant impact on tissue engineering. Non-viral vectors have the potential to reduce immunogenicity and have added benefits, such as ease of production, but come at the cost of efficiency. Substrate topographies are known to be able to modulate cell behaviors including efficiency of non-viral transfection and transdifferentiation. We hypothesized that nanotopographical substrates would be able to increase non-viral transfection efficiency and/or enhance non-viral neuronal transdifferentiation of human mesenchymal stem cells (hMSCs). We screened the effects of 16 different topographies, with different geometries. We found that five of the topographical patterns (nano- and micro-gratings, micro-lenses, micro-holes, and nano-pillars) increased the efficiency of Lipofectamine-mediated transfection. Convex and concave micro-lenses showed opposite effects on transfection efficiency. Neuronal transdifferentiation of hMSCs was induced by non-viral delivery of BAM (Brn2, Ascl1, and Myt1l) transcription factors, using poly(N,N-cystaminebisacrylamide-4-amino-1-butanol) (pABOL) polyplex. The transdifferentiation study showed weak immunofluorescent staining for the neuronal lineage marker, microtubule-associated protein 2 (MAP2) in all samples. We found that the pABOL transfection reagent used during the

transdifferentiation procedure was more toxic to hMSCs than to mouse embryonic fibroblasts or COS-7 cells. Together, our observations suggest that optimization of the specific non-viral transfection reagent used as well as factors affecting cell confluence, such as seeding density or length of transfection phase, might enable the use of nanotopography to enhance non-viral neuronal transdifferentiation of hMSCs for clinically applicable products.

Funding Source: Natural Sciences and Engineering Research Council (NSERC) Discovery (RGPIN-2016-04043, RGPIN-2021-03200).

W1078

PLURIPOTENT STEM CELL-DERIVED NK PROGENITOR CELL THERAPY PREVENTS TUMOUR OCCURRENCE AND ERADICATES MINIMAL RESIDUAL DISEASE

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The lack of persistence and short-term efficacy presents a major challenge for CAR-NK cell therapy. Here, we addressed this issue by developing pluripotent stem cell-derived NK progenitor (iNKP) cell therapy. Engineered to express CXCR4 and chimeric antigen receptors (CAR), the iNKP cells efficiently migrated to the bone marrow and generated CAR-iNK cells persisting in peripheral blood (PB) for over 80 days. Notably, CAR-iNKP cell therapy durably protected animals from tumour occurrence. Furthermore, infusion of CAR-iNKP cells following conventional chemotherapy eradicated minimal residual disease (MRD), leading to long-term complete remission. Our findings present a novel strategy to overcome the limitations of traditional CAR-NK cell therapy and offer a potential breakthrough in immunotherapy for tumour prevention and MRD elimination.

W1080

REACTIVE OXYGEN SPECIES FROM MITOCHONDRIA DRIVE EXCESSIVE DIFFERENTIATION OF NEURAL STEM/PROGENITOR CELLS FOLLOWING NON-CYTOTOXIC EXPOSURE TO CISPLATIN

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Neural stem and progenitor cells (NSPCs) are essential for nervous system development, as they self-renew and differentiate into neurons and glia. These processes are highly sensitive to environmental and chemical factors, including chemotherapeutic agents like cisplatin, an FDA-approved drug that disrupts DNA replication. While cisplatin effectively targets cancer cells, it also induces side effects, such as nephrotoxicity, ototoxicity, and neurotoxicity. Although its cytotoxic effects are well-documented, less is known about its impact at non-cytotoxic levels. This study examined the effects of non-cytotoxic cisplatin (5 μM) on NSPC differentiation and mitochondrial activity, focusing on reactive oxygen species (ROS) as mediators. Mitochondrial function was analyzed via MTT assays, ATP measurements, and flow cytometry for mitochondrial potential (ΔΨm), biomass, and ROS levels. NSPC self-renewal and differentiation were assessed using microscopy and pharmacological ROS inhibition with Mito-TEMPO. After 24 hours of exposure, cisplatin enhanced mitochondrial activity, evidenced by increased ATP production, ΔΨm, and ROS generation, accompanied by reduced cell cycle progression and self-renewal. This led to exaggerated differentiation into neuronal and glial lineages. ROS inhibition reduced differentiation but failed to restore cell cycle progression and self-renewal. These findings suggest that subtherapeutic cisplatin disrupts NSPC integrity by promoting differentiation through a ROS-dependent mechanism, highlighting the vulnerability of NSPCs even at non-cytotoxic doses. Co-administration of antioxidants during chemotherapy may protect NSPCs and prevent long-term developmental and cognitive risks in vulnerable populations, such as neonates exposed to cisplatin indirectly (e.g., via breastfeeding). Safeguarding the neural stem cell niche is critical to mitigating these risks.

Funding Source: FONDECYT Postdoctorado N°3220204.

W1082

RETINOIC ACID MODULATES SELF-RENEWAL AND DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS BY REGULATING GROWTH FACTORS SIGNALING AND APPLICATION

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Retinoic acid (RA) is the major metabolite from vitamin A (retinoid), and plays a central role in mammalian embryogenesis. In in vitro experiments, RA has been reported to be applied in different lineage cell differentiation and enhance the efficiency of reprogramming. The reported RA action in pluripotent stem cell is to interact with its nucleus Retinoic acid receptors (RARs) and alters the stem cell fate by directly interact with pluripotent gene OCT4 in transcriptional level. However, we believe that besides transcriptional level regulation, RA also modulates intracellular signaling that determine cell fate. But the actual molecular mechanisms in human pluripotent stem cell affected by RA is still remained unclear. In our study, we find several essential growth factors have been significantly changed in human embryonic stem cells (hESC) with RA treatment. When treated with RA, hESC shows a significant effect in TGFβ super family, fibroblast growth factor family and WNT signaling. More specifically, BMP4, NODAL and WNT3 are the most affected genes. With long-term RA treatment hESC will be gradually differentiated, we then suppress BMP4 and WNT3 signaling together with RA treatment and find out that hESC maintains its pluripotency and can further maintained over five generations. Moreover, when RA is supplied with differentiation chemicals like CHIR990321 and BMP4, RA strongly enhances their effect on growth factors like

NODAL, BMP4 and WNT3. In current conclusion, we find an essential growth factor signaling network based on BMP4/WNT3 to explain how RA regulates hESC pluripotency and differentiation. Findings of RA effect on BMP4/WNT3 can be applied to different lineage differentiation and increase reprogramming and in vitro somite formation efficiency.

W1084

SPACE-ASSOCIATED RETROELEMENT HALLMARKS OF STEM CELL AGING

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Retrotransposable elements (RTEs) regulate genomic stability and cellular plasticity, particularly in stem cell populations, but their deregulation is implicated in stem cell aging, inflammation-associated diseases, and malignant transformation. RTEs contribute to genomic instability through retrotransposition, primarily by long interspersed nuclear elements (LINEs), or through

epitranscriptomic alterations that activate antiviral inflammatory pathways, particularly via human endogenous retrovirus (HERV) expression. These pathways activate endogenous epitranscriptomic base editors, such as ADAR1 (adenosine deaminase acting on RNA 1) and APOBEC3C (apolipoprotein B mRNA editing enzyme catalytic polypeptide-like type 3C), which evolved to regulate RTE activity in hematopoietic stem and progenitor (HSPC) populations. Notably, both ADAR1 and APOBEC3C overexpression have been linked to HSPC malignant transformation, and their mutational signatures contribute to clonal hematopoiesis and age-associated stem cell decline. Spaceflight represents a unique physiological stressor that may influence RTE expression in a cell-type- and context-specific manner. Here, we quantified retrotransposable element expression in single-cell RNA sequencing data with the scTE pipeline in CD34+-enriched peripheral blood mononuclear cells (PBMCs) from commercial astronauts aboard the Axiom-2 and Axiom-3 missions, sampled before, during, and after spaceflight. Our analysis revealed a transient increase in LINE-1 expression within CD34+ stem cell populations that peaked immediately post-flight and returned to baseline after 21 days. Notably, retroelement activation correlated with longer spaceflight durations, with specific LINE-1 and HERV families differentially expressed in a temporally and cell-type-specific manner alongside base editor activation. These findings provide one of the first insights into RTE activation in HSPCs in response to spaceflight, implicating ADAR1 and APOBEC3C in stress-induced retrotransposable element activity. Understanding how spaceflight influences transposable element regulation may inform strategies to mitigate hematopoietic aging, enhance regenerative medicine applications, and assess long-term health risks associated with space travel.

W1086

STUDY OF MICROENVIRONMENT EFFECTS ON MESENCHYMAL STEM CELL (MSC)-DERIVED SMALL EXTRACELLULAR VESICLES (SEVS) IN 3-DIMENSIONAL AND DYNAMIC CULTURE

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The therapeutic benefits of mesenchymal stem cells (MSCs) are widely believed to be derived from their paracrine signaling, such as extracellular vesicles (EVs). Cell-free therapy using EVs is an active and emerging field in regenerative medicine. Despite the great potential of MSC-derived small extracellular vesicles (sEVs), a major challenge for EV production in manufacturing is the choice of culture system. Among the biophysical strategies, 3D and dynamic cultures are proposed to enhance their therapeutic efficacy, although the mechanisms are not yet fully understood. In this study, we aimed to better understand the effects and mechanisms of these culture microenvironments on MSC-derived EVs during EV production. The experimental results showed that dynamic culture induced higher EVs particles secretion from NTA data, while 3D spheroid culture, in contrast, mitigated EV quantity. Furthermore, more informative biomolecules such as proteins and miRNAs were encapsulated per EV particle with 3D spheroid culture, in both dynamic and static cultures. Our hypothesis is that shear force triggers more EV particles, albeit with less surface marker expression, and the 3D culture environment stimulates more surface marker proteins to be packed on each EV. In summary, the results suggest that MSCs secrete more informative EVs from 3D cultures, and dynamic cultures trigger more EV production but compromise the quality of EVs. This study provides new insights into MSC-EVs obtained from different culture environments, which should be considered and justified in EV manufacturing.

W1088

THE IMPACT OF APOBEC3 ON RNA SPLICING IN AGED BONE MARROW-DERIVED HEMATOPOIETIC STEM AND PROGENITOR CELLS

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Inflammatory cytokine-inducible APOBEC3 cytosine deaminases evolved to protect human genomic integrity by restricting retroviral integration and retrotransposition during stress responses. However, in cancer, APOBEC3-induced mutagenesis drives genomic instability, facilitating tumor initiation and progression in inflammatory microenvironments. By performing whole genome and whole transcriptome sequencing analyses of myeloproliferative neoplasm (MPN) derived hematopoietic stem and progenitor (HSPCs) compared to age-matched controls, we discovered that APOBEC3C overexpression, C-to-T mutagenesis and IL-6ST upregulation, which induces ADAR1p150 splice isoform switching, correlates with MPN progression. Thus, we sought to investigate the role of APOBEC3 activation as a driver of clonal hematopoietic progenitor splicing deregulation and propagation. We cloned human APOBEC3 family genes into pCDH lentiviral vectors and transduced cord blood derived immunomagnetic bead selected CD34+ cells with APOBEC3A, B, C, D, F, G, or pCDH lentiviral backbone controls for whole genome and whole transcriptome analyses. Following lentiviral APOBEC3B, APOBEC3C, and APOBEC3G transduction of CD34+ cells, gene set enrichment analysis (GSEA) revealed splicing pathway alterations along with differential splicing typified by increased exon skipping. Subsequently, CD34+ cells derived from aged normal bone marrow (ABM), were transduced with APOBEC3C for whole genome and whole transcriptome analyses, coupled with functional assays to assess the effects of APOBEC3C overexpression on RNA splicing, which further confirmed splicing pathway alterations. Moreover, we observed that lentiviral APOBEC3C overexpression in ABM-derived CD34+ cells resulted in differential exon usage using splicing reporter assays. Specifically, upon confirmation of APOBEC3C overexpression, we seeded CD34+ derived ABM cells into our 3D biosensing nanobioreactor system to analyze differential exon usage using confocal fluorescence microscopy along with FACS analyses. Together, these findings suggest that detection of APOBEC3-mediated splicing deregulation may inform the development of diagnostic and therapeutic strategies aimed at preventing splicing-mediated MPN progression.

W1090

TRANSCRIPTOMIC COMPARISON OF CANINE BLADDER CANCER IN PATIENT-DERIVED ORGANOIDS AND TISSUES

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Canine bladder cancer (BC) is a malignant tumor that affects the urinary tract of dogs. Canine BC is a spontaneous animal disease model of human bladder cancer since it closely mimics human invasive bladder cancer in terms of molecular characteristics, drug response, and histopathologic appearance. The use of spontaneously occurring canine tumors as a model for research in human cancers is facilitated by the fact that canine cells can be cultured in the same way as human cells, and in the form of advanced in vitro models. Such models, in relation to oncological research, are organoids – 3D cultures reflecting the heterogeneity and spatial orientation of tumor cells. Organoids replicate the functions and genetic mutations of their in vivo counterparts, providing an effective platform for testing novel therapeutics. Canine BC organoids can be cultured from either urine or tissue biopsies from tumors, offering the development of a non-invasive in vitro model (urine). However, there is limited research on the similarities between urine-derived organoids, tissue-derived organoids, and tumor tissue biopsies, which reduces the importance of the dog model. Therefore, the aim of this study was to develop canine patient-derived BC organoids and compare their molecular characteristic with corresponding tissue biopsies from the same tumor. Urine-derived organoids and tissue samples of five patients were used to compare bulk RNAseq-derived transcriptomic data. Cancer cells found in urine were used to establish organoid cultures and were preserved in RNALater. RNA extractions used a Qiagen RNeasy kit, samples were sequenced at Genewiz, quality of reads were checked using FastQC, and Kallisto was used for alignment. Transcriptomic analysis revealed variability of RNA expression patterns in different patients, visible in both tissues and organoids. Furthermore, the organoids were characterized with upregulation of proliferation pathways (G2M Checkpoint, MTORC1_Signaling) while inflammatory pathways (IL2 STAT5_Signaling, Allograft_Rejection) were upregulated in tissues. Principal component analysis revealed strong patient-specific clustering in both organoids and tissues, highlighting their potential as a personalized medicine tool that accurately reflects patient heterogeneity in canine bladder cancer.

Funding Source: 5 R21 CA267372-02; NIH ID 10539328.

Poster Session 1 (EVEN)
5:00 PM – 6:00 PM

TRACK: DISEASE MODELING AND DRUG DISCOVERY (DMDD)

W1092

DEVELOPMENT OF AN AGE-RELATED HEARING LOSS ORGANOID MODEL USING PATIENT-SPECIFIC MIRNA

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Age-related hearing loss (ARHL) is a type of hearing loss that occurs gradually in many individuals as they age. Studying ARHL in clinical research is challenging due to the need for long-term studies. The auditory system is complex, consisting of hair cells, nerve cells, and various supporting cells, which makes it difficult to model ARHL accurately. 'Organoid' technology offers a potential solution to overcome these limitations. However, there has been no successful report of establishing a human ARHL organoid model to date. In this study, we aimed to construct an ARHL disease organoid model using microRNA (miRNA) specifically found in ARHL patients. First, we screened for miRNAs that are highly expressed in the blood of ARHL patients. Next, we cloned the target miRNA into a doxycycline-inducible vector and transduced them into induced pluripotent stem cells (iPSCs) using lentivirus. These iPSCs were then differentiated into inner ear organoids, and doxycycline was administered to induce the overexpression of the target miRNA, thereby modeling ARHL. Target miRNA-induced inner ear organoids exhibited increased expression of senescence-related genes and decreased expression of auditory hair cell-related genes compared to normal inner ear organoids. Immunohistochemistry and flow cytometry results demonstrated that target miRNA overexpression led to a decreased proportion of cells expressing auditory hair cell markers such as MYO7A, and an increased expression of aging markers like P53 and P62. These findings suggest that the induction of target miRNA could potentially create an ARHL model in inner ear organoids. This represents a significant discovery that could contribute to a better understanding of the pathogenesis of ARHL and the development of novel therapeutic strategies.

Funding Source: This research was supported by the Korean Fund for Regenerative Medicine (KFRM) grant funded by the Korea government (the Ministry of Science and ICT, the Ministry of Health and Welfare) (22A0303L1-01).

W1094

HEPATIC ORGANOID-BASED MODEL FOR SEVERE FEVER WITH THROMBOCYTOPENIA SYNDROME VIRUS (SFTSV) INFECTION

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Severe fever with thrombocytopenia syndrome virus (SFTSV or Dabie bandavirus) is a tick-borne virus that causes hemorrhagic fever with a high case fatality rate. It is endemic to East Asia, particularly South Korea, China, and Japan. Also, it has recently raised public health concerns in the United States, where ticks carrying SFTSV have been detected in over 20 states. Despite its

clinical importance, there are currently no effective treatments or vaccines, and the mechanisms of SFTSV remain poorly understood. To this end, many in vitro studies have been conducted; however, challenges remain due to the difficulty of establishing infection and the prolonged initial incubation period. Especially, animal models for SFTSV infection have been challenging to establish, as commonly used mouse models fail to support efficient viral replication and do not fully recapitulate severe symptoms such as high fever, significant weight loss and multi-organ failure. In this study, we developed a pluripotent stem cell-derived hepatic organoid (HO) model to investigate SFTSV infection. The pluripotent stem cell-derived HO showed a SFTSV infection pattern at the genomic level. In addition, immature HO showed higher susceptibility to SFTSV than mature HO. Transcription analysis further revealed differences in gene expression for HO maturation and virus susceptibility. These findings suggest that HO derived from pluripotent stem cells can serve as a promising in vitro model for studying SFTSV infection, providing new insights into the virus-host interactions and potentially aiding in developing antiviral strategies.

Funding Source: This study was supported by the National Institute of Health (NIH) research project (project number 2025-NI-008-00 and 2025-NI-009-00).

W1096

A SENSITIVE QPCR METHOD TRACKING TRANSPLANTED HUMAN IPSC-DERIVED CELLS IN XENOTRANSPLANTATION MODELS FOR PRE-CLINICAL RESEARCH

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Human iPSC-derived cells have emerged as useful sources for cell therapy. After transplanting cells into hosts, observing the distribution and fate of the transplanted cells is an essential step for safety evaluation. It is even more difficult and complicated to track the human iPSC-derived cells in the context of animal models with human cancer due to the genetic homology of human cells. To assess the biodistribution of the human iPSC-derived cells, highly sensitive and specific analytical methods, which can distinguish target cells from animal cells and human cancer cells, must be developed. In this study, we developed a real-time quantitative polymerase chain reaction (qPCR) method that could efficiently track human iPSC-derived cells with high sensitivity, specificity and accuracy for pre-clinical research. By targeting human MHC DNA sequences, primer pairs and a probe were designed and synthesized. A TaqMan probed based quantitative real-time PCR approach was developed after methodology validation using a touchdown PCR protocol. After the animals bearing human cancers received human iPSC-derived cells, major organ tissue and blood samples were collected, and genomic DNA was isolated. The amount of the human genomic DNA was quantified by a Qubit fluorometer. This assay showed high sensitivity, evidenced by its ability to detect 1 human iPSC derived cell in 1.0 x 10^5 animal cells. There was no cross-reaction with

genomic DNA obtained from animal models with human cancers. Using this assay, the biodistribution of intravenous injected human iPSC-derived NK (iNK) cells was investigated in NCG mice bearing human liver cancer cells. The results showed iNK cells accumulated in the lungs first and then reentered into circulation and distributed into other organs tissues after injection. The fate of injected iNK cells was evaluated by comparing the amount of iNK DNA detected in tissues collected at different time points. Our primer-probe set and touchdown qPCR protocol are applicable for the quantitative detection and tracking of human iPSC-derived cells transplanted into animals models of human cancers in preclinical study.

W1098

AUTOMATING HIGH-THROUGHPUT SCREENING OF CARDIAC CONTRACTILITY BY ROBOTICALLY CONTROLLED FUNCTIONAL TESTING OF STEM CELL-DERIVED MICRO-TISSUES IN A 96-WELL PLATE FORMAT

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Human engineered cardiac tissues, which typically comprise a thin strip of cells and supporting matrix suspended between polymeric force sensors, offer a stem cell-based alternative to experimental animals for compound testing and disease modeling, aligned with FDA Modernization Act 2.0. However, a large cell count and time-consuming data acquisition protocols often limit the throughput and efficiency of cardiac contractility screening. To overcome these challenges, our team developed a miniaturized version of our human ventricular Cardiac Tissue Strip (hvCTS), configured in a standard 96-well plate format. Cells suspended in a liquid hydrogel are constrained by surface tension to a hydrophobic channel in each well, allowing tissue self-assembly onto two flexible end-posts with curved anchor points optimized by finite element analysis. The resulting micro Cardiac Tissue Strips (μCTS) are 3-mm long and require only 150,000 cells per tissue, an 85% reduction of cell number from its predecessor. Optical tracking and twitch force measurement is achieved by a custom lid featuring intensity-regulated lighting and carbon electrode pairs to stimulate the tissues for controlling beat rate and promoting cardiomyocyte maturation. The μCTS exhibit > 90% viability, a coefficient of variation < 20%, and a strong signal-to-noise ratio. Validation using six different drug classes (adrenergic agonist, cardiac glycoside, cardiac myosin activator, Ca2+ sensitizer, Ca2+ channel blocker, hERG K+ channel blocker) demonstrated dose-dependent functional responses as expected for human heart muscle. Our robotic cardiac tissue screening system localizes the electrical stimulation and captures data from 16 tissues simultaneously, automatically translating the plate through up to 6 regions and conducting user-defined experimental protocols, thus reducing the screening time to less than 10% of that required for single-tissue readouts. Our next-generation 96-well μCTS system offers increased sample number, fewer cells per tissue, low variability across tissues, decreased overall measurement time, and compatibility with automated liquid handling systems, enabling efficient high-throughput human stem cell-based preclinical screening of cardiac contractility, satisfying the latest regulatory

guidelines.

Funding Source: Hong Kong Innovation and Technology Commission (Enterprise Support Scheme #B/E008/22).

W1100

DECIPHER TEMPORAL CONTROL OF GENE REGULATION DURING HEALTHY AND DISEASED THYMIC ORGANOGENESIS USING 22Q11DS HUMAN INDUCED PLURIPOTENT STEM CELL MODEL

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The main function of the thymus is to mature and select T cells against self and foreign antigens. Failure in this process can lead to autoimmunity or immuno-deficiency. In 22q11 Deletion Syndrome (22q11DS), the most common human copy number variant, patients present with a broad phenotypic spectrum of immune defects. In severe cases, patients are born athymic with high morbidity and mortality. The immune defects in 22q11DS have been largely attributed to the deletion of a copy of transcription factor T-box 1 (TBX1). However, how the monoalleic deletion leading to dysregulation of retionic acid singaling and microRNA processing and perturbs thymic morphogenesis is unknown. In this study, we aimed to decipher the precise temporal control of gene regulation during healthy and diseased thymic organogenesis using a human induced pluripotent stem cell model and multi-omics approaches. Using a platform that generates functional thymic organoids by recapitulating key stages of thymic epithelial differentiation in vitro, we have generated 22q11DS thymic organoids that mimic transcriptomes of 22q11DS thymic differentiation. Single-cell transcriptomic analysis revealed striking differences in the expression of genes regulatory networks governing development, retinoic acid signaling and microRNA processing at the third pharyngeal pouch stage. Our ongoing work aims to study transcriptomic differences caused by dysregulation of retinoic acid signaling and microRNA processing leading to failure of thymic organogenesis.

W1102

ENHANCED DRUG CLASSIFICATION USING MACHINE LEARNING WITH MULTIPLEXED CARDIAC CONTRACTILITY ASSAYS

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Cardiac screening of newly discovered drugs remains a longstanding challenge for the pharmaceutical industry. While therapeutic efficacy and cardiotoxicity are evaluated through

preclinical biochemical and animal testing, 90% of lead compounds fail to meet safety and efficacy benchmarks during human clinical trials. A preclinical model more representative of the human cardiac response is needed; heart tissues engineered from human pluripotent stem cell derived cardiomyocytes offers such a platform. In this study, three functionally distinct and independently validated bioengineered cardiac tissue assays were used: human ventricular cardiac anisotropic sheet (hvCAS) designed for screening cardiac electrophysiology, cardiac tissue strip (hvCTS) designed for cardiac contractility, and cardiac organoid chamber (hvCOC) designed for cardiac pump function. Each assay was exposed to increasing concentrations of known compounds representing 5 classes of mechanistic action, Including adrenergic agonists, calcium channel blockers, cardiac glycosides, hERG potassium channel blockers, and myosin II inhibitors. This created a robust electrophysiology and contractility dataset for training and testing the machine learning model. Combining the weighted results from six individual models, comprising support vector machine (SVM) and random forest (RF) models applied to data from each of the 3 assays, the optimized ensemble algorithm was able to classify the mechanistic action of unknown compounds with 86.2% predictive accuracy, outperforming simpler single-assay and dual-assay models. This study represents the first time such an ensemble machine learning approach has been used to leverage the complementary strengths of a diversity of cardiac screening assays, all built upon the same underlying ventricular cell types. Such screening tools represent the next generation of human-specific stem cell-based assays that are poised to fulfill the promise of efforts such as the FDA Modernization Act 2.0, aiming to improve upon, and ultimately replace experimental animals to efficiently bring safer and more effective drugs to patients in need.

Funding Source: Hong Kong Innovation and Technology Commission (Enterprise Support Scheme Grant #B/E033/18).

W1104

MODERATE SCALE MANUFACTURING OF HUMAN IPSC-DERIVED IMMUNE CELLS

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The advent of reprogramming technologies has revolutionized stem cell research, offering unique opportunities in regenerative medicine and immunotherapy. Among the most promising applications is the scalable generation of functional human iPSC-derived CAR-macrophages (CAR-iMacs). However, achieving reliable, reproducible, and scalable production of iPSCs and their derivatives remains a significant challenge. Critical factors include accurate cell counting and analysis, as well as smooth and efficient processes for the expansion and differentiation of various stem cell populations including iPSCs. For instance, dissociating stem cells—from mild, cluster-preserving methods to more singularized and harsher treatments—poses significant challenges in consistently counting and analyzing cells using a unified method. Addressing these challenges is

essential to ensure reproducibility and standardization in research applications. To tackle these issues, we developed a scalable workflow that integrates an advanced cell counting and analysis method, utilizing cell volume and size measurements, with a parallel bioreactor-based, three-dimensional suspension system for the continuous generation of CAR-iMac with a reproducible phenotype. This platform enables smooth handling of cells regardless of their origin or shape, allowing for efficient management of both homogeneous and heterogeneous populations. The advanced cell counting and analysis method provides a label-free approach to regularly monitor cell quality, such as population homogeneity, without requiring additional sample treatments. This significantly reduces workflow complexity, improves process efficiency, and enhances the overall reproducibility of experiments. This streamlined two-step workflow supports the reliable and reproducible continuous generation of CAR-macrophages using an easy-to-use bioreactor. By addressing critical bottlenecks in stem cell analysis and differentiation, our approach provides an innovative, scalable solution for advancing research in immunotherapy and regenerative medicine.

W1106

RAPID DEVELOPMENT FRAMEWORK FOR THE GENERATION OF PATIENT SPECIFIC, RARE DISEASE IPSCS: THE ZTTK CASE STUDY

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Rare diseases, often genetic in origin, are complex and pose significant challenges in diagnosis and treatment. Zhu-Tokita-Takenouchi-Kim Syndrome (ZTTK) is one such rare condition caused by de novo heterozygous mutations in the SON gene, leading to haploinsufficiency and loss-of-function. It is characterized by intellectual disability and developmental delays. Developing treatments for rare diseases is resource-intensive and hindered by limited scientific understanding and inadequate models. Induced pluripotent stem cell (iPSC) technology offers substantial advantages as patient-specific, genetically accurate disease models for drug study and screening. In this study, we report the first successful generation of iPSCs from a 2-year-old ZTTK patient with a 4-bp (TTAG) deletion in the SON gene, causing a frameshift mutation and nonsense-mediated mRNA decay. Additionally, normal iPSCs were gene-edited to introduce the 4-bp mutation, creating syngeneic control lines. Peripheral blood from a ZTTK-affected infant and a healthy adult donor was reprogrammed using the CytoTune-iPS 2.0 Sendai Reprogramming Kit. iPSCs were successfully generated and banked. The 4-bp mutation was introduced into normal iPSCs via precise genome editing. Three genome-editing approaches—Cas9, Cas12a, and TALENs—were evaluated for optimal target recognition and knock-in efficiency. A streamlined process for clonal isolation and expansion of iPSCs and edited cells was established. Generated ZTTK-iPSCs and edited WT-iPSCs with the 4-bp deletion were characterized to confirm pluripotency, normal karyotype, and mutation presence. The iPSC clones produced in this study will be cryopreserved, deposited in a cell bank, and made available to researchers as valuable models for ZTTK syndrome research.

W1108

"DERIVATION OF LIVER STEM CELLS TO DISSECT MOLECULAR PATHWAYS FOR NON-ALCOHOLIC FATTY LIVER DISEASE"

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Non-alcoholic fatty liver disease (NAFLD) is gaining track as an imminent global pandemic that is estimated to impact up to a quarter of the adult population worldwide. Anticipated to place a significant strain on the healthcare system, NAFLD is expected to have a considerable impact due to its widespread occurrence and the potential health complications associated with obesity and diabetes. NAFLD encompasses a range of disease stages, starting from benign accumulation of fat in the liver (steatosis) to the degeneration, inflammation, and cirrhosis of liver cells. The organoid system is a highly important tool for investigating hepatocyte functions and advancing our understanding of liver biology in NAFLD progression. Our lab has made significant research progress on the generation of liver organoids from pluripotent stem cells (PSCs) and went a step further to develop a one-step protocol for directed differentiation of adult liver stem cells (LSCs) to a hepatocyte-like cell (HLC) lineage. The successful generation of liver organoids from both PSCs and LSCs showcases their versatility, offering promising prospects for their utilization in modeling drug-induced liver injury and fatty liver disease. In addition, our laboratory has also further developed an algorithm that utilizes transcriptomic data from diverse patients across different continents for the continuous staging of patients with NAFLD. In this presentation, we will explore how a multi-disciplinary approach can uncover valuable insights into NAFLD mechanisms and stages, providing promising avenues for tackling this complex disease.

W1110

CHARACTERIZING THE ROLE OF PUTATIVE ENHANCERS IN RETINAL DISEASES USING HUMAN RETINAL ORGANOIDS

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Retinitis Pigmentosa (RP) and Macular Telangiectasia (MacTel) are inherited retinal disorders (IRDs) that cause vision loss. While these diseases have established genetic risks, >30% of cases cannot be explained by protein-coding variants alone. Cis-regulatory elements (CREs) are noncoding DNA sequences that regulate gene expression and mutations in CREs have been linked to several IRDs. However, regulatory variants are difficult to characterize because CREs can be cell-type specific, act at a distance, and are often not conserved across model systems. To address these challenges, human retinal organoids (ROs) derived from induced pluripotent stem cells (iPSCs) have emerged as a powerful model for studying CREs and retinal diseases. Here, we present two complementary single-cell approaches for multiplexed testing of CREs in ROs to assess changes in target gene expression and cell state. The first method pools CRE knockout ROs derived from CRISPR-edited iPSCs, each labeled with a unique probe barcode, and profiled

by single-cell RNA sequencing (scRNA-seq). This approach enables characterization of different CREs in a parallelized and multiplexed manner, facilitating the analysis of up to 16 samples simultaneously. The second method involves electroporating CRISPR/Cas9-expressing plasmids into developing human ROs to target candidate CREs, followed by scRNA-seq of the pooled, electroporated organoid cells. The individual gRNAs serve as barcodes for de-multiplexing, allowing the identification of essential CREs linked to IRDs. Using these high-throughput techniques, we identified CREs essential for expression of the RP-associated gene NRL and the MacTel-associated gene TTC39B. Disrupting an enhancer of NRL significantly decreased the expression of rod-specific transcription factors and shifted cell fate from rod to cone, while disrupting an enhancer of TTC39B demonstrated a cell-type specific decrease in gene expression. These findings uncover novel CRE-mediated disease mechanisms in previously unexplored genomic regions. Together, these methods represent powerful tools for the functional interrogation of CREs in ROs, advancing our understanding of epigenetic regulation in retinal development and disease.

W1112

CRISPR MEETS OPTI-OX: NEXT-GENERATION HUMAN NEURAL CELL MODELS FOR NEUROSCIENCE RESEARCH AND DRUG DISCOVERY

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CRISPR/Cas9 gene knockouts are essential for dissecting gene function but can be challenging to implement effectively in neural cell types due to low transfection efficiency and difficulties generating stable Cas9-expressing lines. To address these limitations, we combined our deterministic cell programming technology (opti-ox™) with CRISPR/Cas9 to develop CRISPR knockout (CRISPRko)-Ready ioCells™ - human induced pluripotent stem cell (iPSC)-derived ioMicroglia, ioOligodendrocyte-like cells, and ioMotor Neurons constitutively expressing Cas9. The integration of opti-ox and CRISPR technologies provides reproducible, scalable, and physiologically relevant neural cells, enabling efficient gene editing workflows via synthetic or

lentiviral guide RNA (gRNA) delivery one day post thaw and at later experimental timepoints. Cas9 functionality has been rigorously validated across all cell types, consistently achieving knockout efficiencies above 70%. Importantly, each of the CRISPRko-Ready ioCells retains the phenotypic and functional characteristics of its wild-type counterpart, ensuring reliable experimental outcomes. The optimised performance of these cells positions them ideally for CRISPR screening workflows. As a proof-of-concept, a pooled single cell CRISPR knockout screen in ioMicroglia successfully identified genetic modulators of microglial polarisation between homeostatic (M0) and activated (M1) states, illustrating the practical utility of these cells for functional genomics. Overall, CRISPRko-Ready ioCells provide researchers with a robust and flexible research tool to accelerate gene function studies, streamline target identification, and advance drug discovery in neuroscience.

Funding Source: bit.bio.

W1114

CRISPR-READY IOGLUTAMATERGIC NEURONS FOR VERSATILE GENE MODULATION AND DRUG TARGET DISCOVERY

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CRISPR technology has transformed the study of gene function, enabling precise manipulation through multiple modalities: knockout (CRISPRko), interference (CRISPRi), and activation (CRISPRa). While CRISPRko provides robust gene disruption, CRISPRi and CRISPRa offer nuanced, transcriptional modulation - downregulating or upregulating gene expression without genome editing. Implementing these approaches in post-mitotic human neurons remains challenging due to low transfection efficiency and the complexity of stable line generation. To overcome these barriers, we developed CRISPR-Ready ioGlutamatergic Neurons, human induced pluripotent stem cell (hiPSC)-derived neurons stably expressing either active Cas9 (CRISPRko) or catalytically inactive Cas9 (dCas9) fused to transcriptional effectors. These cells enable efficient, rapid gene perturbations via lentiviral or lipid-based guide RNA delivery immediately after thawing. Crucially, these hiPSC-derived neurons maintain key neuronal markers (MAP2, TUBB3, VGLUT2), form functional networks within days, and exhibit high lot-to-lot consistency. Leveraging our CRISPRko-Ready ioGlutamatergic Neurons, we established a single-cell CRISPR screening platform to identify genes involved in neurological diseases. A proof-of-concept pooled scCRISPR

screen targeting neurodegeneration-associated genes identified genes implicated in Charcot-Marie-Tooth Disease. Furthermore, CRISPRi/a-Ready ioCellsTM allow precise perturbation of gene networks, facilitating comprehensive analyses of complex regulatory interactions. CRISPR-Ready ioGlutamatergic Neurons simplify experimental workflows, accelerate target identification and validation for drug discovery, and broaden possibilities for functional genomic studies in neurological disorders.

Funding Source: bit.bio.

W1116

GENERATION OF MACROPHAGE-INTEGRATED 3-DIMENSIONAL CARDIAC SPHEROIDS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Research on three-dimensional (3D) cardiac models utilizing cardiomyocytes derived from human induced pluripotent stem cells (hiPSCs) is advancing rapidly, enhancing our understanding of heart development, disease mechanisms, and drug responses. While in vitro models can provide valuable insights, they lack to replicate the complexity of the cardiac microenvironment, particularly regarding the roles of immune cells. In this study, we present a novel 3D cardiac spheroid model that incorporates hiPSCs-derived cardiomyocytes (CMs), endothelial cells (ECs), cardiac fibroblasts (CFs), and macrophages (Mφ). The successful differentiation and spatial integration of heart-consisting cell types were confirmed through immunofluorescence analysis. Furthermore, this study suggests a macrophage-integrated 3D cardiac spheroid as a next-generation model for investigating cardiac diseases with immune involvement. By bridging the gap between cardiac physiology and immunology, this system could provide a more physiologically relevant platform for disease modeling, precision medicine, and drug discovery. Future work will focus on optimizing immune-cardiac interactions and scaling the model for high-throughput screening applications.

Funding Source: This research was supported by the Bio and Medical Technology Development Program of the National Research Foundation (NRF) and funded by the Korean government (MSIT) (No. RS-2023-00261905, 2022M3A9E4017151).

W1118

NOVEL VASCULARIZED NEURAL SPHEROIDS FACILITATE REPAIR IN THE SPINAL CORD INJURY

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Induced pluripotent stem cells (iPSCs)-based therapy holds great potential for substituting neural loss in the spinal cord injury (SCI), however, conventional cell therapies face the challenges of poor survival, inadequate neuronal differentiation, as well as the lack of vasculatures that supplies nutrients and regulates neurogenesis. Here, we employed self-assembling Nucleic-Acid-nanostructures-decorated-living-Cells (NACs) to create novel vascularized neural spheroids by combining hypoimmunogenic human motor neurons induced from iPSCs with human umbilical vein endothelial cells. Transplantation of vascularized neural spheroids into completely transected SCI mouse models results in a significant repair, as evidenced by their long-term survival and efficient differentiation into MNs, as well as function recovery of the bladder and hind limbs. Notably, these vascularized spheroids exhibited successful graft-host vascular connection, and more importantly, improved neurogenesis and functional restoration in mice compared to non-vascularized neural spheroids. Furthermore, these vascularized neural spheroids were transplanted into semi-transected SCI pig models and significantly improved motor functions, allowing their treatment in large animal models. Thus, this study reported a novel strategy to create vascularized neural spheroids and highlights the beneficial therapy of vascularization for SCI repair.

Funding Source: The National Natural Science Foundation of China (32400806).

W1120

PRE-CLINICAL TARGETING OF CANCER STEM CELLS VIA ADAR1 INHIBITION WITH REBECSINIB IN TNBC

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Cancer stem cells (CSCs) promote relapse and therapy resistance in aggressive cancers. ADAR1, an RNA editing enzyme, supports CSC survival and immune evasion through splicing and self-renewal. Rebecsinib (17S-FD-895), a selective splicing modulator, inhibits ADAR1 to block these pathways. We evaluated its effects using xenografts, spaceflight-based models, and single-cell RNA sequencing (scRNA-seq). $CD34^+$ hematopoietic stem/progenitor cells (HSPCs) were co-cultured with ADAR1-reporter metastatic breast cancer (MBC) cells under spaceflight and ground conditions aboard Axiom Mission 3. ADAR1 activity and CSC clustering were assessed via live-cell imaging. scRNA-seq of six MBC samples (including space-exposed) profiled heterogeneity and ADAR1 expression. Rebecsinib (10 mg/kg IV or 15 mg/kg oral, twice weekly × 2 weeks) was tested in TNBC CDX and PDX models (MBC009, MBC013). CSC burden was evaluated via IVIS imaging and flow cytometry ($CD44^+$, $ADAR1^+$). Spaceflight increased ADAR1 activity and CSC clustering, indicating niche reprogramming under microgravity. scRNA-seq showed expansion of myeloid progenitors and $CD44^+CD47^+$ CSC-like cells with elevated ADAR1 linked to immune escape and transcriptomic plasticity. Rebecsinib significantly reduced $ADAR1^+$ and $CD44^+$ CSCs while sparing normal HSPCs. Rebecsinib effectively targets CSCs by inhibiting ADAR1-mediated RNA editing. Spaceflight models highlight microgravity-induced CSC plasticity and ADAR1 dynamics, supporting Rebecsinib as a promising CSC-directed therapy.

Funding Source: This work was supported by Research Foundation, and NASA NRA NNJ13ZBG001N.

W1122

TARGETING THE RNA-BINDING PROTEIN HUD IN HUMAN IPSCS TO CONTROL ALS DISEASE

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HuD is an RNA-binding protein encoded by the ELAVL4 gene, expressed in the nervous system and playing a crucial role in neurodevelopment. Recent evidence suggests its involvement in Amyotrophic Lateral Sclerosis (ALS), a neurodegenerative disease characterized by death of Motor Neurons (MNs). Notably, an upregulation of HuD mRNA and protein has been observed in MNs derived from human-induced pluripotent stem cells (hiPSCs) carrying FUS P525L mutation, which is responsible for a severe familial form of ALS. This alteration leads to changes in transcriptome and phenotype of MNs and demonstrated to exacerbate cell-autonomous effects of the FUS P525L variant, leading to defects in neuromuscular junctions' establishment and apoptosis phenomena in co-cultures of hiPSC-derived MNs and skeletal muscle (SKM), with a rescue of the phenotypes

after siRNA against HuD treatment. However, very little is still known about the role of HuD in MNs and how its deregulation contributes to these phenotypes. To further investigate the role of HuD in FUS P525L ALS and with the intention of downregulate the target using possible therapeutic molecules, we designed RNA-based molecules such as miRNA mimics and Antisense Oligonucleotides (ASO) Gapmers specific for HuD. These molecules exhibit high biodistribution and tolerability and are increasingly being tested in clinical trials, with some already commercialized. As a model system, hiPSCs offer a closer approximation to human physiology. We tested a few Gapmers candidates in SK-N-BE neuroblastoma cell line, evaluating different concentrations and optimizing the transfection method. Next, we moved to the model system of hiPSCs-derived P525L MNs, and we tested the selected Gapmers using different transfection protocols, reagents, and molecule concentrations. The same approach has been applied for miRNA mimic 375, which has the sequence of the endogenous miR-375, that targets HuD and is downregulated in FUS P525L MNs. These molecules will be transfected into mutant MNs and wild-type SKM cocultures to assess phenotypic rescue and validate their efficacy in prospective of deeper investigations on the role of HuD. The use of RNA-based molecules, studied and applied for therapeutic purposes, may represent a forward-looking approach for modulating the expression of HuD in ALS.

Funding Source: CAMPIONE NAZIONALE 3 finanziato dal PNRR Missione 4 su PNRR MISSIONE 4 – COMPONENTE 2 - INVESTIMENTO 1.4.

W1124

CARDIAC PROTECTIVE STRATEGY AGAINST DOXORUBICIN-INDUCED CARDIAC TOXICITY

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Doxorubicin (DOX) can effectively suppress cancer but cause serious damage to heart tissues. Dexrazoxane (DRZ) is the only FDA-approved treatment for DOX-induced cardiotoxicity but has been shown to interfere with the antitumor effects of DOX. Our previous work has shown that ICG-001, an inhibitor of β-catenin/CBP, can alleviate toxicity, but the latter has questionable translational potential. In this project, we investigate the potential of PRI-724, an analogue of ICG-001 which has been shown to well-tolerated in patients, to inhibit DOX-induced cardiotoxicity. Using patients-derived iPSC-CM and mouse models, we showed that PRI-724 alleviated CM damage induced by DOX in vitro and in vivo and its cardioprotective effects were comparable to the conventional treatment by DRZ. PRI-724 also showed cancer suppressive effects while DRZ did not. We concluded that PRI-724 is effective against DOX-induced cardiotoxicity in vitro and in vivo, and our results support future clinical studies of this compound to improve the safety and efficacy of DOX treatment to improve patient outcome.

W1126

COOPERATIVE ANTIBACTERIAL EFFECT USING LACTONASE EST816-AG POLYMER NANOCOMPOSITE

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To synthesize an advanced polymer nanocomposite, quorum quenching lactonase Est816-Ag nanocomposite, and investigate its physicochemical property and antibacterial against Porphyromonas gingivalis (P. gingivalis) co-cultured with Streptococcus sanguis (S. sanguis). Est816-Ag nanocomposite was synthesized using a one-pot reaction and then its physicochemical property was characterized using dynamic light scattering, circular dichroism, fluorescence spectroscopy, and X-ray photoelectron spectroscopy. The morphology, viability, and ultrastructure of P. gingivalis co-cultured with S. sanguis treated by Est816-Ag nanocomposite were assessed using scanning electron microscopy (SEM), confocal laser scanning microscopy (CLSM), transmission electron microscopy (TEM). No treatment was included as a negative control and group treated with Est816 or nanosliver was included as positive control for comparison. Chemical characterization analysis indicated an Ag-N coordination between silver and the polymer/protein in the Est816-Ag nanocomposite with an average particle diameter of 102 nm and an enzymatic activity of 6 U/ml. SEM demonstrated scattered biofilm growth in Est816-Ag group, in contrast to confluent growth in the control. The live cell ratio under CLSM for Est816-Ag treatment and control were 15±5 and 85±8, respectively ($p < 0.01$). TEM revealed damaged cell and cytoplasmic membranes after Est816-Ag nanocomposite treatment, in contrast to the intact cell morphology observed in the control. The novel approach of integrating quorum quenching lactonase Est816 with silver nanoparticles provides provides stable physicochemical properties and enhanced antibiofilm efficacy against oral biofilm.

W1128

DECODING ADENOMYOSIS PATHOGENESIS USING AN ASSEMBLOID MODEL

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Adenomyosis is a challenging gynecological condition to study due to the absence of in vitro models that accurately replicate endometrial tissue dynamics across the menstrual cycle. In this study, we established an assembloid model that simulates cycle-dependent endometrial responses and mirrors the cellular and molecular features of adenomyosis, including lesion-specific epithelial and stromal heterogeneity. Single-cell transcriptomic revealed that ectopic epithelial cells exhibit a

luminal-dominant and glandular-deficient profile during the secretory-like phase following minimal differentiation medium (MDM) treatment. These changes were linked to MDM-induced stromal shifts, characterized by the loss of BMP4+ stromal cells and an increase in CRYAB+IL15+ stromal cells, disrupting stromal-epithelial BMP signaling and enhancing WNT signaling pathways. Additionally, ectopic epithelial and stromal cells demonstrated increased immunity and angiogenesis activities. This assembloid model provides a robust platform for studying adenomyosis pathogenesis and highlights WNT signaling as a potential therapeutic target, paving the way for more targeted and effective treatments.

Funding Source: National Key Research and Development Program of China (2022YFC2702200, 2023YFA1801800, 2023YFA1800300), NSFC (82471684, 32330030, 32270840, 32270908), Science and Technology Commission of Shanghai Municipality 23JC1403700.

W1130

DERIVATION OF MACROPHAGES FROM PORCINE EXPANDED POTENTIAL STEM CELLS FOR STUDYING HOST-PATHOGEN INTERACTIONS AND ANTI-VIRUS DISCOVERY

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African Swine Fever (ASF) is highly contagious and lethal disease in domestic pigs. Pulmonary alveolar macrophages (PAMs), which have long been adopted for host-pathogen interaction studies, are known typical host cells for virus infection and propagation, particularly for African Swine Fever virus (ASFV). However, limited lifespan and batch-to-batch variation of PAMs hinder experimental reproducibility and scalability, highlighting alternative models are needed. In this study, we utilized porcine expanded potential stem cells (pEPSCs) to establish a renewable in vitro supply of functional and experimentally tractable monocyte-macrophages that share similar phenotypes and features with ex vivo PAMs. This culture system is a serum- and feeder-free system involving the generation of embryoid bodies (EBs) from porcine EPSCs, followed by the formation of mesoderm and hematopoietic specification. Transcriptional profile revealed mesoderm fate transition for porcine EPSC differentiation under bone morphogenetic protein (BMP), WNT, transforming growth factor-beta (TGF-β), and VEGF signalling, enabling the acquisition of hemogenic-like signatures. The derived hemogenic intermediate allowed for the directed differentiation of hematopoietic progenitor cells (HPCs), which can be further differentiated into the myeloid lineage. The efficient and robust gene editing capacity of porcine EPSCs offers new opportunities to investigate the functional genetics and molecular dynamics of host-pathogen interactions. Overall, our newly identified culture system supports the derivation of cells closely resembling ex vivo porcine primary macrophages, providing a valuable and renewable cell source for viral studies and informing potential therapeutic strategies against porcine viruses.

Funding Source: This project is supported by Health@InnoHK, Innovation Technology Commission, HKSAR.

W1132

DIFFERENTIAL EFFECTS OF MONONUCLEAR PHAGOCYTES FROM HEALTHY INDIVIDUALS AND AGE-RELATED MACULAR DEGENERATION PATIENTS ON THE TRANSCRIPTOME OF HESC-DERIVED RETINAL PIGMENT EPITHELIUM CELLS

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Reticular pseudodrusen (RPD) are present in a subset of individuals with age-related macular degeneration (AMD) and are associated with an increased progression risk to late AMD. The underlying mechanisms for this association remain unclear. This study evaluated whether peripheral blood mononuclear cells (PBMCs) from individuals with intermediate AMD and RPD (iAMD/RPD+) uniquely influenced the transcriptome of retinal pigment epithelium (RPE) cells compared to PBMCs from individuals with iAMD without RPD (iAMD/RPD-) or healthy controls. PBMCs were collected from patients with iAMD/RPD+ (N=9), those with iAMD/RPD- (N=9), and age-matched healthy controls (N=9). H9 human embryonic stem cells were differentiated into RPE cells and cultured for 90 days. CD14+ monocytes were enriched from PBMC samples and co-cultured with RPE cells for 24 hours. Following co-culture, monocytes were removed, and total RNA was extracted from the RPE for bulk RNA sequencing (Illumina NovaSeq 6000). Transcriptomic data were normalised to adjust for any adherent immune cells and analysed using gene set enrichment analysis. Co-culturing RPE cells with PBMCs from healthy controls, compared to untreated RPE cells, resulted in 5015 differentially regulated genes (FDR< 0.05), including 12 genes critical for retinoid metabolism and RPE function. Co-culturing RPE cells with PBMCs from individuals with iAMD/RPD+ or iAMD/RPD- showed significant enrichment of gene sets related to inflammation compared to healthy controls, including those involved in chemotaxis (CXCL1, IL6, ICAM1), and cytokine/chemokine production. Finally, co-culturing RPE with PBMCs from iAMD/RPD+ resulted in distinct gene set enrichment differences compared to iAMD/RPD-, including negative enrichment for genes involved in epithelial mesenchymal transition. Our results suggest that PBMCs from individuals with iAMD induce molecular changes in the RPE. Furthermore, PBMCs from individuals with iAMD and RPD influence the RPE in a distinct manner compared to iAMD patients without RPD. These data implicate circulating innate immune cells in influencing the RPE transcriptome, causing differential RPE dysfunction between AMD patients with and without RPD.

W1134

HEALTH CONFERRED BY EPITHELIAL CELLS AND THEIR ROLE IN PARAPHAGY PROMOTED CHOLESTEROL CLEARANCE AND LIPID METABOLISM DURING EPIDIDYMAL SPERM MATURATION

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Mammalian sperm undergo functional maturation in the epididymis, preparing themselves for the long and challenging journey to begin a new life by successfully fertilizing an oocyte. Defects in

epididymal sperm maturation are closely related to male infertility and paternal health and even the health of offspring. In the epididymis, the halted translational machinery in spermatozoa while maturing in a quiescent state prompts them to immensely dependent on epithelial cells for nutritional supports and information exchange. Thus, epididymal epithelial function and luminal microenviroment play crucial roles during epididymal spermatozoa maturation. We have previously reported that infertile male mice lacking the functional gene for epithelial tight-junction protein occludin resulted in impaired sperm lipid metabolism and defects in epididymal-dependent early embryo development. However, the underlying mechanism remains unclear. Our indepth study discovered that occludin promotes paracellular junctional endocytosis and luminal cholesterol clearance and lipid metabolism. This occludin-dependent junctional endocytosis involves the formation of double-membrane phagosomes from the epithelial apical paracellular membranes, which we called paraphagy. We found that paraphagy involves interactions of surface receptor FCGR2B and apical absorption of APOJ from the lumen. We propose that occludin-promoted paraphagy ensures epididymal function and sperm maturation, and the epididymal-dependent reproductive events including embryo development.

Funding Source: This work is supported by NNSFC (82071704), SMCST (19140903400), ShanghaiTech University, and University of Saint Joseph.

W1136

IDENTIFICATION OF STRESS-RESILIENCE GENE AS A NOVEL TARGET OF ALZHEIMER'S DISEASE

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Chronic stress has been implicated in the etiology of neurodegenerative disorders such as Alzheimer's disease (AD) and brain aging, primarily by disrupting the hypothalamus-pituitary-adrenal (HPA) axis. This dysfunction leads to hyperactivation of the HPA axis, resulting in elevated glucocorticoid levels, which are commonly observed in AD patients. However, individual responses to stress vary, with stress-resilient individuals showing no significant neurodegenerative phenotypes with normalized HPA axis despite similar stress exposure. Thus, identifying the stress resilience targets that contribute to stress resilience under diverse stress conditions, including prenatal and adulthood stress, is crucial for developing targeted therapeutic strategies for AD and brain aging. In this study, we used a chronic unpredictable mild stress (CUMS) protocol in a mouse to distinguish between stress-resilient and stress-susceptible groups. Mice were exposed to either prenatal or adult stress, and their cognitive and depressive-like behaviors were assessed. Resilient mice exhibited normal cognition and mood. We then performed RNA sequencing of the brain to identify potential resilience genes. Furthermore, we also performed amplicon sequencing and LC/MS on stool to find the potential resilience-related microbiome. Our findings highlight the importance of stress resilience targets in modulating the effects of chronic stress on neurodegeneration. The significance of these genes or molecules is being further validated in AD models.

Funding Source: National Research Foundation of Korea grant funded by the Korea government (MSIT) (RS-2024-00345307) Korea Basic Science Institute (National Research Facilities and Equipment Center) grant funded by the MSIT (RS-2024-00403028).

W1138

INDUCING MATURATION OF HUMAN AMELOBLASTS FOR TOOTH REGENERATION AND DISEASE MODELING OF AMELOGENESIS IMPERFECTA

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Enamel, the hardest material in the human body is required to protect our living organ, tooth. However, over 90% of adults have lost or damaged enamel and cannot regenerate the protective structure due to lack of enamel producing cells, ameloblasts. IPSC derived Ameloblasts (iAM) have promise in future regenerative dentistry however, today it is not known why iAM maturation requires intimate contact with the dentin producing cell type, odontoblast. Here we reveal that the critical signaling ligand emanating from odontoblasts for ameloblast maturation is Delta. We further developed a computer designed soluble Notch activator that can mature iAM organoids in a precedented manner, without interactions with odontoblast layer. This novel maturation procedure allows us to analyze the detailed requirement of Dlx3 function in ameloblasts, independent of its known function in odontoblasts. We show Dlx3 is required cell autonomously in Ameloblasts for Enamel and MMP20 expression.

Funding Source: ISCRM.

W1140

INVESTIGATING THE SYNERGISTIC EFFECTS OF QUERCETIN AND VITAMIN C ON HUMAN CHONDROPROGENITORS: A CELLULAR AND MOLECULAR APPROACH

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Knee osteoarthritis is a burdensome condition since it affects the quality of life and is also immoderately expensive for operative management. In a quest for effective alternative solutions, nutraceutical supplementation has been extensively investigated over the past decade due to its ability to attenuate reactive oxygen species (ROS) formation within chondrocytes, a critical step in

the pathogenesis of this disease. Recently, there have been some tangible advances in the development of natural nutraceutical supplements, such as quercetin and vitamin C as the substitute for growth factors to repair cartilage defects. Understanding the possible protective mechanisms by supplementation of quercetin and vitamin C will have clinical implications in the treatment of OA. The present was conducted to examine the effect of quercetin and/or vitamin C on cell vitality and phenotypic properties of human chondroprogenitors (CPs) and to determine the effect of quercetin and/or vitamin C supplementation on the differentiation potential of chondroprogenitors into chondrocytes. FACS analysis of CPs was confirmative for the phenotypic expression of positive markers (CD73, CD90, CD105, CD146, CD166, CD271, collagen type II, aggrecan), and negative markers (CD45 and HLA-DR). CPs supplemented with a combination of quercetin (10 μM) and vitamin C (100 μM) exhibited decreased ROS activity, least apoptotic cells and superior chondrogenesis as confirmed by the mRNA expression of Collagen type II α1, Aggrecan, Collagen type XI α1, Collagen type X, SOX9, Cartilage oligomeric matrix protein (COMP), transforming growth factor-β (TGF-β), fibroblast growth factor-2 (FGF2), Cartilage-derived retinoic acid-sensitive protein (CD-RAP) by qPCR. The present study results are conclusive of the synergistic potential of quercetin (a multiple signaling inhibitor) and vitamin C (an antioxidant agent) in possible prevention of cellular damage and enhanced cartilage regeneration. However, preclinical investigations are required priorly, keeping in view of the transplantation approaches of quercetin and vitamin C supplemented CPs as a clinical source for cartilage tissue regenerative therapies.

Funding Source: Nitte (Deemed to be University).

W1144

POLYCYSTIC KIDNEY ORGANOIDS FOR DISEASE MODELLING AND DRUG SCREENING

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Polycystic kidney disease (PKD) is the most common genetic cause of kidney disease and the 4th leading reason of kidney transplantation worldwide. To offer insights into this condition, we developed organoid cultures from adult stem cells of human polycystic kidneys. With ethical approval and informed consent, organoids were established directly from the cyst-lining epithelium of three polycystic kidneys or the cortex of three transplant-declined healthy control kidneys. Cultures were grown in basement membrane extract (BME) in the presence of growth factors and expanded for more than ten passages. They were then characterised and compared. Both PKD and healthy kidney organoids developed into 3D structures expressing markers of various renal tubular compartments (Epcam, GATA3, CDH1, SLC4A1, ZO-1, Integrin 6 etc.) and renal stemness (PAX8). Bulk RNA-seq revealed that PKD organoids retain transcriptomic signature of PKD tissue, upregulating fibrosis, inflammation, and loss of barrier integrity as well as altered metabolism markers as compared to healthy organoids and tissue. Additionally, after orthotopic transplantation into immunodeficient mice, PKD and healthy organoids showed successful engraftment under the murine kidney capsule. Overall, these findings support this model as a valuable tool to understand PKD. Expression of key tubular markers, maintenance of tissue-of-origin transcriptomic signature as well as successful murine orthotopic engraftment enables use of PKD organoids (and healthy controls) as an exciting platform for in vitro and in vivo disease modelling and drug screening. To further enhance this model, we are currently in the process of utilising CRISPR-Cas9 editing to achieve a double knock-out of the two disease-causing genes of PKD (PKD1 and PKD2) on healthy kidney organoids. We are hoping that this three-armed organoid platform (PKD-derived,

healthy, and PKD1+PKD2 CRISPR-Cas9 knock-out) will enable us to further understand this disease as well as to interrogate novel drug targets to improve the lives of many patients.

W1146

SELECTIVE VULNERABILITY OF CEREBRAL VASCULAR SMOOTH MUSCLE CELLS TO NOTCH3 VARIANTS IN AN IPSC MODEL OF GENETIC SMALL VESSEL DISEASE CADASIL AND A NOVEL THERAPEUTIC TARGET

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NOTCH3 variants underlie CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), the most common genetic small vessel disease (SVD) and a leading cause of vascular dementia. Despite its prevalence, CADASIL is often underdiagnosed due to its late-onset nature. Clinically, CADASIL is characterized by recurrent strokes, migraines with aura, mood disturbances, progressive cognitive decline, and ultimately vascular dementia. NOTCH3 is primarily expressed in vascular smooth muscle cells (VSMCs), but the molecular mechanisms remain poorly understood, leaving no specific or effective treatments available. Although CADASIL affects small vessels systemically, its clinical manifestations are predominantly brain specific. To investigate this brain-selective vulnerability, we developed human induced pluripotent stem cell (iPSC) models harbouring the NOTCH3-R153C and NOTCH3-C224Y variants. These iPSCs were differentiated into VSMCs through three developmental lineages: neuroectodermal (NE), lateral plate mesodermal (LPM), and paraxial mesodermal (PM), representing brain, cardiac, and peripheral VSMCs, respectively. Intriguingly, only NE-VSMCs from CADASIL iPSCs exhibited significant abnormalities, including increased proliferation and migration, decreased contractility, disorganized focal adhesion and actin cytoskeleton, and elevated cell death. These phenotypes were absent in LPM-VSMCs and PM-VSMCs, highlighting the heightened susceptibility of brain VSMCs to NOTCH3 variants. Further analysis of NE-VSMCs using RNA sequencing, qRT-PCR, and immunostaining revealed an upregulation of extracellular matrix (ECM)-related genes and a downregulation of contractile genes, indicating a phenotypic switch from contractile to synthetic states. RNA sequencing also identified dysfunction in a pathway, the manipulation of which successfully reversed the abnormal behavioural and functional changes in VSMCs, suggesting a promising drug target. Our findings provide critical insights into the brain-specific mechanisms of CADASIL and pave the way for the development of targeted therapies for this devastating condition.

Funding Source: The British Heart Foundation; National Centre for 3Rs in Research.

W1148

THE CARDIOTOXICITY OF METABOLIC SYNDROME VLDL IN INDUCING CARDIOMYOCYTE MITOCHONDRIA DYSFUNCTION

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Metabolic syndrome, characterized by abnormal lipid metabolism and elevated cardiovascular risk, involves lipoprotein abnormalities that may impair mitochondrial function and contribute to mitochondrial-related diseases. Among these, very-low-density lipoproteins (VLDL) are implicated in mitochondrial dysfunction, though the underlying mechanisms remain unclear. This study aimed to elucidate the effects of VLDL on cardiomyocyte mitochondria using human induced pluripotent stem cell-derived cardiomyocytes (iCMs). Differentiation of iCMs from mesodermal induction to progenitor formation was confirmed, and VLDL-induced lipotoxicity was investigated through assessments of intracellular ROS and Ca^{2+} handling dysfunction. RNA sequencing revealed 27 upregulated and 97 downregulated differentially expressed genes (DEGs) associated with VLDL exposure, highlighting significant pathways linked to mitochondrial impairment. KEGG pathway mapping pointed to SIRT1-associated mechanisms as a critical contributor to VLDL-induced mitochondrial dysfunction. Functional assays, including Seahorse analysis, demonstrated a 23–36% reduction in mitochondrial oxygen consumption rates (OCR) following VLDL exposure, confirming its adverse impact on mitochondrial respiration. Immunofluorescence further indicated intra-mitochondrial modifications linked to VLDL lipotoxicity. Together, these findings provide new insights into the role of VLDL in disrupting mitochondrial function and metabolism in cardiomyocytes, offering mechanistic explanations for the progression of cardiovascular disease in patients with metabolic syndrome.

Funding Source: KMUH113-3M06.

W1150

UNLOCKING THE POTENTIAL OF BRAIN ORGANOIDS FOR UNDERSTANDING AND TREATING NEURODEVELOPMENTAL DISORDERS

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Pluripotent stem cell-derived organoids represent a significant advance in modelling human brain development and disease in vitro. However, the application of organoids to understand and find new therapies for brain diseases has been impeded by the scarce reproducibility and scalability of these models, and the challenge of identifying reliable pathological phenotypes suitable as readouts in high-throughput drug screenings. We addressed these issues by establishing a new highly standardised protocol that generates brain organoids with consistent morphology, encompassing shape, size, structure, and cellular composition. Bright-field imaging, immunohistochemistry, and single-cell RNA-sequencing analysis of individual organoids showed remarkable reproducibility across different organoids, cell lines and experimental batches. Over time, organoids generate a large collection of cell types resembling those found in the developing human forebrain. These include diverse types of neural progenitors, excitatory projection neurons, inhibitory interneurons, as well as late-born astrocytes and oligodendrocytes. We leveraged the

reproducibility of this new organoid model to set up a fully automated system for the generation and high-throughput screening of brain organoids. By using this automated platform, we showed that forebrain organoids haploinsufficient for the histone methyltransferase KMT5B, which is associated with various neurodevelopmental disorders, show increased size recapitulating megalencephaly observed in patients, highlighting the potential of our organoid platform to identify reliable disease phenotypes. Finally, high-throughput screening of forebrain organoids treated with a library of FDA-approved epigenetic compounds revealed that individual drugs induce reproducible alterations in organoid growth and development, further validating the robustness of our new brain organoid platform for large-scale drug screenings. Overall, our work offers new understanding of the role of epigenetic regulation during normal forebrain growth and development and paves the way to implement brain organoids into future drug discovery programs to identify potential therapies for neurodevelopmental disorders.

Funding Source: This research is supported by The Novo Nordisk Foundation Center for Stem Cell Medicine, reNEW, funded by the Novo Nordisk Foundation grant NNF21CC0073729.

W1152

ALMS1 MUTATION IMPAIRS CILIARY DISASSEMBLY AND PROMOTES CELL CYCLE EXIT IN ALSTROM SYNDROME BRAIN ORGANOIDS

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Alström Syndrome (AS) is an autosomal recessive rare disorder caused by mutations in ALMS1, a protein localized to centrosomes and basal bodies of cilia, which are pathologically affected in AS patients. Although previously demonstrate normal intelligence, increasing evidences indicate involvement of ALMS1 mutation in brain symptoms, including autism-spectrum behavior, seizure, obsessive compulsive, psychotic behaviors and so on. However, the underlying molecular mechanisms underlying the neurologic pathologies have never been elucidated. Here, we established two human induced pluripotent stem cell (hiPSC) lines with ALMS1 mutations from AS patients. We also generated isogenic control with CRISPR-Cas9-mediated mutant gene correction and ALMS1 gene knockout lines. The hiPSCs and control lines were then differentiated into cerebral organoids. We identified that ALMS1gene mutation caused complete loss of ALMS1protein, resulting in abnormally elongated primary cilia in human neural progenitor cells (hNPCs), which was rescued by gene correction. By establishing starvation and recovery method, patient NPCs exhibited obvious disruption of cilium disassembly. These NPCs, subsequently displayed reduced cell proliferation and increased cell cycle exit due to shorter cell cycle length. Single-cell transcriptomic profiling of patient derived brain organoid demonstrated significantly decreased autophagy and ubiquitination in the neuroblasts caused by ALMS1 mutation, suggesting abnormal development of patient derived NPCs. Our work elucidates the pivotal role of ALMS1 in human brain development, providing an important foundation for the treatment of this disease.

Funding Source: National Natural Science Foundation.

W1156

CHRONIC ETHANOL EXPOSURE DRIVES AMYLOID BETA DEPOSITION AND IMPAIRED NEURONAL MATURATION IN HUMAN IPSC-CORTICAL NEURONS AND CEREBRAL ORGANOIDS

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Ethanol exposure is a widely recognized environmental factor that disrupts neuronal function and has been implicated in neurodegeneration processes. The COVID-19 pandemic has further exacerbated concerns regarding alcohol consumption, with studies reporting increased alcohol use as a response to heightened psychological distress and social isolation. Given the chronic risks associated with excessive alcohol intake, understanding its impact on the nervous system is critical. Chronic alcohol consumption has been associated with cognitive decline and an increase of Alzheimer's diseases (AD), yet the precise mechanisms remain incompletely understood. In particular, the effects of ethanol on neuronal maturation, synaptic integrity, and amyloid-beta (Aβ) accumulation in human neurons have not been fully defined. This study utilizes human iPSC-derived cortical neurons and three-dimensional cerebral organoid models to investigate the impact of ethanol exposure on key neurobiological mechanisms. We first confirmed that ethanol exposure at physiologically relevant concentrations dose not compromise neuronal viability, as assessed by CCK-8 assays and Ki67 expression. However, ethanol exposure led to a significant reduction in neuronal maturation markers, including MAP2, FOXG1, TBR1 and FZD9, at both the protein and transcriptional levels Additionally, ethanol exposure resulted in increased Aβ accumulation, accompanied by upregulation of BACE1 and downregulation of ADAM10, suggesting ethanol-mediated alterations in amyloidogenic processing. These effects were further validated in three-dimensional spheroid and organoid models, where ethanol exposure impaired neurite outgrowth, reduced dendritic complexity, and exacerbated Aβ deposition. Together, these findings provide evidence that ethanol disrupts neuronal differentiation and synaptic stability while promoting amyloidogenic pathways, confirming its potential role in accelerating neurodegeneration. By integrating two- and three- dimensional human neuronal models, this study offers new insights into the molecular consequences of ethanol exposure, highlighting its relevance in neurodegenerative disease progression and the need for further research into alcohol-related cognitive decline.

Funding Source: This work was supported by the National Research Foundation of Korea (NRF) and ministries including Science and ICT, Trade, Industry and Energy, Health and Welfare, and Food and Drug Safety (RS-2023-KH142779, RS-2024-00512348).

W1158

CORE MODELS: NEXT-GENERATION CEREBRAL ASSEMBLOIDS FOR BRAIN CANCER MODELING AND DRUG DISCOVER

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The treatment of primary and secondary brain cancers remains a major challenge, with median survival averaging only 10 months, despite advanced multimodal therapies. This highlights an urgent need for improved treatment strategies. However, drug development faces a critically low success rate of just 3–7%, largely due to inadequate preclinical models that fail to accurately replicate neurotoxicity and tumor microenvironment (TME) dynamics. To address this gap, we present a novel, highly modular murine ex vivo platform based on adult neural stem cells (aNSCs),

designed for scalable, medium-throughput drug testing and mechanistic studies. This system integrates three distinct assembloid models: 1) Assembled Model – pre-grown cancer spheroids fuse with cerebral organoids to study tumor invasion and migration. 2) Cancer Core Model – distinct solid tumor cores embedded within healthy neural shells, closely mimicking in vivo-like tumor architectures. 3) Advanced Cancer Core Model – with immune cells, such as microglia, to investigate tumor–immune interactions within the TME. These assembloids form within 72 hours, offering a rapid, reproducible and ethical alternative to in vivo models. Their defined cellular composition and structured tissue architecture enhance translatability, overcoming batch variability in iPSC-derived organoids. Notably, the Cancer Core and Advanced Cancer Core Models are the first to feature a distinct, reproducible tumor core fully embedded in healthy neural tissue, enabling physiologically relevant studies of tumor growth, invasion and therapy response. By faithfully modeling aspects of the tumor–host interactions, this system supports in-depth investigations of tumor biology, drug efficacy and mechanisms of action. The immune-integrated Advanced Cancer Core Model further enables research on tumor–immune crosstalk and immunotherapy testing. Bridging in vitro and in vivo studies, this platform aligns with 3R principles, reducing reliance on animal models while maintaining biological complexity. By enhancing the predictive power of preclinical drug testing, these assembloids represent an innovative tool for brain cancer research, poised to accelerate bench-to-bedside translation.

Funding Source: Supported by Cenibra GmbH (AW, VA), Friedrich-Ebert-Stiftung, Joachim Herz Foundation and Bayer Foundation (AW), M3 Research Center, EXC2180 (iFIT), HECTOR Foundation. Thanks to OMNI Life Science for CERO 3D.

W1160

DECIPHERING THE REGENERATIVE CAPACITIES OF UC-MSCS AND BM-MSCS: A FOCUS ON CYTOKINE AND FIBROSIS-RELATED MIRNA PROFILES

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Mesenchymal stem cells (MSCs) are powerful mediators of tissue repair through multipotent differentiation, immunomodulation and paracrine signaling. Their therapeutic efficacy varies by tissue origin. Umbilical cord-derived MSCs (UC-MSCs) offer non-invasive accessibility, faster proliferation, and immunological naïveté, making them promising alternatives to bone marrow-derived MSCs (BM-MSCs). However, their molecular and functional roles in fibrosis regulation remain unclear. This study compares their cytokine secretion and fibrosis-associated miRNA profiles—two key determinants of MSC-mediated regeneration. To achieve this, UC-MSCs and BM-MSCs were compared via cytokine profiling (antibody array) to quantify secreted factors and miRNA analysis via qPCR-based fibrosis miRNA panels. Fibrotic and antifibrotic miRNAs scored as -1 (fibrotic) or +1 (antifibrotic). A scratch wound closure assay assessed migratory potential, measuring wound coverage at multiple time points. Using a high-sensitivity cytokine array, UC-MSCs exhibited higher levels of IL-8 (5.33-fold), BDNF (3.16-fold), TIMP-1 (1.40-fold), and TIMP-2 (1.98-fold), suggesting enhanced matrix remodeling and fibrosis regulation. Additionally, ENA-78,

Gro α/β/γ, and Gro-α associated with inflammatory and fibrotic responses—were highly expressed in UC-MSCs but at low levels in BM-MSCs. In contrast, BM-MSCs exhibited higher levels of SDF-1α, VEGF-α, IGFBP-2, IGFBP-3, and IGFBP-4, indicating a more pronounced role in angiogenesis rather than fibrosis resolution. Fibrosis-related miRNA profiling revealed a 318-fold higher anti-fibrotic miRNA score in UC-MSCs compared to BM-MSCs, underscoring their potential in fibrosis resolution. Ongoing analyses aim to correlate cytokine secretion with miRNA expression patterns. Functionally, UC-MSCs exhibited superior migration, covering 71.29% of the wound area within 24 hours, whereas BM-MSCs required 32 hours to reach 65.28% closure. These study suggests that UC-MSCs posssess enhanced wound healing potential, likely driven by their distinct cytokine and miRNA profiles. The differences in regenerative and anti-fibrotic properties between UC-MSCs and BM-MSCs highlight UC-MSCs as a more promising candidate for cell-based therapies targeting fibrotic diseases.

Funding Source: This study is funded by TUSEB-2022-B-03-27707.

W1162

DEVELOPMENT OF A NON-HUMAN PRIMATE MODEL MIMICKING HUMAN PERFORATOR INFARCTION FOR THERAPEUTIC APPLICATIONS

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Stroke often results in sequelae such as paralysis, aphasia, and higher brain dysfunction, imposing a significant societal burden. While thrombolytic therapy has shown success in acute-phase treatment, current pharmacological therapies and rehabilitation remain insufficient for post-acute treatment, highlighting the need for novel therapeutic strategies. Despite promising results in preclinical rodent models, translating these findings to human clinical trials has often proved unsuccessful. To bridge this gap, studies in non-human primates, which have closer anatomical and functional parallels to humans, are essential. In this study, we successfully established a model of perforator infarction in Macaca fascicularis by surgically ablating the perforating branches of the middle cerebral artery, closely replicating human perforator infarction. In human cases, perforator infarction often leads to severe paralysis while preserving higher cognitive functions, making it a potential target for future cell transplantation therapies. Our model exhibits strong paralysis similar to human cases, demonstrating its utility as a valuable platform for drug development.

Funding Source: This work was supported by Grants in research center network for realization of regenerative medicine and gene therapy, Japan Agency for Medical Research and Development (AMED) and JSPS KAKENHI Grant Number 22K09230.

W1164

DIVERGENT LINEAGE TRAJECTORIES AND GENETIC LANDSCAPES IN HUMAN GASTRIC INTESTINAL METAPLASIA ORGANOIDS ASSOCIATED WITH EARLY NEOPLASTIC PROGRESSION

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Gastric intestinal metaplasia (IM) represents a critical pre-cancerous stage, characterized by a morphological spectrum that is currently inadequately represented by human cell line models. Understanding the progression of IM along the cancer spectrum is pivotal in early detection, prevention and treatment of gastric cancer. However, the lack of suitable cell models for IM has hindered our understanding of its pathogenesis and neoplastic progression. To address this significant gap, we have successfully generated a large cohort of human gastric IM organoids (IMOs) that closely recapitulate the cellular and molecular features of IM in vivo. Leveraging the power of single-cell RNA sequencing (scRNA-seq), we comprehensively characterized the stem/progenitor populations within these IMOs and delineated their lineage trajectories. Our analysis showed that lineage plasticity in IM goes beyond gastric or intestinal fates and demonstrated a reversion to a fetal phenotype. We observed that IMO cells spanned a spectrum from hybrid gastric/intestinal to advanced intestinal differentiation, and uncovered lineage trajectories that connected different cycling and quiescent stem and progenitors, highlighting their differences in the gastric to IM transition. Lastly, we found that cell populations in gastric IM and cancer tissues were highly similar to those derived from IMOs and exhibited a fetal signature. In conclusion, our findings provide crucial insights into the lineage trajectories and genetic landscapes of IM, demonstrating that our IM organoid models are ideal for studying early gastric neoplastic transformation and chemoprevention. This research advances our understanding of IM pathogenesis and its neoplastic progression, potentially paving the way for the future development of targeted therapeutic strategies and improved patient outcomes.

Funding Source: The theme-based research grant (project no. T12-710/16-R) from the Research Grants Council of Hong Kong SAR, China; Health@InnoHK Initiative funded by the Innovation and Technology Commission of Hong Kong SAR, China.

W1166

FEASIBILITY OF 3-DIMENSIONAL HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED OSTEOBLAST USING AIR-LIQUID INTERFACE METHOD FOR SPINAL FUSION IN A RAT MODEL; PRELIMINARY REPORT

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Spine fusion (SF) is one of the most common strategies for the treatment of various spine pathologies, such as degenerative disc diseases, trauma, tumor or infection. To achieve fusion of mobile vertebral segments, bone graft materials play an important role. However, harvesting autologous bone can cause morbidities at donor sites, and its amount is limited. The aim of this study was to investigate the efficacy of bone formation after transplantation of 3-Dimension iPSC derived osteoblast differentiation in Air-Liquid interface (3D iOB) in a spinal fusion animal model. Differentiation of 3D iOB using an Air-Liquid interface method in vitro. Spine fusion rat model was generated using 7-week-old adult male Sprague-Dawley rats. Those were allocated into four

groups based on the graft materials: (1) SF only, (2) SF+Collagen Ⅰ without cells, (3) SF+demineralized bone matrix (DBM), and (4) SF+3D iOB. Transplantation of 3D iOB in the spinal fusion rat model improved bone volume and trabecular thickness at 8- and 12-weeks post transplantation. The results of this study suggest that iPSC-derived osteoblasts can be a powerful cell source for spinal fusion. In addition, the possibility of promoting bone formation using 3-Dimension iPSC derived osteoblast differentiation in Air-Liquid interface as well as its utility as a graft scaffold.

Funding Source: This work was supported by the National Research Foundation of Korea (NRF) and ministries including Science and ICT, Trade, Industry and Energy and Drug Safety (RS-2023-KH142779, RS-2019-NR040058).

W1168

GENERATION OF LGE-COMMITTED NEURAL PROGENITOR CELLS WITH AFFINITY TOWARD DRD2+ MEDIUM SPINY NEURON POPULATION FROM HUMAN STEM CELLS USING A NOVEL SMALL MOLECULE-BASED RECIPE

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Medium spiny neurons originate from the LGE region of developing forebrain and later migrate to the striatum where they mature into DRD1 and DRD2 neuronal subtypes. Loss of DRD2+ cells due to HTT gene mutation is the cause of Huntington's disease (HD). HD is a devastating neurodegenerative disease with life altering symptoms including motor, cognitive and psychological disorders. To this day there is no cure for HD disease. Effective differentiation of human stem cells to DRD2+ subtype in vitro can be the key to finding a cure for Huntington's disease by providing access to correct cell type with minimal contamination from other fates that can faithfully reflect the mechanisms involved in the onset and advancement of the disease, hence, helping the researchers to gain better understanding of the disease and facilitate drug discovery efforts. Moreover, stem cell derived medium spiny neural progenitors can be transplanted into patients to replace the lost cell population. Here, we implemented HD-DoE technology to develop a small molecule-based differentiation protocol that can target optimization of specific genes at each stage of differentiation, closely following embryonic development. We successfully developed a 10-day protocol comprised of 3 chemically defined small molecule-only recipes that guide the hiPSCs to LGE-committed neural progenitor cells. These cells express forebrain markers FOXG1, SIX3 and OTX2, ganglionic eminence markers DLX5 and ASCL1 and LGE specific markers FOXP1 and FOXP2. The purity of culture was evaluated by immunocytochemistry, and it's estimated that more than 80% of cells are committed to LGE region. When NPCs were differentiated for an additional three days, we were able to confirm expression of DRD2 protein along with early neuronal markers such as TUBB3 and DCX in the culture, while majority of cells express SIX3 and CTIP2 proteins. Importantly, expression of NKX2-1 protein, which is a MGE marker, is successfully suppressed. Bulk RNA sequencing was performed during differentiation, and it was confirmed that generated cells express DRD2 gene as early as day 10 while DRD1 gene is absent in culture. The significant

advantage of this novel protocol is that it can be translated to large scale production of cells in a cost-effective way that makes the cells easily accessible.

W1170

HUMAN BREAST MILK PRESERVES FATTY TRANSPORT AND INTESTINAL INTEGRITY IN MODELING NECROTIZING ENTEROCOLITIS USING HUMAN SMALL INTESTINAL ORGANOIDS

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Necrotizing enterocolitis (NEC) is a deadly disease for preterm neonates, with mortality up to 50% and significant long-term complications, despite many modern medical and surgical advancements. Although human breast milk reduces the incidence of NEC and appears to promote intestinal adaptation, the underlying molecular mechanisms are not fully understood nor replicated by formula feeds. Moreover, enteral feeding is often restricted in neonates with NEC due to ileus, bowel rest, or perioperative consideration. As there are a lack of specific treatments for NEC, elucidating these adaptive processes may reveal novel therapeutic targets that mimic the benefits of breast milk, offering a promising strategy to reduce NEC-associated morbidity and mortality. Human organoids are established models of both the intestinal system and the NEC disease process. Mature iPSC-derived human small intestinal organoids were cultured in growth media supplemented with either human breast milk, Enfamil A+ formula or 1X D-PBS (control) at 1:10 ratio. To simulate NEC-inducing inflammatory conditions, groups were exposed to a 24 h treatment of TNF-alpha and LPS, administered before or after exposure to the supplemented media. Immunocytochemistry assessed the integrity of tight-junctions, enterocyte abundance, and preservation of goblet cells, all key components of epithelial barrier function. Concurrent RT-qPCR analysis focused on the expression of critical fatty acid transport proteins. Our results demonstrate that when compared to formula, human breast milk uniquely upregulates lipid transport-associated genes FABP1, SCARB2, and FATP4. Goblet cell populations and tight junction integrity is also better preserved in breast milk-treated NEC organoids, as compared to formula-fed ones. These molecular adaptations offer insight into the potent protective effects of breast milk, underscoring its role in promoting intestinal homeostasis under inflammatory stress. These results expose promising molecular targets for drug discovery, such as fatty acid transporters, laying the groundwork for the development of targeted therapies capable of replicating the adaptive benefits of human breast milk. Such strategies may be leveraged to improve intestinal function in neonates afflicted by intestinal diseases.

Funding Source: University of Saskatchewan Department of Surgery, Resident Research Award.

W1172

INCREASED NATURAL KILLER RESISTANCE IN P53-DEFICIENT OSTEOSARCOMA AND MESENCHYMAL STEM CELLS DUE TO UPREGULATED NF-KAPPA BETA-IFNB-HLA SIGNALLING

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Growing evidence had demonstrated important immunomodulatory functions of p53 (encoded by TP53), in addition to its other well-known roles in tumor suppression. Tumor cells with different p53 statuses often manifest complicated immune evasion capability relevant to various mechanisms. Here, we found that osteosarcoma cell lines with p53 loss had lower natural killer (NK) sensitivity than those with wild type p53, which was restored following ectopic expression of TP53 in the cells. Then, we elucidated the correlation of p53 and NK sensitivity in human osteosarcoma. Via single cell RNA-seq analysis, we identified mesenchymal stem cells (MSCs) as a source-of-origin for osteosarcoma, in which cells with high scores of p53 functions had more signal communications with NK cells than cells with low scores. Then, we modeled the preneoplastic stage of osteosarcoma using TP53-/- MSCs constructed through TP53 knockout. TP53-/- MSCs showed lower NK sensitivity than the wild-type control and TP53-/- MSC-derived osteocytes displayed osteosarcoma-like characteristics. The reduction in NK sensitivity was associated with NF-κB-IFNβ-mediated upregulation of NK-inhibitory ligands, mainly type-Ia human leukocyte antigens (HLA-Ia). Consistently, clinical data mining also demonstrated the correlation of p53 functions negatively with the HLA-Ia level and type I IFN signaling. Thus, these findings suggest that NK resistance increases in p53-deficient MSCs and osteosarcoma cells via NF-κB-IFNβ-mediated upregulation of HLA-Ia, revealing a novel role of p53 during tumorigenesis.

Funding Source: Macau Science and Technology Development Fund grants [0002-2021-AKP, 0071-2022-A2], University of Macau Research Committee funds [MYRG2022-00044-FHS], China National Natural Science Foundation [32270842].

W1174

INNOVATIVE WORKFLOW FOR DRUG RESPONSE STUDIES ON SINGLE 3-DIMENSIONAL CELL MODELS COMBINING SPHEROONE AND INCUCYTE

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Complex three-dimensional (3D) in vitro cell models, including spheroids, tumoroids, and organoids, are becoming indispensable in various fields, aiding drug discovery and personalized medicine. However, transitioning from traditional monolayer cell cultures to 3D cell models poses significant challenges in sample handling, assay read-out, and overall assay reproducibility. This study presents an innovative workflow for high-throughput preparation of large quantities of spheroids in microcavity microplates, sorting and isolating homogeneous 3D models using spheroONE, and real-time live-cell analysis of drug response using the Incucyte® Live-Cell Analysis System. In this proof-of-concept, thousands of Human Embryonic Kidney (HEK293) spheroids were prepared in microcavity plates. After 3 days of culture, spheroids with diameters

ranging from 190 to 260 µm were sorted and isolated as single spheroids per well in 96-well plates using spheroONE. Immediately following spheroid isolation, various concentrations of camptothecin were applied, and drug response was monitored over 7 days of culture through live-cell imaging and analysis using the Incucyte® Live-Cell Analysis System. Workflows combining spheroONE and Incucyte® live-cell imaging and analysis are adaptable to a variety of microphysiological systems (including spheroids, tumoroids, and organoids) and provide researchers with powerful solutions for accelerating toxicology and drug development studies.

W1176

INTERPLAY BETWEEN IRE1-ALPHA AND CARDIAC EXCITATION-CONTRACTION COUPLING IN STEM CELLS-DERIVED CARDIOMYOCYTES

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Cardiovascular disease (CVD) is the leading cause of death worldwide with no cure identified so far, the current clinical treatment method primarily relies on early diagnosis. Activation of the endoplasmic reticulum (ER) stress is associated with CVD or it may be triggered by CVD. Therefore, targeting ER stress signalling is a promising therapeutic direction. We previously showed that in isolated adult mouse cardiomyocytes, IRE1α (the most conserved ER stress sensor) is localized to the junctional sarcoplasmic reticulum (jSR) responsible for the regulation of cardiac excitation-contraction (E-C) coupling. In this study we used mouse embryonic stem (mES) cells with deletion of the IRE1α gene and showed delayed cardiac differentiation with weak beating activities, indicating an important role of IRE1α in cardiac development and cardiomyocyte function. We also discovered that IRE1α is in close proximity with the Cav1.2 (the α subunit of L-type calcium channel) and Cavβ2 (the β subunit of L-type calcium channel) in human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes. These findings indicate that IRE1α plays a novel role in supporting cardiac E-C coupling through regulating the L-type calcium channel and controlling calcium homeostasis. Nonetheless, IRE1α showed activation in response to thapsigargin and tunicamycin-induced ER stress indicating that IRE1α also plays a canonical role in responding to ER stress in the heart through the IRE1α-XBP1 signalling pathway. Since the dysfunctions of L-type calcium channel and excessive ER stress give rise to cardiac defects, IRE1α may serve a cardioprotective role in the heart as a L-type calcium channel regulator and an ER stress sensor. As the generation of iPSC provides a patient-specific platform for thorough understanding of the CVD pathology, further investigating the mechanism of IRE1α as a component of cardiac E-C coupling, specifically as a regulator of the L-type calcium channel points towards a new direction for CVD drug development.

Funding Source: Canadian Institutes of Health Research (CIHR) China Scholarship Council (CSC).

W1180

MODELING PURA SYNDROME IN CEREBRAL ORGANOIDS USING PURA KO AND PATIENT IPSCS

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Heterozygous de novo mutations in the PURA gene cause PURA syndrome, a neurodevelopmental disorder characterized by neurodevelopmental delay, intellectual disability, hypotonia and epileptic seizures. Besides its nucleic acid binding ability, PURA's molecular role in cells is poorly understood. To model PURA syndrome in vitro, homozygous knock-out (KO) iPS cell lines and reprogrammed patient iPS cell lines harboring the most recurrent PURA mutation (Phe233del) were generated as well as their respective isogenic controls. The iPSCs were differentiated into cerebral organoids to study the impact of PURA's absence and the effect of Phe233del mutation during early neurodevelopment. While morphological and size analysis of the organoids showed no difference between KO, patient cells, and their isogenic controls, multi-omic approaches revealed significant cellular changes in early development upon loss of PURA.

Funding Source: DFG - Deutsche Forschungsgemeinschaft.

W1182

MULTI-REGIONAL NEURAL ORGANOID PANEL FOR SCREENING OF NEURODEVELOPMENTAL DEFECTS

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Neural organoids have been successfully used for modeling brain functions, studying neurodevelopmental disorders, and advancing drug development. However, traditional models often focus on limited brain regions, failing to capture the effects of entire brain regions. This can be particularly problematic in disease modeling and toxicity studies, as it limits the understanding of broader pathological phenomena that may occur across multiple brain regions. To address this issue, we established a simple and scalable method to generate a Multi-regional Neural Organoid Panel (MrNO). MrNO was produced through a streamlined three-stage culture process (neural induction → neural patterning → neural maturation), incorporating different cocktails of patterning factors to achieve anteroposterior and dorsoventral axis-dependent production of multiple organoids simultaneously. MrNO demonstrated high batch-to-batch reproducibility, validated by both morphological and transcriptomic analyses. This approach can be expanded to optimize conditions to produce other regionally specified organoids. Furthermore, through large-scale production, we faithfully identified toxic substances that induce primary and secondary microcephaly, revealing region-specific differential toxic responses. This platform extends single-

region models to multi-region systems, facilitating the study of various brain regions and enabling a more holistic evaluation of pathological phenomena in neurodevelopmental defect screening.

Funding Source: This study was supported by the Bio and Medical Technology Development Program of the NRF (RS-2023-00225239). This research was also supported by a grant of the Korea Dementia Research Project through the KUCRF (RS-2024-00467876).

W1184

NEUROGENOMICS STUDY OF STEM CELL-DERIVED NEURAL MODELS IDENTIFIES FUNCTIONAL RISK VARIANTS FOR BRAIN DISORDERS AND IMPLICATES MICROGLIA-SPECIFIC ROLE OF PICALM IN ALZHEIMER'S DISEASE

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Despite genome-wide association studies (GWAS) of late-onset Alzheimer's disease (LOAD) having identified many genetic risk loci 1-6, the underlying disease mechanisms remain largely unknown. Determining causal disease variants and their LOAD-relevant cellular phenotypes has been a challenge. Leveraging our approach for identifying functional GWAS risk variants showing allele-specific open chromatin (ASoC) 7, we systematically identified putative causal LOAD risk variants in human induced pluripotent stem cells (iPSC)-derived neurons, astrocytes, and microglia and linked PICALM risk allele to a previously unrecognized microglia-specific role of PICALM in lipid droplet (LD) accumulation. ASoC mapping uncovered functional risk variants for 26 LOAD risk loci, mostly specific to microglia. At the microglia-specific PICALM locus, the LOAD risk allele of rs10792832 reduced transcription factor (PU.1) binding and PICALM expression, impairing the uptake of amyloid beta (Aβ) and myelin debris. Interestingly, microglia carrying the PICALM risk allele showed transcriptional enrichment of pathways for cholesterol synthesis and LD formation. Genetic and pharmacological perturbations of microglia further established a causal link between reduced PICALM expression, LD accumulation, and phagocytosis deficits. Our work elucidates the selective LOAD vulnerability in microglia for the PICALM locus through detrimental LD accumulation, providing a neurobiological basis that can be exploited for developing novel clinical interventions.

Funding Source: R01AG063175 (J.D. and G.T.), R01AG081374 (J.D.), RF1AG079141 (G.T.). R01 AG073260 (H.J.B.), and U01 AG072439 (H.J.B.). A.S. is supported by a BrightFocus fellowship. R01MH116281 and R01MH106575 (to J.D.).

W1186

PATHOLOGICAL ANALYSIS OF AGE PROGRESSION USING ORGAN ORGANOIDS DERIVED FROM AGED MICE FOR ELUCIDATION OF CANCER-RELATED DISEASE PATHOGENESIS

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Multi-stage carcinogenesis theory is known as the process by which healthy cells repeatedly accumulate genetic mutations and transform into malignant tumors. Still, genetic mutation varies among individuals and cancers, and in some cases, carcinogenesis can occur without mutation. Therefore, carcinogenesis factors may have been also related to the senescence of stem cells in the organs in old age. Previous studies reported experimental models of carcinogenesis induced by chemical substances or cancer-related gene mutations, but there has not been an established carcinogenesis model using aged animal-derived cells. Therefore, the current study focused on establishing aged (19-month-old) mice organoids (AMO) and comparing their histopathological characteristics with young (4-weeks-old) mice organoids (YMO). Kidney, lung, gall bladder, and bladder tissue were extracted from aged or young mice and used to generate organoids. The relationship between stem cells and carcinogenesis between AMO and YMO in different organ organoids was genetically evaluated. RNA sequencing was performed using AMO and YMO to explore signaling pathways that were specifically activated in AMO compared to YMO. Changes in morphology and aging-related gene markers were assessed by HE staining and IHC. Organoids were successfully derived and the morphological differences were evident between AMO and YMO, especially for gallbladder and bladder organoids. Expressions of MUC3, and CK19 in gallbladder organoids, and UPK3A, CK7, and CK5 in bladder organoids were downregulated in AMO, compared to YMO. RNA sequencing revealed that some age-related signaling pathways were significantly more activated in AMO than in YMO. It would be expected that organoids derived from aged mice will have usefulness in the future to elucidate the mechanism of aging and the process of age-related carcinogenesis.

W1188

PROTEOMIC PROFILING OF ENDURANCE EXERCISE REVEALS INFLAMMATORY AND STEM CELL-ASSOCIATED RESPONSES USING DRIED BLOOD SPOTS

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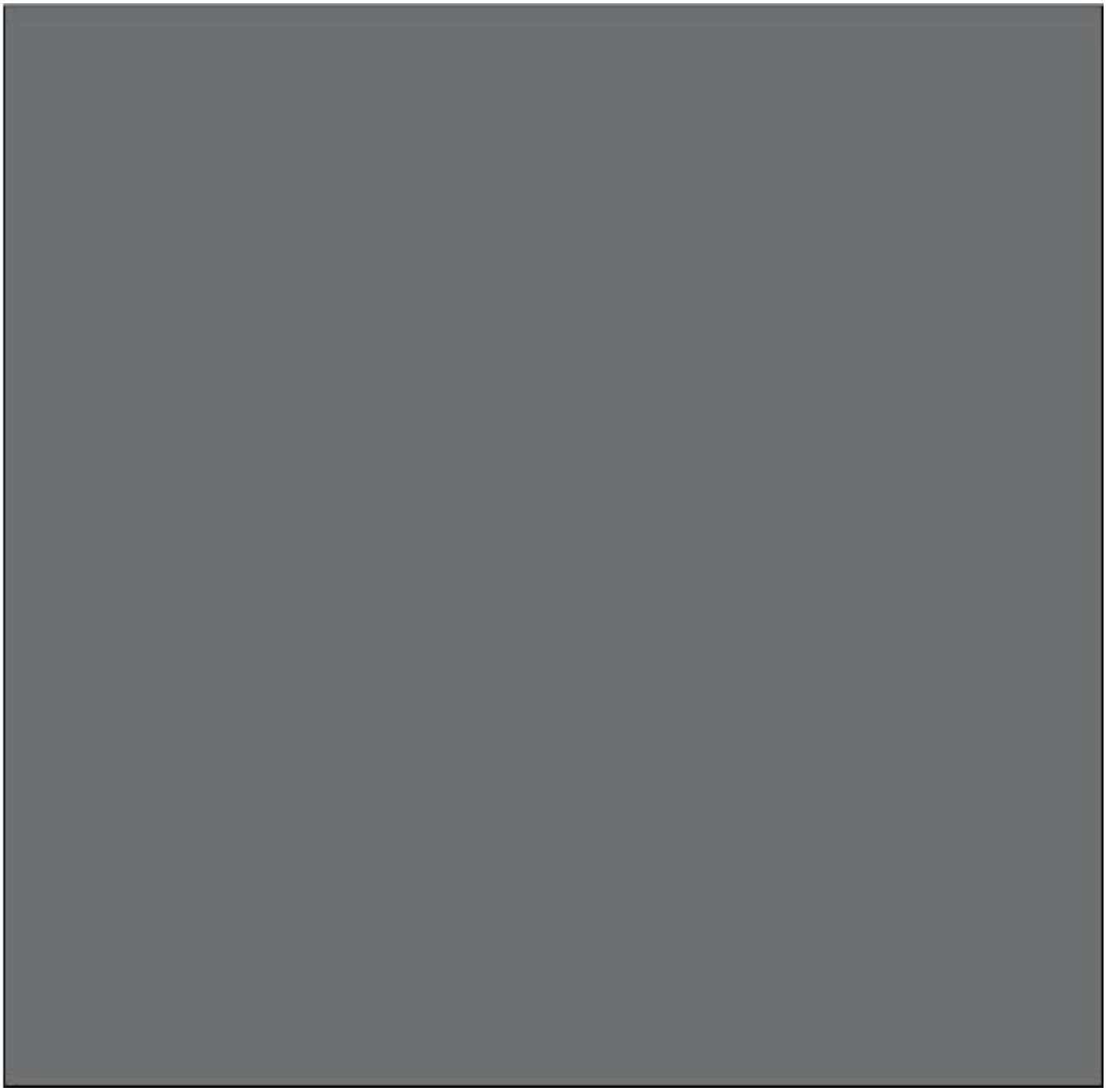
Dried blood spots (DBS) have emerged as a minimally invasive biosampling method for proteomic analyses, offering novel insights into the inflammatory and regenerative processes associated with endurance exercise. This study leveraged the NULISAseq 250-plex Inflammation Panel to evaluate the proteomic shifts in endurance athletes before, during, and after a half-marathon. Our findings indicate a dynamic inflammatory response, characterized by significant changes in key cytokines and growth factors implicated in tissue repair, immune modulation, and cellular regeneration. Notably, biomarkers such as IL-6, VEGFD, and TNFRSF8 exhibited time-dependent fluctuations, suggesting their potential role in stem cell activation and recruitment during exercise-induced stress and recovery. Given the established link between systemic inflammation and stem cell

mobilization, our data support the hypothesis that endurance exercise may serve as a physiological stimulus for endogenous stem cell activity. Furthermore, the ability to capture these proteomic signatures using DBS underscores the feasibility of field-based, high-throughput biomarker discovery for regenerative medicine applications. These findings highlight the potential of integrating exercise physiology with stem cell research to optimize recovery strategies and therapeutic interventions for tissue repair and regeneration.

Funding Source: WorldQuant Initiative for Quantitative Prediction.



I

W1192

REDUCTION OF THE MITOCHONDRIAL COMPLEX I SUBUNIT AMELIORATES CELL DEATH IN HUMAN IPSC-DERIVED NEURONS

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Neurons depend heavily on ATP production via the mitochondrial oxidative phosphorylation (OXPHOS) pathway. Dysfunction of mitochondrial respiratory complex I (MCI), a key component of OXPHOS, has been observed in multiple neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD). A recent study showed that disruption of MCI in dopaminergic neurons in mice leads to decreased dopamine production and PD-like symptoms. However, how MCI reduction is related to the degeneration of human neurons remains poorly understood. Here, we investigated the relationship between MCI and neurodegeneration by reducing MCI subunit expression using CRISPR interference (CRISPRi) or CRISPR-Cas9 in human iPSC-derived neurons. We found decreasing MCI subunit expression in hiPSCs did not affect their differentiation into neurons or the viability and morphology of the resulting neurons under normal culture conditions. Compared with control neurons, those with reduced MCI subunit expression showed upregulation of the glutathione synthesis pathway, an essential antioxidant defense mechanism. In addition, when treated with a cell death inducer, MCI-deficient neurons exhibited higher survival rates, maintained mitochondrial membrane potential for longer, and produced lower levels of ROS than controls. Our results demonstrate that reducing MCI subunit expression enhances glutathione-mediated antioxidant defenses and reduces ROS generation, thereby ameliorating cell death. Thus, this study offers a new perspective that reducing the mitochondrial complex I subunit may lead to a promising neuroprotective strategy, in contrast to the previous view that MCI inhibition leads to neuronal death. Furthermore, it underscores the potential clinical application of this approach to slow or prevent neuronal loss in AD, PD, and other neurodegenerative disorders.

Funding Source: Masason Foundation California Institute for Regenerative Medicine (CIRM) The on-site laboratory initiative launched by Kyoto University Funding from Mr. Hiroshi Mikitani and Mr. Marc Benioff National Institutes of Health (NIH).

W1196

THE DYNAMIC OF MAMMALIAN PLACENTA METABOLITES AND THEIR IMPACTS ON PLACENTATION, PLACENTAL FUNCTION, AND EMBRYONIC DEVELOPMENT

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Mammalian placental metabolism is crucial for placental and embryonic development. Yet the stage-specific metabolic dynamics governing placentation remain poorly characterized. Here, we conducted integrated metabolomic-transcriptomic profiling of 501 mouse placentas across embryonic days (E) 8.5-14.5 and revealed the key metabolic characteristics of placentation. Our multi-omics approach revealed three distinct developmental phases (E8.5, E9.5-10.5, E11.5-14.5) with transitional periods at E8.5-9.5 and E10.5-11.5. Phase-specific metabolic signatures emerged: ubiquinone biosynthesis dominated E8.5, riboflavin/phenylalanine pathways marked E9.5-10.5, while glycine/serine/nucleotide metabolism prevailed post-E11.5. Based on a series of metabolite and enrichment analyses, we identified 1,4-dihydronicotinamide adenine dinucleotide (NADH) as a central regulator. Using IVC embryos, we found that NADH promoted body length extension. Mechanistically, presomitic mesoderm (PSM) -like cells treated with NADH showed an elevated total NAD+/NADH ratio, increased generation of reactive oxygen species (ROS), and accelerated cell proliferation. Together, our findings reveal the role of placental metabolites, which might provide mechanistic insights for optimizing placenta organoids and IVC embryos or embryoids.

Funding Source: This work was financially supported by the National Natural Science Foundation of China (82402032), the Postdoctoral Fellowship Program of CPSF (GZB20240738).

W1198

USING IPSC-DERIVED NEURONS TO INVESTIGATE PATHOGENIC MECHANISMS OF APP-V717I MUTATION IN FAMILIAL ALZHEIMER'S DISEASE

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Alzheimer’s disease (AD) affects over 55 million people worldwide. Familial AD, which accounts for approximately 1–5% of all AD cases, is characterized by early-onset cognitive decline, accelerated brain atrophy, and rapid disease progression compared to AD. Familial AD is primarily caused by single pathogenic mutations in 1 of 3 genes: APP (amyloid precursor protein), PSEN1 (presenilin 1), and PSEN2 (presenilin 2). The APP-V717I mutation is one of the most common APP mutations worldwide. However, the mechanisms by which this mutation induces neurodegeneration remain unclear. Here, we utilized patient-derived induced pluripotent stem cells (iPSCs) carrying the APP-V717I mutation and differentiated them into cortical neurons as a model to investigate the underlying mechanism. Compared to control, conditioned media from these iPSC-derived patient neurons has elevated levels of Aβ42 level and Aβ42/40 ratio as well as a lower sAPPα/β ratio. In addition, these neurons exhibited alteration of gene expression linked to cell stress and stress response pathways. Furthermore, neurons carrying the APP-V717I mutation exhibited an increase in reactive oxygen species after induction, suggesting impairment of anti-oxidative stress function. We then used the CRISPR/Cas9 gene editing system to disrupt the APP-V717I mutation, which rescued the alterations in APP processing, including Aβ42 level, Aβ42/40 ratio, and sAPPα/β ratio. Moreover, we noted a reversal of stress-related transcriptomic changes and restoration of anti-

oxidative stress function in neurons differentiated from genome-edited iPSC. These findings elucidate how the APP-V717I mutation disrupts APP processing and exacerbates neuronal stress. The rescue of molecular and functional deficits through CRISPR editing underscores its potential as a targeted therapeutic strategy for familial AD.

Funding Source: RGC HK: CRF (C6027-19GF), TRS (T13-605/18-W), GRF (HKUST16103122; HKUST16104624), AoE (AoE/M-604/16) ITC: InnoHK (INNOHK18SC01), Grant (ITCPD/17-9) NSFC: NSFC/RGC JRS (32061160472) SIAT-HKUST joint lab, CAS: (JLFS/M-604/24).

W1200

A COMPARATIVE STUDY OF CRISPR-BASED IN VIVO GENE KNOCK-IN STRATEGIES FOR HAEMOPHILIA B TREATMENT AND EDITING OUTCOMES IN GENOME

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CRISPR-Cas9-mediated insertion of exogenous sequences into a targeted genomic locus showed promising potential to provide novel gene therapies to treat a wide range of inherited disorders. However, low integration rates and diverse editing outcomes remain the major hurdles for clinical translation. In this study, haemophilia B was employed as a disease model to assess the therapeutic potential of in vivo targeted insertion strategies. We performed a multi-parameter knock-in analysis, to identify efficient and high-fidelity gene knock-in strategy for advancing gene insertion-based therapy. A systematic and quantitative framework using Illumina RNA-seq and long-read sequencing was employed to analyze diverse knock-in outcomes. In this study, we performed a comparative analysis of multiple parameters involved in knock-in process. The results revealed that break configurations, choice of DNA repair pathways and donor template play an important role in the targeted insertion. Cas9-induced DSBs are necessary for efficient knock-in. Nickase-induced SSB (single-stranded break) and no cutting groups showed low or even undetectable insertional efficiency. Surprisingly, we found that leveraging distinct DNA repair pathways yields comparable insertional efficiency in mouse liver. Surprisingly, we found that only 20bp-microhomology arms in MMEJ (microhomology-mediated end joining) donor could support insertion as efficient as the widely demonstrated HDR (homology-directed recombination) donor and NHEJ (non-homologous end joining) donor. The chimeric donor containing only one homology arm showed suboptimal performance. Besides, we observed that ssAAV vector outperformed the scAAV as a donor template for targeted insertion over scAAV, no matter combining with NHEJ donor or MMEJ donor. In conclusion, a comparative analysis of parameters highlights the role of break configurations, DNA repair pathways and donor template in the targeted insertion. Future research will leverage long-range sequencing to analyze diverse insertional outcomes influenced by these parameters.

W1202

A MOUSE LIVER TUMOR-DERIVED ORGANOID PLATFORM FOR UNDERSTANDING DRIVER-DEPENDENT LIVER CANCER AND RESPONSE TO THERAPY

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In recent years, multiple drugs have been approved for the treatment of hepatocellular carcinoma (HCC). However, the clinical benefit is far from optimal, extending the survival of patients for a few months only. This is due to the complex, heterogeneous nature of different HCC patients, in which cancer cells harbor a mix of driver and passenger mutations. Traditional cell lines and animal models lack a clean background in which the effects of individual driver mutations can be studied in detail in the absence of passenger mutations. Here, we utilized HCC tumors generated by the hydrodynamic tail vein injection of proto-oncogene combinations in mice together with the tumor organoid platform to study the characteristics of HCC driven by different driver mutations and their response to therapeutics. We have successfully established HCC tumor organoids with specific driver mutations (loss of TP53, Axin1, and PTEN, and CTNNB1 activating mutation in the background of MYC overexpression) and validated their gene mutations at both genomic and proteomic levels. Using a high-throughput screening approach, with the screen consisting of over 600 FDA-approved drugs for drug repurposing, we have identified ERBB family inhibitors as therapeutic targets for CTNNB1 mutation-driven HCC (Δ90CTNNB1/MYC). Multi-omics analysis through chromatin accessibility, transcriptomics, and phosphoproteomics revealed activation of the ERBB signaling pathway in Δ90CTNNB1/MYC models via increase transcription of related genes, Egft and Tgfa, and phosphorylation of ERBB family, EGFR and ERBB2. Activation of this pathway in HCC patients with CTNNB1 activating mutation was further confirmed with clinical data extracted from publicly available datasets. Finally, the sensitivity of CTNNB1 mutation-driven HCC towards ERBB family inhibitors was validated using an animal model. In conclusion, we successfully established an HCC organoid platform with specific driver mutations and discovered a novel therapeutic target for CTNNB1 mutation-driven HCC. We further elucidated the distinct pathways that potentially drive the sensitivity towards ERBB family inhibitors in CTNNB1 mutation-driven HCC. Our findings provide the foundation for future repurposing of FDA-approved drugs for the treatment of HCC patients with CTNNB1 mutation.

Funding Source: This project is supported by Research Grants Council of Hong Kong - Research Impact Fund 2020/21 (R7022-20F), Research Fellow Scheme (RFS2122-7S05) and Croucher Foundation – Croucher Senior Research Fellowship to Stephanie Ma.

W1204

A NOVEL HUMAN FETAL LUNG-DERIVED ALVEOLAR ORGANOID MODEL FOR INVESTIGATING AT2 CELL PHYSIOLOGY AND DISEASE MECHANISMS

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Alveolar type 2 cells play a vital role in lung homeostasis as stem cells and producing pulmonary surfactant. Disorders in AT2 cell physiology affects development of various lung diseases, including interstitial lung diseases in human. Particularly, certain inherited forms are linked to the mislocalization of surfactant protein C (SFTPC) variants. Modelling SFTPC mutations and investigating underlying pathogenic mechanisms has been challenging due to difficulties in deriving and expanding human AT2 cells in vitro. In this study, we show the development of mature, expandable AT2 organoids from human fetal lungs. These organoids are phenotypically stable, capable of differentiating into AT1-like cells, and amenable to genetic modification. Using this organoid model, we tested key regulators of SFTPC maturation identified in a forward genetic screen, including the E3 ligase ITCH. Overall, this study introduces a novel human alveolar organoid model, which we used to identify essential factors in SFTPC maturation required for AT2 cell function.

Funding Source: K.L. is supported by a Korea University grant and by the National Research Foundation of Korea (NRF) grant funded by Korea government (MSIT) (RS-2024-00342036 and RS-2023-00221182).

W1206

A NOVEL MRNA-LIPID NANOPARTICLE PLATFORM TO RAPIDLY GENERATE FUNCTIONAL FOREBRAIN NEURONS FROM HUMAN PLURIPOTENT STEM CELLS USING NGN2

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Differentiation of human pluripotent stem cells (hPSCs) into neuronal cell types has become a cornerstone of neurological research by providing unique cellular models of human diseases. Lentiviral delivery of the transcription factor neurogenin-2 (NGN2) has been widely adopted over the past decade to rapidly convert hPSCs into neurons. However, this method faces challenges such as genomic integration risks, variability, labor-intensive steps and viral safety requirements. To address these limitations we developed the STEMdiff™-TF Forebrain Induced Neuron Differentiation Kit, an integration-free system that simplifies the generation of highly-pure hPSC-

derived neurons using mRNA-lipid nanoparticle (LNP) delivery of NGN2. hPSCs from four independent cell lines (H9, H1, WLS-1C, and SCTi003-A) were seeded at a density of 7.5 x 10^4 cells/cm2 into 24-well plates. Synthetic NGN2 mRNA encapsulated in LNPs was then added to the culture for three consecutive days. By day 6, we observed efficient neuron conversion, with 96% ± 3% of cells expressing neuronal marker TUJ1, and a yield of 7.6 × 10^5 ± 3 × 10^3 (mean ± SD; n = 8, 4 cell lines) neurons per 24-well plate. After two weeks of maturation using BrainPhys™ hPSC Neuron Kit, 94% ± 2.6% of neurons expressed MAP2, and 90% ± 4.9% expressed the synaptic vesicle marker synapsin I (n = 8, 4 cell lines). Transcriptomic profiling revealed a mostly forebrain identity, with high expression of FOXG1, TBR1 and SLC17A7. To characterize the electrophysiological properties of these neurons, hPSCs were seeded into 96-well plates, and their electrical activity was assessed using a multiwell microelectrode array over several weeks. Spontaneous electrical firings were detectable on day 8 (mean firing rate [MFR] = 0.11 Hz ± 0.03 Hz), peaked on day 21 (MFR = 1.5 Hz ± 0.5 Hz) and persisted for > 48 days in culture (n = 8, 4 cell lines), demonstrating sustained functional activity. In summary, STEMdiff™-TF Forebrain Induced Neuron Differentiation Kit provides a user-friendly, efficient, and integration-free method for generating functional forebrain neurons from hPSCs using NGN2. This system enables rapid acquisition of electrical activity, providing a platform for studying human neurons and advancing neurobiological research.

W1208

A PLATFORM FOR MULTIPLEXED RNA-BASED CELL ENGINEERING TO DRIVE DIFFERENTIATION IN HUMAN STEM CELLS VIA MECHANOPORATION

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The ability to generate diverse functional cell types rapidly and efficiently from induced pluripotent stem cells (iPSCs) holds transformative potential for disease modeling, drug discovery, and regenerative medicine. Protocols have been derived to differentiate iPSCs into several different cell types; however, these methods come with limitations such as low efficiency, variability between batches, and long production times. Herein we describe a novel mechanoporation technology that allows for cytosolic delivery of cargos (e.g. nucleic acids, proteins, peptides) by means of rendering cell membranes temporarily permeable through mechanical stress. The technology has demonstrated compatibility across multiple cell types and materials, while having minimal effects on endogenous gene expression. Our approach is scalable, reproducible, and minimizes some of the safety concerns associated with other delivery methods. Our results show that mechanical delivery of mRNA encoding Neurogenin 2 (NGN2) can enable differentiation of iPSCs into neuronal precursors via increased expression of early neuronal markers and a decrease in pluripotency markers. These data illustrate the potential to overcome current limitations associated with differentiation while maintaining a viability of 75% at a RNA expression level of over 70%. Additionally, we show that our technology enables delivery of circular RNA, antibodies, and other impermeable cargos, such as DNA-encoded libraries, which can be used for diverse cell engineering and drug discovery applications. At a larger scale for clinical processes that meet GMP

requirements, we have demonstrated delivery of over 1 billion cells per minute. By continuing to simplify stem cell modification, we aim to further unlock the biological potential of iPSCs while simultaneously reducing manufacturing complexity and safety concerns associated with other delivery modalities.

W1210

A POTENTIAL REJUVENATION FACTOR C IMPROVES METABOLIC DYSFUNCTIONS AND MITIGATES ATHEROSCLEROSIS IN LDLR -/- MICE

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Atherosclerosis, an age-related disease with metabolic dysfunction and chronic inflammation, remains a major contributor to cardiovascular disease-mediated mortality worldwide. Recent advancements in regenerative medicine have focused on uncovering extrinsic anti-aging factors showing immunomodulation and metabolic homeostasis to mitigate atherosclerotic progression. A potential rejuvenation factor C (PRFC refer as an alias), a member of the IL-6 cytokine family, utilizes gp130 in its receptor complex to mediate anti-inflammatory and metabolic regulatory effects. Considering its rejuvenation potential and anti-inflammatory capacity, we hypothesized that PRFC overexpression could alleviate atherosclerosis and metabolic disorders. To evaluate this hypothesis, LDL receptor knockout (Ldlr-/-) mice, after being treated intravenously with either AAV-Ctrl or AAV-Prfc, were fed a Western diet for 20 weeks. Overexpression of PRFC led to significant metabolic and vascular improvements. By analyzing DEXA and indirect calorimetry, PRFC-overexpressing mice exhibited reduced body weight gain and fat mass and enhanced energy expenditure despite no differences in food intake. Atherosclerotic plaques in the aortic arch and aorta were significantly reduced in PRFC-overexpressing mice compared to controls. PRFC overexpression decreased the expression of senescence-associated secretory phenotype-related genes, including Mmp13 and Spp1, and ameliorated pro-inflammatory biomarkers in plasmas. These findings provide that PRFC is a novel extrinsic anti-aging factor entailing immunomodulation and metabolic homeostasis, suggesting it might be a promising candidate for developing regenerative therapy for age-related diseases, such as atherosclerosis.

Funding Source: This study was supported by the KRIBB Research Initiative Program, by the NST grant (No. GTL24021-100), by the ABC project grant (RS-2024-00433755), by the Basic Science Research Program (NRF-2021R1I1A2056805).

W1212

A STUDY OF SEVERE CATECHOLAMINERGIC POLYMORPHIC VENTRICULAR TACHYCARDIA WITH IPSC-CARDIAC MODEL DERIVED FROM PEDIATRIC PATIENTS

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Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a genetic cardiac condition causing severe risk for sudden cardiac death. Structure of a patient’s heart is normal, and no arrhythmias are detected at rest. When the heart rate increases due to emotional stress or exercise

severe arrhythmias occur causing symptoms like loss of consciousness, and even sudden death. Approximately 30% of the patients receive symptoms before age of 10 and prognosis of death before age of 30 is 30-35%. We have derived iPSC lines from the blood samples of six patients from 10 to 18 years old carrying severe form of the CPVT. These iPSC lines are differentiated into cardiomyocytes and their electrical properties are measured with calcium imaging method. We have now studied calcium release from the calcium storages which are essential for beating of the cells and at the moment we are optimizing increasement of the beat rate so we could observe electrical events on a moment where beat rate starts to elevate. As the severe clinical phenotype of the patients predicts there are also challenges in a cell model when the beat rate increases. Previously we have studied cardiomyocytes derived from adult CPVT patients in 3D bioprinted cardiac structures which showed normal functionality, Ca2+ handling properties and disease phenotypic response to adrenaline treatment. This cardiac iPSC model could be used to determine why the clinical phenotype, especially with these patients, is so life threatening and in addition to test drugs to treat arrhythmias caused by increased beat rate.

W1214

ABCA7 DEFICIENCY DISTURBS IMMUNE RESPONSES IN HUMAN IPSC-DERIVED MICROGLIA-LIKE CELLS

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Alzheimer's disease (AD) is the most common form of dementia and the sixth leading cause of death in the United States. Despite extensive research on AD pathogenesis, no effective treatment to cure AD or prevent its progression due to unclear mechanism. ABCA7 is an ATP-binding cassette (ABC) transporter sub-family A member that regulates the distribution of lipids and other lipophilic molecules across cellular membranes. Several loss-of-function variants in the ABCA7 gene are genetically associated the early-onset and late-onset AD. However, the pathogenic mechanism caused by ABCA7 loss of function remains elusive. We generated isogenic ABCA7 knockout iPSCs using CRISPR)/Cas9 technology as an ABCA7 loss of function model. We differentiated the iPSCs into microglia-like cells (iMGLs), and comprehensively assessed functions of ABCA7 knockout iMGLs using RT-qPCR, western blotting, immunofluorescent staining, and scRNA-seq. We also co-cultured the iMGLs with iPSC-derived neurons to investigate effect of ABCA7 deficiency in iMGLs on synaptic functions using microelectrode array (MEA). The scRNA-seq identified seven clusters, including human leukocyte antigen (HLA), homeostatic microglia (HM), and disease-associated microglia (DAM). Geno ontology (GO) analyses identified pathways related to lipid biosynthetic process and antigen processing and presentation pathways in ABCA7 knockout iMGLs compared to the controls. RT-qPCR showed that ABCA7 deficiency diminished the LPS-induced increases of IL6 and IL1B expression in iMGLs. We also found less immunopositivity of microglia activation markers CD68 and gelectin-3 in ABCA7 knockout iMGLs than the controls after Aß exposure. Western blotting found the compromised expressions of RAB5A and EEA1. MEA analysis revealed that ABCA7 knockout iMGLs repressed spike frequency in the neurons compared to the control iMGLs. We demonstrated that ABCA7 loss of function

compromises proper immune activation in iMGLs. These findings should provide novel mechanistic insight into how the loss of ABCA7 function impacts the AD pathogenesis.

W1216

ADIPOSE-DERIVED STROMAL CELL SECRETOME: A PROMISING CELL-FREE ALTERNATIVE FOR TREATING TENDINOPATHY

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Tendinopathy, a painful condition arising from tendon overuse and aging, currently lacks effective treatments. This study explores the efficacy of adipose-derived stromal cells (ADSCs) and their secretome—comprising extracellular vesicles and soluble factors—on tendon healing in a rat model of degenerative tendinopathy. We aimed to optimize the dosage and injection frequency of both ADSCs and their secretome, examining their effects in rat tendons, and in tendon-derived stem/progenitor cells (TDSCs) isolated from healthy tendons (nTDSCs) and tendinopathic tendons (pTDSCs) of patients. ADSCs and their secretome were prepared by Rohto Advanced Research Hong Kong Ltd. nTDSCs and pTDSCs were treated with ADSCs or their secretome, with or without IL-1β stimulation, and the mRNA expression of inflammatory markers, matrix remodeling markers, and lineage differentiation markers was assessed using qRT-PCR. The effect of secretome on cell viability and migration was evaluated through Alamar Blue assays and scratch wound healing assays. In the rat model, animals received various doses of ADSCs and secretome at different frequencies following collagenase injection. Tendon samples were collected for histological, immunohistochemical, and mRNA analysis at specified intervals. Results indicated that ADSC secretome significantly enhanced the viability and migration of inflammatory nTDSCs and pTDSCs, counteracting the effects of IL-1β on the expression of inflammatory cytokines, matrix remodeling enzymes, and multilineage markers, with the secretome exhibiting superior efficacy in suppressing inflammation, matrix-degradation and promoting tenogenesis. High doses of ADSCs and a single equivalent dose of secretome exhibited comparable benefits in tendon repair over time, while multiple secretome injections did not provide additional advantages. Both treatments effectively suppressed inflammatory cytokines and matrix-degrading enzymes, promoting tenocyte marker expression. The similar in vivo effects of ADSCs and their secretome, coupled with superior in vitro results of the secretome, suggest that it is a promising cell-free alternative for treating tendinopathy.

Funding Source: This project was supported by Innovation and Technology Commission, HKSAR, China, with half of the funding provided by Rohto Advanced Research Hong Kong Ltd. (ARHK) (PRP/007/22FX).

W1218

AGE-EQUIVALENT 3D IN CONSTRUCTS TO STUDY AGE-DEPENDENT PATHOLOGY AND RESILIENCE MECHANISMS IN ALZHEIMER'S DISEASE

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Aging is the primary risk factor for neurodegenerative diseases such as Alzheimer's Disease (AD), which exclusively affects the elderly. Despite its critical role, our understanding of the intersection between aging and neurodegeneration remains incomplete due to the limitations of existing models: postmortem studies do not allow for mechanistic studies, and animal models fall short in capturing the interplay of human biological aging and genetics. While iPSC-derived models provide valuable patient-specific insights, they are rejuvenated cells that lack the physiological aging signatures, and represent fetal brain characteristics instead. To address these gaps, a more authentic 3D model that integrates human aging-related molecular disease mechanisms with pathological AD signatures in a patient-specific manner is desirable. Here, we harness the potential of induced neurons (iNs), directly reprogrammed from patient fibroblasts, which uniquely retain critical aging signatures, exhibit adult 3R/4R Tau splicing, and replicate aging-associated AD phenotypes. However, traditional 2D iN cultures lack the 3D complexity and dynamic interactions—such as cell-extracellular matrix signaling—essential for replicating the aging human brain's microenvironment and extracellular AD pathology. Here, we developed a 3D iN construct system that integrates iNs with synthetic microcarriers. These microcarriers are engineered to support complex neuronal networks, promote synaptic connectivity, and sustain long-term cell viability in a physiologically relevant 3D environment. Notably, our system successfully models the endogenous production of extracellular matrix proteins, including the buildup of toxic protein (such as beta amyloid) in cultures derived from control, sporadic AD, and familial AD patients. This demonstrates the potential of our platform to uncover key mechanisms underlying aging and neurodegeneration. By combining the aging signatures of iNs with the architectural sophistication of 3D biomaterials, this model offers a transformative approach to studying age-related mechanisms in AD. Furthermore, it provides a robust platform for therapeutic discovery, enabling the identification of interventions that reduce pathological burden and promote neuronal resilience.

W1220

ALMS1 DEFICIENT HIPSC DERIVED RETINAL ORGANOIDS MODELING ALSTROM SYNDROME LOSS PHOTORECEPTORS

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Alström syndrome (AS) is a rare syndromic monogenic recessive disorder characterized by hearing and vision loss, obesity, type 2 diabetes mellitus, dilated cardiomyopathy and progressive renal dysfunction. Visual symptoms develop within a few weeks after birth, progressively leading to blindness. Currently, no cure is available. ALMS1 gene is responsible of this disorder. The protein coded by this gene localizes at the centrosome and within the basal bodies of ciliated cells and has suggested roles in intraciliary transport, cell migration, extracellular matrix production, and in endosomal trafficking. Our work aims to model visual symptoms in a human cell model of AS with the goal to understand molecular mechanisms underlying AS and to identify novel therapeutic targets. To this end, we generated different human induced pluripotent cells (hiPSC) lines using CRISPR/Cas9 base editing approaches to induce selected non-sense mutations within ALMS1's sequence. The different cell lines obtained were qualified to confirm their pluripotency. The lines were then differentiated into retinal organoids to assess the consequence of gene mutations. All ALMS1 mutated hiPSC differentiated into retinal organoids lost photoreceptors compared to isogenic wild type controls after 150 days in culture, recapitulating photoreceptor loss in AS patients. Future work on these retinal organoids will allow deciphering molecular mechanisms involved in this photoreceptor loss in order to identify a molecular target suitable for drug testing.

Funding Source: I-Stem is part of the Biotherapies Institute for Rare Diseases supported by the Association Française contre les Myopathies-Téléthon. This work was funded by Retina FRANCE.

W1222

ALTERED MATRIX ENVIRONMENTS ENHANCE THE IMMUNOMODULATORY ACTIVITY OF MESENCHYMAL STROMAL/STEM CELLS THROUGH TNF-ALPHA/NF-KAPPA B AND TNF-ALPHA/JNK/AP1 SIGNALLING PATHWAYS

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Mesenchymal stromal/stem cells (MSCs) are recognised for their role in modulating immune cells during tissue repair. Given the dynamic nature of the extracellular matrix (ECM) in such situations, this study aimed to identify the signalling pathways in MSCs that are influenced by the ECM and how this impacts their immunomodulatory activity. Human MSCs were cultured in a monolayer on tissue culture plastic (TCP) or encapsulated in three 3D ECM matrices (Fibrin, Collagen Type 1 and Collagen Type 1-Fibrin). After 24 hours of subjecting them to a low-dose pro-inflammatory environment (2ng/µl TNFα and 5ng/µl IFNγ), transcriptional profiles were analysed using bulk RNA sequencing (RNAseq). The significant transcriptional changes were validated using RTqPCR, ELISA and Western blot. RNA silencing and pharmacological inhibition were employed to investigate key signalling pathways. Previously, we showed that human MSC expression of TNFAIP6 and CXCL10 in 3D environments is significantly upregulated in response to pro-inflammatory stimuli. Here, RNA-seq analysis identified 2,085 genes that were significantly upregulated in 3D matrices compared to TCP, with over 90% of the highly expressed genes (including FOSB, FOS, and TNFAIP6) being consistently shared across all hydrogel scaffolds. In response to cytokine treatment, subsequent gene ontology analysis highlighted the TNF signalling pathway as one of the most enriched. Protein-protein interaction predictions revealed that the hydrogel environment differentially influenced the TNF-alpha/NF-kappa B and AP1 pathways.

Pathway inhibition studies showed that 3D environments regulate the immunomodulatory activity of MSCs treated with TNFα and IFNɣ through the TNFα/NF-kappa B and TNFα/JNK/AP1 signalling pathways. These findings highlight that MSCs exhibit distinct immunomodulatory responses in 3D environments compared to traditional monolayer cultures. This underscores the importance of 3D models in more accurately mimicking (patho)physiological conditions for studying MSCs and their therapeutic potential.

Funding Source: King Abdulaziz City for Science and Technology.

W1224

AMELIORATING SYMPTOMS OF MULTIPLE SCLEROSIS BY TARGETING CD19+ B-CELLS AND SECRETING TGF-BETA1 USING CAR-MICROGLIA

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Multiple Sclerosis (MS) is an unpredictable and chronic autoimmune disease that gravely affects the central nervous system (CNS). It is often characterised by neuroinflammation and demyelination of neurons. Currently, there is no cure for MS, and patients suffer from various degrees of physical incapacitation and cognitive impairment. Recent advancements in chimeric antigen receptor (CAR) technology and immunotherapy have provided an opportunity for engineering immune cells for MS treatment. This study will engineer lineage-negative hematopoietic stem and progenitor cells (Lin- HSPCs) with a CAR construct to generate CD19-targeting and TGF-β1 secreting CAR-microglia. The functions of the CAR construct will be validated in vitro using murine microglia, murine B-cell, and HEK-Blue TGF-β reporter cell lines. Subsequently, an experimental autoimmune encephalomyelitis (EAE) mice model induced by myelin oligodendrocyte glycoprotein (MOG) will be used to study the CAR-microglia's effects in vivo. The MOG-induced EAE model is one of the most common mouse models for MS, and the MOG(1-125) peptide should be used for B-cell-dependent therapies. After myeloablative conditioning with Busulfan, injecting the engineered Lin- HSPCs into the CNS of EAE mice can allow cells to repopulate as microglia in the brain environment. The CAR-microglia will aim to phagocytose CD19+ autoreactive B-cells that play a pathogenic role in MS and simultaneously maintain an anti-inflammatory CNS environment. Since microglia are the resident macrophages of the CNS and may play a protective role in MS, equipping them with a CD19-derived single-chain variable fragment (scFv) and anti-inflammatory TGF-β1 payload will be a novel and beneficial therapy for MS. This method can be further adapted by modifying the antigen-binding domain and the payload of the CAR construct for different autoimmune and neurodegenerative diseases. This study hopes to contribute to the immunology and neuroscience fields by developing a tailorable and novel therapeutic approach using CAR-microglia.

W1226

ANALYSIS OF ENTEROVIRUS A71 INTESTINAL INFECTION USING HUMAN ES/IPS CELLS AND MICROPHYSIOLOGICAL SYSTEMS

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Enterovirus A71 (EV-A71), one of the viruses responsible for hand, foot, and mouth disease, infects and replicates in the human intestine. While EV-A71 infection is known to cause the damage of the central nervous system (CNS), the pathophysiology of intestine including the enteric nervous system (ENS) remains unknown. Additionally, there are no therapeutic drugs to treat EV-A71 infection. In this study, we developed intestinal models with enteric neurons and infected them with EV-A71 to elucidate the intestinal pathophysiology of EV-A71 infection and to evaluate whether these models can be used for pharmaceutical research. We previously generated an intestinal model by differentiating human ES/iPS cells in microfluidic devices (micro-intestine system). The single-cell RNA-sequencing (scRNA-seq) analysis revealed that this model contained not only intestinal epithelial cells but also enteric neurons. Thus, we used the micro-intestine system to study EV-A71 infection. EV-A71 genome was detected in the culture supernatant of virus-infected systems, suggesting that EV-A71 can efficiently infect the micro-intestine system. RNA-seq analysis showed that the expressions of neuron and epithelial markers in the micro-intestine system were downregulated by EV-A71 infection. Histological analysis also indicated that Tubulin Beta 3 (TUBB3)-positive neurons were decreased, and the epithelial layer was disrupted by EV-A71 infection, indicating that EV-A71 infection caused the neural and epithelial damage. We evaluated the anti-viral effect of rupintirivir, which is one of the 3C protease inhibitors, using micro-intestine system. The viral genome copy number in the cell culture supernatant of the infected micro-intestine system was decreased by rupintrivir treatment. This result suggested that this model is useful for evaluating the effectiveness of drugs. In conclusion, the micro-intestine system with enteric neurons is a valuable tool for elucidating the detailed pathophysiology of EV-A71-infected intestine and evaluating the effect of therapeutic drugs.

W1228

APPLICATION OF TONSIL-DERIVED STEM CELL SPHEROID FOR RECOVERING VOLUMETRIC MUSCLE LOSS

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Volumetric muscle wasting (VML) is a traumatic injury characterized by irreversible removal of skeletal muscle, resulting in loss of tissue function and lifelong disability. However, treatments for VML are limited to scar tissue removal and autologous muscle transplantation. Skeletal muscle can regenerate after injury, but in VML, when tissue loss is severe, the natural regenerative capacity of muscle tissue is impaired. In this study, we evaluated the potential of spheroids derived from mesenchymal stem cells (MSCs) to treat VML, where muscle regeneration is poor. In previous studies, we have confirmed that tonsil-derived mesenchymal stem cells (TMSCs) can be

differentiated into skeletal muscle cells (SKMCs), and these cells have characteristics of skeletal muscles. Spheroids were formed using TMSC-derived skeletal muscle cells (TMSC-SKMC-spheroids) in microwell. To evaluate the treatment effects, grip test, gait analysis and nerve conduction study were performed after treated the mixture with TMSC-SKMC-spheroids and medical collagen into a VML rat model. The VLM model treated with TMSC-SKMC-spheroids showed improved motor function of the hindlimb and regeneration of the gastrocnemius muscle. These regeneration of the muscle after treatment was confirmed by changes in the expression of myokine markers. Taken together, these results indicate the therapeutic potential of TMSC-SKMC-spheroids mixed with collagen for VML. Furthermore, TMSC-SKMC-spheroids are expected to be developed as one of the promising therapeutic resources for hereditary, degenerative muscle diseases.

Funding Source: This study was supported by a Basic Science Research Program through the NRF funded by the Ministry of Education (2022R1I1A01064295) and KFRM funded by Ministry of Science and ICT, and Ministry of Health and Welfare (23A0201L1).

W1230

AUTOMATED DEVELOPMENT OF IPSC-DERIVED 3D NEURAL ORGANOIDS AND FUNCTIONAL ANALYSIS OF CALCIUM OSCILLATION ACTIVITY

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Growing Neural 3D organoids from human induced pluripotent stem cells (iPSC) is a rapidly developing technology with great potential for understanding brain development, neuronal diseases, as well as impact of different genetic backgrounds. However, the formation of neural organoids is a complex and lengthy process, making it difficult for compound screening. Here we demonstrate automated protocol for development of forebrain neural organoids using CellXpress.ai cell culture system and Dorsal Forebrain organoid differentiation media. The CellXpress.ai has liquid handling, imager, automated incubator as well as process controlling scheduling software. The device enables automation of most repetitive tasks, including culture and expansion of iPSC, formation of organoids in AggreWell plates, then transfer and culture 3D organoids in 24well low attachment plates with periodic media exchanges, agitation, and monitoring organoid development by imaging. After 50 days of culture and maturation of 3D organoids functional characterization of spontaneous neural activity was measured by recording and analysis of calcium oscillations. Calcium oscillations were recorded after addition of calcium sensitive dye on a FLIPR instrument that measured fast kinetic changes in calcium signal. Oscillation patterns were analyzed by software for multiple parameters including peak count, amplitude, and peak width. Organoids demonstrated spontaneous calcium oscillation activity, with consistent rate of oscillations. Morphological characterization of 3D organoids was done by imaging. Organoids diameters ranged from 1800-2000 µm, and the expression of neural markers including TUJ1 and GAFP was detected with fluorescently labeled antibodies. For pharmacological characterization, several compounds were used to show the appropriate functional responses. AMPA and 4-AP addition resulted in dose-dependent increase of frequency of calcium oscillations, while GABA and caused decrease in oscillation frequencies. Taken together, this biological system of iPSC-derived 3D neural organoids

paired with process automation and detailed analysis of calcium oscillations demonstrates a promising tool for compound testing.

W1232

BRAIN-SPECIFIC DYSTROPHIN ISOFORMS HAVE A FUNCTION IN RADIAL GLIA DURING HUMAN CEREBRAL ORGANOID DEVELOPMENT

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Duchenne muscular dystrophy (DMD) is a neuromuscular disease chiefly characterised by progressive muscle weakness due to loss of structural protein, dystrophin (Dp427). Numerous cognitive comorbidities, like autism spectrum disorder (ASD), are observed in 30% of DMD patients due to the cumulative loss of Dp427 and embryonic, brain-specific dystrophin variant, Dp140. Though previous studies in adult mice have linked brain dystrophin to modulating synaptic transmission, its precise cellular localisation and developmental function remain unclear. This is due to the scarcity of embryonic human brain tissue, particularly from DMD patients, and disparities between human-mouse cerebral cortex. To address these challenges, we generated DMD patient-derived induced pluripotent stem cell (iPSC) cerebral organoids to model disease-specific cortical architecture. Bulk qRT-PCR showed that DP140 expression peaks at 100 days of WT organoid culture, mirroring non-disease human mRNA profiling data. Super-resolution microscopy of WT organoids showed distinct localisation of Dp427 and shorter dystrophin in neuroepithelial rosettes, a previously unreported phenotype. DMD iPSC-cerebral organoids exhibited both reduced bulk DP140 and altered dystrophin expression in neural progenitor cells. Additionally, publicly available single cell RNA sequencing data from human embryonic brain showed DMD transcript enrichment in radial glial cells. Together, this suggests a potential role for dystrophin during neurogenesis, whose loss may affect the onset of cognitive comorbidities in DMD. We are currently analysing temporal cortical layer thickness and synaptic markers in DMD patient iPSC-cerebral organoids. We aim to perform cell and axonal migration studies to examine potential disease phenotypes arising from defects in neurogenesis. Single cell RNA sequencing paired with spatial transcriptomics will be done to assess DMD isoform expression and pathway analysis across cell types in our organoids. This will allow us to gain a deeper understanding of human-specific, early molecular defects associated with Dp140 deficiency, potentially opening avenues for novel therapies.

Funding Source: Young Researchers Grant, KAKEN Japan (2024-2027) Defeat Duchenne Canada, The Jesse Davidson Foundation (2024-2027).

W1234

CARDIAC SYMPATHETIC DYSREGULATION IN CATECHOLAMINERGIC POYLMORPHIC VENTRICULAR TACHYCARDIA

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Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmogenic disorder that can lead to life-threatening arrhythmias and sudden cardiac death, particularly in young individuals. Stress-induced activation of the sympathetic nervous system is viewed as the primary driver, yet the molecular mechanisms governing neuronal-cardiac signalling in CPVT remain incompletely understood. Existing studies often rely on animal models that don't fully recapitulate the human phenotype. To address this, we developed hiPSC-derived cardiomyocytes (CMs) and sympathetic neurons (SNs) from patients with CPVT and their isogenic controls in both 2D co-cultures and 3D microtissue platforms. The 3D models, fabricated with an innovative droplet-printing technique, mimic the complex tissue architecture and electrophysiological properties of human neuronal-cardiac interactions. Our 2D co-culture studies revealed that CPVT hiPSC-CMs and SNs exhibit abnormal calcium signalling, increased cAMP levels, heightened excitability, and enhanced norepinephrine release from CPVT hiPSC-SNs, leading to disrupted electrophysiological activity. Using 3D microtissues, we further contextualized neuronal-cardiac interactions in a physiologically relevant setting, demonstrating that CPVT SNs exacerbate arrhythmogenic phenotypes in healthy CMs. Single-cell RNA sequencing identified critical transcriptomic changes, suggesting novel therapeutic targets for regulating neuronal-cardiac signalling. Advancements in 3D extracellular matrix design enhanced tissue integration and functionality. These findings provide new insights into the pathophysiology of CPVT and highlight the potential of targeting sympathetic excitability as a therapeutic strategy to personalized treatments.

Funding Source: British Heart Foundation SP/F/22/150027.

W1236

CELL INTRINSIC AND EXTRINSIC OF ASTROCYTES LIPID METABOLISM IN SPINAL MUSCULAR ATROPHY

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Spinal muscular atrophy(SMA) is an autosomal recessive neuromuscular disorder resulting from reduced Survival Motor Neuron(SMN) protein levels due to SMN1 mutations. SMA patients are categorized into types I-IV, who experience extensive spinal motoneuron (MNs) degeneration and death within weeks or years of birth. Current FDA-approved therapies via restoring SMN levels have limited effectiveness, suggesting an incomplete understanding of disease mechanisms. Recent evidence indicates that SMA could be systemic and developmental defects rather than postnatal spinal MN malfunction, with extrinsic influences from glial supportive cells that may contribute to disease onset and severity. Astrocytes are key bioenergetic cells in the central nervous system(CNS) responsible for synthesizing and transporting lipid metabolites to fuel and detoxify neurons during development and homeostasis. Although astrocyte abnormalities are reported in SMA, the etiology and actions of astrocytes in SMA remain controversial, with undefined molecular mechanisms. Here, we have successfully established disease modeling by generating patient-specific neuromuscular organoids from induced pluripotent stem cells(iPSCs) derived from both healthy and different types of SMA patients, along with isogenic control, which are further benchmarked with human fetal datasets via Single-cell RNA sequencing analysis. Building on this, we found that SMA astrocytes exhibited varying degrees of developmental defects, which are highly associated with lipid metabolism deficiency, positively correlating with SMA severity. Furthermore, we identified reduced SREBF2 in SMA astrocytes., a key regulator for lipid metabolism responsible for cholesterol and fatty acids synthesis, which contribute to abnormal astrocyte formation and functional impairment, negatively impacting MN development and homeostasis, leading to increased disease susceptibility and reduced resilience. Restoring SMN cannot rescue deficient lipid pathways mediated by SREBF2 in both SMA astrocytes and SMA mouse models. Our study revealed an SMN-independent disease mechanism and further research is warranted to explore whether restoring astrocytic SREBP2 could enhance therapeutic efficacy when combined with current treatment for SMA patients.

W1238

CHARACTERISATION OF IPSC-DERIVED MACROPHAGES SUBTYPES IN AGE-RELATED MACULAR DEGENERATION

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Age-related macular degeneration (AMD) is a complex disease and the leading cause of blindness in the western world. During macular degeneration, retinal pigment epithelium (RPE) cells are lost due to stress and aging, resulting in damage to the retinal and in central vision loss. The immune system plays an important role in the development of AMD. Damage to the RPE triggers an

immune response and chronic inflammation. Investigating the factors that lead to this local inflammation and immune response in AMD will contribute to a new understanding of AMD pathology, providing an opportunity to develop new treatments that could avoid vision loss in patients. Macrophages are immune cells that can promote inflammation and phagocytose foreign and unhealthy cells. Macrophages also play an important role in the aging process and the development of age-associated diseases, such as AMD. Samples from post-mortem tissue from AMD patients show an accumulation of macrophages around macular RPE, highlighting the importance of these cells in the development of AMD. In order to study macrophages characteristics, and behaviour, a stem cell-based AMD model system was developed. iPSC were generated from AMD, age-matched and young control patient samples, and iPSC were differentiated into macrophage subtypes (M0), and further differentiated into “proinflammatory” (M1) and anti-inflammatory (M2) subtypes. Immune-related gene and protein expression was examined by Q-PCR and Flow analysis, the secretome was assessed using cytokine arrays and functionality was tested by phagocytosis of bioparticles. Our data suggests that macrophage characteristics are altered in healthy ageing and disease. In the future, these cells will be used to study the activation of macrophages by inflammatory cytokines realised by RPE in vitro. This would help provide further understanding of the crosstalk between RPE and the immune system during the development of AMD.

Funding Source: The Uren Foundation.

W1240

CHARACTERIZATION OF EXTRACELLULAR VESICLES (EVS) DERIVED FROM UMBILICAL CORD MESENCHYMAL STROMAL CELLS (UC-MSCS) LOADED WITH MIR-365A-5P ON CD4+ MEMORY T CELLS

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Autoimmune and inflammatory diseases exhibit an imbalance between pro-inflammatory and regulatory CD4+ T cells, making immune response regulation a persistent challenge. Mesenchymal Stromal Cells (MSCs) are a promising therapeutic option due to their immunosuppressive capacity, mainly attributed to their secretion of extracellular vesicles (EVs). However, clinical outcomes are divergent, leading to proposals for enhancing their therapeutic potential by reprogramming MSCs towards glycolysis. Additionally, studies suggest that miRNAs within EVs may influence immunomodulation. Our studies with glycolysis-reprogrammed UC-MSC-derived EVs (EVs-glyco) demonstrated enhanced immunosuppressive activity on memory CD4+ T cells compared to non-reprogrammed EVs (EVs) and a high expression of miR-365a-5p. Therefore, we characterized EVs internalization through C. elegans miR-39 expression and evaluated the effect of miR-365a-5p on memory CD4+ T cells. EVs and EVs-glyco were isolated by ultracentrifugation. They were subsequently loaded with miR-39 via electroporation (EVs-miR-39) and characterized by nanoparticle tracking analysis and FACS. The internalization of miR-39 in EVs and treated memory CD4+ T cells was analyzed by qPCR. To assess the effect of miR-365a-5p, EVs were loaded with this miRNA, and its levels were detected by qPCR. Memory CD4+ T

cells were treated with EVs, EVs-glyco, and EVs-miR-365a-5p, followed by FACS analysis to determine Th1, Th17, and Treg phenotypes. The characterization of EVs-miR39 showed no significant differences in size or surface markers CD9/CD63/CD81 when compared to non-electroporated EVs. qPCR confirmed miR-39 presence in EVs-miR-39 and its internalization into memory CD4+ T cells. Analyses showed elevated levels of miR-365a-5p in EVs-miR-365a-5p compared to non-electroporated EVs. In memory CD4+ T cells treated with EVs-miR-365a-5p, a similar trend of immunosuppression was observed as that seen with EVs-glyco on Th1, Th17, and Treg subpopulations. It is suggested that the electroporation process does not alter the essential characteristics of EVs, and effective transfer of miR-39 as well as miR-365a-5p was demonstrated. This opens possibilities for further investigation into the role of miR-365a-5p in the immunosuppressive function of EVs-UC-MSC.

Funding Source: ANID - Project Advanced Technology - IDEA grant N°TA24I10054.

W1242

COILED-COIL DOMAIN-CONTAINING PROTEIN141 IS ESSENTIAL FOR CARDIOMYOCYTE DEVELOPMENT AND FUNCTION

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Heart diseases are the leading cause of death worldwide. These diseases overwhelm the healthcare systems and exert an enormous economic burden on society. The development of more effective therapeutic strategies for managing and treating heart diseases is militated by knowledge gaps in our understanding of the development and function of the heart. Currently, the broad view of the development and function of the heart is known. However, the nitty-gritty of the processes involved is not known in sufficient detail. For instance, many genes are abundantly expressed in cardiomyocytes, the parenchymal cells of the heart, but their function in cardiomyocytes is largely obscure. Notably, we have found that coiled-coil domain-containing protein141 (CCDC141) is abundantly expressed in cardiomyocytes of vertebrates from the heart development period up to adulthood. To study the function of CCDC141 in cardiomyocyte differentiation, we generated a CCDC141 knockout human embryonic stem cell line (CCDC141-KO hESC) using the clustered regularly interspaced short palindromic repeats (CRISPR) associated protein 9 (CRISPR-Cas9). We further differentiated the CCDC141-KO hESC into cardiomyocytes to examine the effects of loss of CCDC141 in cardiomyocyte development and function. Our results showed that the loss of CCDC141 did not affect the commitment of hESC to cardiomyocyte lineage during in vitro differentiation. The transcriptomic analysis of CCDC141-KO hESC showed altered myofibril and extracellular matrix. Additionally, the loss of CCDC141 function induced the downregulation of genes associated with oxidative phosphorylation with normal mitochondrial abundance. The molecular underpinning of the role of CCDC141 in cardiomyocyte biology is ongoing.

W1244

COMPREHENSIVE CHARACTERIZATION OF MESENCHYMAL STROMAL CELLS UNVEILS FEATURES PREDICTIVE OF IMMUNE MODULATION CAPACITY

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Mesenchymal stromal cells (MSCs) offer enormous therapeutic potential for immune-mediated disorders through their profound immunosuppressive effects. However, their potency varies, which may be associated with specific phenotypic and molecular features. This study characterizes resting and IFNg primed clinical-grade MSCs from adipose (ADSC) and decidua (DSC) and integrates the predictive phenotypic, genetic, and epigenetic markers linked to better potency. The immunosuppressive potential of MSCs was evaluated by co-culturing them with PBMCs (n=4) at various concentrations. Key metrics included activation marker expression, proliferation indices, and Tregs analyzed by FACS, alongside cytokine levels measured by Luminex. These data were integrated using PCA and PLSDA analysis, with the area under the curve (AUC) of PC1 and LV1 calculated across MSC concentrations to represent PBMC functional activity and hence, of the MSC immunosuppressive potential. The phenotypic and molecular features of MSCs predictive for AUC were determined using integrative analysis of morphology markers (n=84), cytokines (n=46), gene expression markers (n=25), miRNA markers (n=800) and surfaceome (n=361). Scores were determined by statistical analysis via mixOmics R package on experimental set (n= 6: 3 ADSCs and 3 DSCs). Our results showed lower AUC for ADSCs compared to DSCs and for primed versus resting MSCs, indicating greater potency of ADSCs and primed MSCs. We identified five highly correlated scores distinguishing between ADSC and DSC; these included the morphology score composed of cytoplasmic eccentricity and zernike_8_0 and 1_1and nuclear zernike_5_5 and 4_2; surface markers scores - EphA2, CD274, CD49c, MSC and CD99; RNA score- IFIT1, CD83, PDL1and VCAM1; miRNA score-miR99b.5p, miR23b.3p, let7i.5p, miR148a.3p and miR29c.3p; cytokines score- GROb, PDGF-AB/BB, PDGF-AA, EGF and IP-1. RNA and cytokines scores were validated with high accuracy in the validation set. We additionally determined scores distinguishing between high and low performing ADSCs in the experimental set, which are being tested in the validation set (n=32). Through an extensive characterization of clinical grade products, we identified scores that can guide selection and manufacturing of MSC products with enhanced immune suppressive potency.

Funding Source: Qatar National Research Fund (QNRF).

W1246

CONTINUAL COLLECTION OF CONDITIONED MEDIUM FROM QUIESCENT ADIPOSE TISSUE-DERIVED STROMAL CELLS EMBEDDED IN GELS TO SUPPLY MATERIALS TO ENHANCE WOUND HEALING IN DIABETIC MICE

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Mesenchymal stem cell (MSC)-based cell therapies for diabetic ulcers have attracted much attention, but have not become widespread. One of the reasons for this is that chronic inflammation in diabetes causes premature senescence of MSC, which significantly impairs their proliferative and differentiation potential as well as their immunomodulatory functions. We have previously reported that quiescence of adipose tissue-derived stromal cells (ADSC), one type of

MSC that has been utilized to develop MSC-based cell therapies, induced by culturing them in three-dimensional gels with the stiffness of adipose tissue, eliminates premature senescence of ADSC caused by high-glucose treatment. We have also reported that transplanting quiescent ADSC in gels promotes wound healing in diabetic mice. In this study, we investigated the effect administering conditioned medium derived from quiescent ADSC (Q-CM) on diabetic ulcers and attempted to construct a highly efficient method to produce Q-CM for future clinical application. To this end, Q-CM was collected every 3 days from the same quiescent ADSC in gels and used to culture cells seeded on plastic culture plates or intradermally injected into streptozotocin-induced diabetic mice (STZ mice) after removal of skin on their back. Compared to regular culture medium, fibroblast migration and tube formation by vascular endothelial cells were enhanced, when Q-CM was administered. A cohort of Q-CM exhibited a similar level of restoration of proliferation and tumor necrosis factor-alpha/interferon-gamma-stimulated indolamine 2,3-deoxygenase secretion by aged ADSC. Q-CM also accelerated wound healing in STZ mice. These results suggest that it is possible to continually collect CM from quiescent ADSC with maintained therapeutic effects on diabetic wound healing.

W1248

CORTICAL ORGANOIDS FROM INDUCED PLURIPOTENT STEM CELLS EXHIBIT SPIKING AND FIELD POTENTIAL ACTIVITY REFLECTIVE OF SLEEP-LIKE STATES AND PHARMACOLOGICAL MODULATION

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Cortical organoids derived from human induced pluripotent stem cells (iPSCs) provide a powerful model to study neurodevelopment and network activity. While spiking activity in organoids is well-documented, field potential activity, potentially representing synchronized oscillations akin to in vivo sleep-like states, remains less understood. Pharmacological interventions targeting neural states further expand these models' potential for studying drug effects on neural circuits. We generated human cortical organoids from iPSCs and assessed their electrophysiological activity using the Biocam Duplex multielectrode array (MEA) platform. We analyzed both spiking and field potential activity to investigate network synchronization. To modulate neural activity, 4-aminopyridine (4AP) was applied to induce small depolarizations, followed by midazolam and xylazine, drugs associated with sleep modulation. Electrophysiological data were analyzed for network-level changes in response to these compounds. Spiking activity emerged by day 120, indicating early network formation and robust field potential activity was observed, demonstrating synchronized oscillatory patterns associated with neural maturation. Following 4AP-induced depolarization, administration of midazolam and xylazine led to significant, expected changes in field potential frequency and amplitude, consistent with their known pharmacodynamic effects. These findings suggest that cortical organoids can recapitulate key neural oscillations, including those linked to sleep-like states. Our study shows that cortical organoids develop spiking and field potential activity, paralleling in vivo network synchronization. The ability to pharmacologically modulate these neural states further supports their physiological relevance. These findings underscore the potential of cortical organoids for investigating neural development, sleep-associated processes, and drug effects on synchronized neural networks.

W1250

CRAFTING MESENCHYMAL STEM CELL-DERIVED EXTRACELLULAR VESICLES TO CATALYZE THERAPEUTIC BREAKTHROUGHS FOR LIVER DISEASES

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Liver fibrosis (LF) results from the excessive accumulation of fibrous connective tissue due to chronic liver inflammation. As LF advances, it can lead to deathly conditions such as cirrhosis and hepatocellular carcinoma (HCC). Currently, no FDA-approved drugs specifically target LF, leaving liver transplantation as the only option in advanced cases—a procedure fraught with limited donor availability. This highlights the critical need for innovative therapies to prevent LF from advancing to cirrhosis or HCC. Extracellular vesicles (EVs), particularly those originating from mesenchymal stem cells (MSC-EVs), hold significant potential as tools for regenerative medicine. In our previous research, we developed a novel MSC-EV system designed to target non-phagocytic cells, specifically hepatic stellate cells (HSCs)—the primary drivers of LF. This work, published in Nature Nanotechnology, demonstrated the creation of an albumin sponge on the EV surface, which enhanced MSC-EVs' targeting capabilities toward HSCs and hepatocytes. This breakthrough opened new avenues for refining EV systems to improve their targeting efficiency and therapeutic applicability. Building on this foundation, our current study aims to enrich EVs with antifibrotic cargo while optimizing the albumin sponge layer. To achieve this, we employ a Design of Experiments approach, allowing simultaneous evaluation of multiple optimizable factors at varying levels. These factors include hypoxic conditions with different oxygen levels, albumin types and concentrations, and incubation durations. This model enables the identification of optimal culturing parameters to produce EVs with a refined albumin layer, confirmed by measuring albumin content and evaluating in vivo cellular uptake in liver subpopulations. These EVs also exhibit potent anti-inflammatory effects, demonstrated by their impact on LPS-treated macrophages, and antifibrotic properties through the suppression of HSC activation in vitro. Future efforts will focus on validating these EVs' therapeutic activities in vivo and unraveling their mechanisms of action. This will involve analyzing their transcriptomic and proteomic signatures, leveraging bioinformatics tools, and investigating intracellular trafficking to identify potential molecular targets.

W1252

CRISPR-CAS9 KNOCKOUT SCREENING UNVEILS ESSENTIAL GENES IN HUMAN TROPHOBLAST DIFFERENTIATION

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The recent derivation of human trophoblast stem cells (hTSCs) offers a scalable in vitro model system for studying human placental development. However, the molecular mechanisms underlying hTSC differentiation into extravillous trophoblasts (EVT) remain unclear. In this study,

we initially induced hTSC differentiation into EVT in vitro and collected RNA samples at day 0, 2, 4, and 6 for high-throughput sequencing to identify key genes involved in the differentiation process. We then employed a genome-wide CRISPR-Cas9 knockout screening to systematically narrow down the critical genes in hTSCs, selecting the top five percent for further functional validation. Currently, we are performing lineage-directed EVT differentiation on both wild-type and several gene knockout hTSCs . Overall, our research will contribute a valuable resource for understanding the molecular regulation of human placental development and associated diseases.

W1254

DAMAGE TO BONE MARROW STROMA INDUCED BY GAMMA-RADIATION OF A RECIPIENT IS A PREREQUISITE FOR ENGRAFTMENT OF DONOR'S MSCS INJECTED INTRAVENOUSLY

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Transplantability of allogeneic mesenchymal stem cells (MSCs) as a stable engraftment and participation in physiological renewal of bone marrow (BM) stroma remains an open question. Successful MSCs transplantation could be beneficial to patients with leukemia, in which BM stroma is modified by leukemic cells, supports their survival and provide them drug resistance. Replacement of BM stroma with healthy one from a donor could also be a saving opportunity for those with genetic disorders of connective tissues such as osteogenesis imperfecta. We hypothesized that a necessary condition for successful MSC transplantation is the prior significant damage to the recipient's BM stroma. To test this, BM derived from male B10 mice was injected intravenously into syngeneic female mice irradiated with 6.5 Gy and 13 Gy, and unirradiated individuals. We measured donor chimerism 30 days after irradiation in recipients' bones by RQ-PCR and in BM CFU-Fs by ddPCR. The ratio of PCR signals from single-copy Y chromosome-linked Prssly gene and autosomal Gapdh gene was used to determine donor chimerism. None of the non-irradiated recipients had donor cells in the subpopulation under study. In the group of recipients irradiated at a dose of 6.5 Gy, donor cells were detected at 0.16% in BM of only one animal. Increasing the radiation dose resulted in a significant increase in donor chimerism among BM stromal cells: it was 11% and 14% in two survived animals of this group. Donor chimerism in the bones of unirradiated recipients was not observed in any of the 15 samples. It was detected in only 2/18 bones of recipients irradiated with 6.5 Gy (0.8% and 1%). The median donor chimerism in bones of recipients irradiated with 13 Gy was 15%. We conclude that the condition for successful engraftment of donor stromal progenitor cells including MSCs is prior damage to the recipient's BM stroma.

Funding Source: This study is supported by Russian Science Foundation (project No 22-25-00459).

W1256

DECIPHERING THE ROLE OF TUBULIN ISOFORMS IN NEURODEGENERATION AND AGEING

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Microtubules are polarized tubular structures composed principally of two proteins, the α-tubulin and the β-tubulin. Several isoforms of α and β-tubulins exist, and their expression could vary according to the cell type and the developmental stage. In this project, we study the function of tubulin isoforms such as tubulin α4A in neurons. TUBA4A cannot be tyrosinated, which was shown in-vitro to confer a higher stability to the microtubules. We hypothesize that TUBA4A could be important for neuronal development and maintenance. During ageing and neurodegenerative diseases, TUBA4A integration within the lattice might be increased to repair and maintain the stability of the neurite microtubules. In this project, we are using enhanced pluripotent stem cells (EPSC) and induced neural stem cells (iNSCs) derived neurons to examine the role of tubulin isoforms in neurogenesis, ageing, and the motor neuron disease amyotrophic lateral sclerosis (ALS). With single-molecule super-resolution microscopy, we will study the expression and integration of specific isoforms within microtubules and examine ALS pathologies in neurites and microtubule integrity in ageing. Through next-generation stem cell and organoid models, we aim to decode how tubulin isoforms contribute to neural development, neurodegeneration, and disease.

W1258

DEFICIENCY OF ASTROCYTE LIPID METABOLISM CONTRIBUTES TO SPINAL MUSCULAR ATROPHY

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Spinal muscular atrophy (SMA) is a leading inherited cause of infant and childhood mortality, caused by mutations in the gene encoding survival motor neuron(SMN) 1, resulting in reduced SMN protein levels. SMA patients experience progressive spinal motoneurons(MNs) degeneration, leading to declined motor functions and death within weeks or years of birth, depending on the severity of the disease. Despite three licensed SMA therapies representing a pioneering breakthrough, SMN protein replacement is not a cure for the disease. In addition, many patients are poor responders for the treatments, highlighting the incomplete understanding of the disease mechanisms. Recent evidence points out that SMA is a systemic and developmental disorder that beyond postnatal spinal MNs malfunction, with many extrinsic influences, such as astrocytes, contributing to MN degeneration. By single-cell analysis of spinal neuromuscular organoids (SNOs) derived from SMA patients and their isogenic control, we have identified lipid metabolism defects in SMA astrocytes during development. Notably, dysregulation of the critical sterol regulatory

element-binding protein, SREBP2, in SMA astrocytes results in deficiencies in cholesterol synthesis, potentially impeding astrocyte maturation and functionality. In addition, SMA astrocytes with cholesterol deficiency impaired neurite outgrowth and reduced resilience of MNs, leading to increased disease susceptibility and severity. Our proposed research will define a new signaling pathway adding to current SMA disease mechanisms, providing multiple avenues for improving therapeutics and care for SMA patients in the future, and may allow for tailored medicine in the future.

Funding Source: C1008-24GF(RGC).

W1260

DELINEATING THE NEUROBIOLOGICAL MECHANISMS INVOLVED IN SETBP1-HAPLOINSUFFICIENCY DISORDER USING HUMAN NEURAL ORGANOIDS AND TRANSCRIPTOMICS

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Haploinsufficiency of the SETBP1 gene causes a highly heterogeneous neurodevelopmental syndrome (SETBP1-haploinsufficiency disorder) with the main phenotypic features including moderate-to-severe speech and language impairments, and wide variability in intellectual functioning. The precise functions of SETBP1, encoding the SET-binding protein, are yet to be discovered. Therefore, the neurobiological pathways by which rare loss-of function SETBP1 variants cause a neurodevelopmental disorder remain largely unknown. By employing induced pluripotent stem cell (iPSC)-derived neural organoids and transcriptomic approaches, we aim to dissect the underlying aetiological pathways. We have generated iPSCs from three patients carrying heterozygous de novo truncating variants and sex-matched parents as controls, and SETBP1-knockout iPSCs with CRISPR/Cas9 gene-editing. These iPSC lines were differentiated into un-patterned neural organoids and their transcriptomic profiles were analysed at whole organoid and single-cell levels. Morphological examination, cell-type specific differential gene expression analysis and cell lineage tracing were performed at two selected developmental timepoints. Both patient-derived and SETBP1-knockout organoids consistently showed gross morphological differences, rosette and transcriptomic anomalies during early organoid development, suggesting aberrations in cell fate commitment during embryonic brain development. Gene ontology analyses demonstrated that dysregulated pathways were related to brain morphogenesis, cilia organisation, axon projection and synaptic function. Together, our work is, to our knowledge, the first mechanistic investigation using iPSCs and organoids derived from SETBP1-haploinsufficiency disorder patients. This work promises to offer valuable insights into the

fundamental understanding of the currently unknown neurodevelopmental roles of SETBP1 and aetiological mechanisms that go awry in SETBP1-haploinsufficiency disorder.

Funding Source: SETBP1 Society Pilot Grant Award 2019, UPenn Orphan Disease Center Million Dollar Bike Ride Grant Award 2023.

W1262

DERIVATION, CHARACTERIZATION AND CRYOPRESERVATION OF PRIMARY HUMAN FETAL ORGANOIDS FROM SECOND AND THIRD TRIMESTER AMNIOTIC FLUID

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Fetal tissue-derived organoids are a powerful tool for modeling developing organs, studying congenital diseases and advancing translational therapies. However, ethical and legislative constraints restrict fetal tissue collection for research in several countries. The amniotic fluid (AF) can be collected through routine clinical procedures, such as amniocentesis, amniodrainage and fetal surgery, performed throughout the second and third trimester of pregnancy. Through single-cell mapping and organoid culture, our team has recently demonstrated that tissue-specific epithelial stem/progenitor cells can be isolated from the AF. These AF-derived cells consistently generate primary epithelial lung, kidney and small intestinal organoids. This study outlines a streamlined approach for establishing, characterizing and cryopreserving clonal AF-derived organoids (AFOs). This includes a rapid and cost-effective immunofluorescence method for identifying AFO's tissue of origin. Through this approach, we derived over a thousand clonal AFO lines from 67 AF samples spanning 16 to 39 weeks of gestation including the isolation of AFOs from samples obtained at term via c-section, extending the window of applicability of our protocol. AFOs recapitulate the differentiation potential, transcriptomic profile, and functional features of their fetal tissue of origin. They also provide a unique opportunity to study fetal human epithelia during gestation, facilitating research on late human development, when tissue biopsies to derive organoids are hardly accessible. The AF offers a minimally-invasive and ethically accessible alternative to derive primary epithelial organoids from continuing pregnancies, with fewer regulatory constraints. Although restricted to the fetal epithelia, AFOs derived from the third trimester allowed to access a developmental window previously untapped. As a consequence, AFOs could find application to model congenital conditions and develop prenatal regenerative medicine strategies personalised for the fetus.

Funding Source: This work was supported by the Academy of Medical Science, UCL Therapeutic Acceleration Support, GOSH-CC, Kidney Research UK, CDH-UK, NIHR GOSH BRC and Rosetrees Trust. GD is supported by an EMBO Scientific Exchange Grant.

W1264

DEVELOPMENT OF A DRUG NEUROTOXICITY EVALUATION PLATFORM USING NEURAL ORGANOIDS AND MACHINE LEARNING

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The development of reliable preclinical models for assessing drug-induced toxicity is essential for improving the clinical and economic efficiency of drug development while ensuring patient safety. Human induced pluripotent stem cells (hiPSC)-derived neural organoids are considered effective resources for personalized medicine and neurotoxicity assessment due to their physiological similarity to human neural cells. In this study, we established a system that can evaluate drug toxicity in real time using patient hiPSC-derived neural organoids. To achieve this, we investigated 1) generating two cell lines capable of monitoring apoptosis in real time, 2) developing a high-efficiency system for producing hiPSC-derived neural organoids, 3) conducting neurotoxicity assessments of 12 FDA-failed drugs within a microfluidic-concave chip, and 4) incorporating AI-based machine learning algorithms to predict drug neurotoxicity using the generated database. As a result, the neurotoxicity predictions obtained from hiPSC-derived neural organoids exhibited over 80% concordance with clinical outcomes. Furthermore, neurotoxicity predictions of iPSC-derived neural organoids demonstrated over 80% similarity to clinical outcomes, proving to be a rapid, effective and cost-efficient system that can be applied to new drug development and safe patient-specific therapies.

Funding Source: This research was supported by the National Research Foundation (NRF) (No. RS-2023-00220207) and the BK21 FOUR funded by the MOE of Korea.

W1266

DEVELOPMENT OF A LIVER-ON-A-CHIP MODEL TO SIMULATE LIVER INFLAMMATORY RESPONSE

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Inflammation is a common response observed in liver diseases and if uncontrolled, can lead to irreversible damage and contribute to liver failure. In view of the limitations of current in-vitro models, organ-on-a-chip platforms, which can better mimic human biology, have recently been

employed to investigate disease mechanisms and perform high-throughput drug testing. In this project, we aimed to develop a 3D liver-on-a-chip model to investigate liver inflammation. HepG2 cells were cultured alone or together with non-parenchymal liver cells (NPCs), including liver sinusoidal endothelial cells (LSECs) and hepatic stellate cells (HSCs), in 2D and 3D for liver modelling. To simulate inflammation, the different liver cells were exposed to conditioned medium (CM) containing inflammatory cytokines (e.g., IL-6, IL-1β, TNF-α) collected from induced human monocyte cells (ThP-1 cells). After CM treatment, HepG2 cells cultured in both 2D and 3D showed decrease in cell viability and albumin levels. An increase in inflammatory markers such as C-reactive protein and reactive oxygen species levels was observed. For NPCs, LSECs showed a decrease in cell viability and expression of cell specific markers after CM treatment. Meanwhile, increased expression of fibrotic markers was seen in HSCs after CM treatment. When HepG2, LSECs and HSCs were co-cultured in 3D, better cell viability and functionality was observed when compared to 2D co-culture and monoculture. Next, a microfluidic chip was fabricated to perform the co-culture on chip which will be used for testing anti-inflammatory drugs. This liver-on-a-chip platform will serve as a valuable tool for high-throughput screening of potential therapeutics for treating liver inflammatory diseases.

Funding Source: National Key Research and Development Program of China; Research Grants Council of Hong Kong; The Charlie Lee Charitable Foundation – Regenerative Immunology Technologies for the Treatment of COVID-19 and Related Diseases– RMG01.

W1268

DEVELOPMENT OF MICROFABRICATED DEVICES FOR IN VITRO RECONSTRUCTION OF SENSORY NEURAL NETWORKS INDUCING HYPERSYNCHRONY BY STIMULATION OF SINGLE NEURONS

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While normal pain perception is essential for the survival of living organisms, chronic pain, a persistent form of pain, significantly reduces the quality of life of patients. Chronic pain is a persistent and difficult-to-treat condition. The rising treatment costs due to the growing number of patients have become a significant global concern. Pain is perceived when nociceptive stimuli trigger electrical activity in dorsal root ganglion (DRG) neurons, which transmit signals to the brain via the spinal cord. Alterations in the spinal cord's pain-processing circuitry can lead to chronic pain, such as nociplastic pain. Conventional animal model studies have limitations in investigating the complex mechanisms underlying changes in neural network function. In particular, tracking the progression of these changes over time is challenging. An experimental system capable of tracking changes in spinal cord networks in real time is essential for drug screening and the development of effective treatments tailored to the progression of chronic pain. In this study, we aimed to establish an experimental system to reconstruct the DRG-spinal cord network in vitro. Using the proposed co-culture method, we attempted functional changes in the spinal cord network, where changes in

the input frequency from DRG neurons increased the synchronous activity of the spinal neurons. To establish the experimental methodology, microdevices were fabricated for co-culturing rat-derived DRG and spinal cord neurons on an electrode array. Electrical activity was induced by optogenetic stimulation of DRG neurons, and extracellular potentials of spinal cord neurons were recorded using high-density microelectrode arrays. Spinal cord neurons exhibited synchronous activity throughout the network before stimulation. During DRG neuron stimulation, the frequency of synchronous activity increased, a change that lasted for at least 20 minutes after stimulation. These results suggest that synaptic input from DRG neurons enhances the activity of spinal cord neurons. In conclusion, the sensory neural network reconstructed in this study offers a valuable platform for investigating the functional changes in the spinal cord network induced by sensory inputs.

Funding Source: This work was supported by JSPS, Grant Number 24K03248, and JST SPRING, Grant Number JPMJSP2108.

W1270

DIRECT REPROGRAMMING OF URINE CELLS INTO CRANIAL NEURAL CREST-LIKE CELLS

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The inaccessibility of the human fetus has led to the development of in vitro methods for generating human embryonic neural crest cells (NCCs), providing valuable insights into their cellular and molecular characteristics. This approach enables the modeling of neural crest defects for mechanistic studies and identification of therapeutic targets. While previous efforts have involved invasive procedures to directly reprogram human blood cells and fibroblasts into NCCs, this study explores a non-invasive approach using urine cells for reprogramming into NC-like cells by lentiviral-mediated overexpression of two different polycistronic cassettes, BRN2-KLF4-SOX2-ZIC3 (BKS2Z) and BRN2-KLF4-SOX17-ZIC3 (BKS17Z). The results show that BKS17Z is more effective than BKS2Z in generating NC-like cells from urine cells. Moreover, the addition of the small molecule DAPT enhances the reprogramming efficiency of BKS17Z compared to direct differentiation of induced pluripotent stem cells into NC-like cells. The reprogrammed NC-like cells exhibit expression of cranial neural crest markers, migratory capacity and the ability to differentiate into neural crest derivatives such as chondrocytes. Altogether, we have established a non-invasive strategy to reprogram urine cells into cranial NC-like cells, paving the way for modeling craniofacial disorders.

Funding Source: Research Grants Council and University Council of Hong Kong (GRF_17102420 and GRF_17114619).

W1272

DYSREGULATION OF METHIONINE METABOLISM OF HEPATOCYTES INDUCED EPITHELIAL-MESENCHYMAL TRANSITION OF HEPATOCYTES AND ABNORMAL GROWTH OF CHOLANGIOCYTES IN BILIARY ATRESIA

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Biliary atresia (BA) is the most prevalent serious neonatal biliary obstructive disorder characterized by rapidly progressive biliary and liver fibrosis. However, the patho-mechanisms underlying the disease initiation and progression of BA are not known. A heterozygous de novo G to A mutation in exon 8 of the MAT1A gene was identified in a BA patient, which generated a frameshift and a premature stop codon. The mutant MAT was prone to degradation, and the level of MAT in patient's liver was only 70% of that in normal control livers. MAT1A gene encodes Methionine adenosyltransferase enzyme 1A in hepatocytes, which catalyzes the biosynthesis of S-adenosylmethionine (SAMe) from methionine and ATP. Pluripotent stem cells (PSCs) generated from this patient's peripheral blood (BA638C) exhibited an epithelial-mesenchymal transition (EMT) during hepatocyte differentiation, accompanied by mitochondrial membrane potential disruption and elevation of oxidative stress. These effects could be reversed by supplementing SAMe and glutathione, or by MG132, which inhibited the mutant MAT degradation. When healthy control PSC-derived cholangiocyte organoids were co-cultured with BA638C PSC-derived hepatocytes, their growth was inhibited, resulting in fewer and smaller organoids. Additionally, organoids exhibited altered morphology and elevated expression of hepatic marker genes. Bulk RNA sequencing of the organoids revealed cell cycle arrest, reduced proliferation, and stress response, with transcriptomics resembling those of BA liver tissue-derived organoids. In conclusion, this study indicates that (i) the MAT1A mutation induced EMT-like behavior of hepatocytes, potentially promoting liver fibrosis in disease progression; (ii) patients' hepatocytes induced abnormal cholangiocyte development, which suggests that hepatocyte metabolic dysfunction can induce cholangiocyte/bile duct injury in the disease initiation of BA.

Funding Source: Theme-based Research Scheme (T12-712/21-R) RGC Hong Kong SAR Government, Hong Kong SAR, China.

W1274

EFFECTS OF A NATURAL COMPOUND ON CARDIOMYOCYTE DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELLS FROM A PATIENT WITH BARTH SYNDROME

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Barth syndrome (BTHS) is a rare genetic disorder caused by a mutation in the Tafazzin (TAZ) gene on the X chromosome, affecting approximately 1 in 300-400,000 people worldwide. TAZ is an acyltransferase enzyme responsible for catalyzing the remodeling of cardiolipin, a phospholipid found in the inner mitochondrial membrane. Defects in cardiolipin biosynthesis and remodeling, caused by a mutant TAZ gene lead to excessive oxidative stress and mitochondrial dysfunction. In this study, I observed the morphogenesis of induced pluripotent stem cells (iPSCs) derived from somatic cells obtained from a patient with BTHS and differentiated them into BTHS cardiomyocytes (BTHS-CMs). BTHS-CMs have a significant reduction in mitochondrial elongation and cardiac structure formation for differentiation. When BTHS-CMs were treated with Compound A(CA) for 48 hours, mitochondrial fusion was significantly increased in 30-day BTHS-CMs. Furthermore, CA led to a significant increase in the expression of autophagy markers, such as Beclin-1, ATG5, SQSTM1/P62, and LAMP1. Additionally, CA treatment significantly upregulated the expression of PGC1a, a marker of mitochondrial biogenesis, and significantly increased the expression of markers associated with mitochondrial dynamics. In addition, long-term treatment for 1 week,

myocardial-specific structure and mitochondrial morphology were restored. In conclusion, the results demonstrate that activation of mitophagy by CA can ameliorate mitochondrial dysfunction in BTHS-CMs. These results suggest that CA has a novel therapeutic potential for BTHS patients.

W1276

ELECTROPHYSIOLOGY AND MOLECULAR PHENOTYPE SCREENING PLATFORM FOR THE DEVELOPMENT OF NEW RETT SYNDROME THERAPIES

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Rett syndrome (RETT) is a rare neurodevelopmental disorder, with an estimated prevalence of 1 in 10000, mostly affecting females. RETT is linked to sporadic mutations in the neurodevelopment-relevant gene Methyl-CpG-binding protein 2 (MECP2) on the X chromosome. RETT is characterized by an early period of apparently normal development, which is then followed by a sudden loss of acquired psychomotor skills. There is currently no cure for RETT. Emerging treatment strategies aim to target MECP2 expression levels and their downstream pathways. To better understand how mutations in the MECP2 gene relate to neurodevelopmental impairment, neuronal function, and the observed range of symptoms in patients, we set out to develop an integrated multimodal phenotypic screening approach. Human induced pluripotent stem cell (hiPSC)-derived neural cultures were derived from control/MeCP2-mutant lines and assessed in 2D and 3D culture systems. Cellular phenotypes were identified using a combination of functional and molecular assays, including high-throughput high-density microelectrode array (HD-MEA) measurements and gene and protein expression analyses. Preliminary data showed that MeCP2-deficient cultures exhibited higher levels of the mature neuronal marker microtubule-associated protein 2 (Map2), as well as early hyperexcitability in comparison to its WT counterpart, which may indicate MeCP2-linked neuronal differentiation deficiencies. Network activity improvement and better long-term maintenance of the 2D cultures in the presence of rat astrocytes evidenced the significance of glial cells in the context of this neurodevelopmental disorder. Neuronal networks developed more robustly in 3D-spheroid cultures with higher expression of Map2 in WT-MeCP2 cultures and spontaneously appearing astrocytes during differentiation. Further work is aimed at genetically modulating MeCP2 expression in MeCP2-mutant cultures and to assess its effect on neural culture development and electrophysiological characteristics.

W1278

ENDOTHELIAL PRMT1 IS ESSENTIAL FOR VASCULAR REGENERATION AND CARDIAC REPAIR POST MYOCARDIAL INFARCTION

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Vascular regeneration, encompassing angiogenesis and vasculogenesis, is crucial for cardiac repair and recovery following myocardial infarction (MI). Protein arginine methyltransferase 1 (Prmt1) is a key enzyme implicated in various cellular functions, including those within the cardiovascular system. Despite its importance, the role of Prmt1 in endothelial cells (ECs), particularly in the context of vascular regeneration, remains poorly understood. This study aimed to elucidate the role of Prmt1 in ECs during vascular regeneration by employing murine MI models, in vitro 2D systems, and human-induced pluripotent stem cell (iPSC)-derived vascular organoid (VO) models. Prmt1 expression is upregulated in ECs within the ischemic zone 7 days post-MI in both human and murine cardiac tissues. EC-specific Prmt1 depletion in MI mice worsens cardiac function, increases fibrosis, impairs angiogenesis, and disrupts redox balance. Similarly, CRISPR-Cas9-mediated Prmt1 knockdown in human iPSCs reduces sprouting, EC markers, and increases mural cell markers in VOs. Pharmacological inhibition of Prmt1 with Furamidine decreases tube formation, proliferation, and sprouting while increasing oxidative stress in hypoxia-treated ECs. These findings underscore Prmt1's critical role in vascular regeneration and cardiac repair post-MI.

W1280

ENGINEERING HUMAN GLOMERULUS-ON-A-CHIP USING HUMAN INDUCED PLURIPOTENT STEM CELLS

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The glomerular filtration barrier (GFB), composed of podocytes, glomerular basement membrane (GBM) and fenestrated endothelial cells, is a highly specialized structure responsible for blood filtration in the kidney. Reconstructing the GFB in vitro has long been a challenge due to its intricate three-dimensional architecture and unique biochemical and mechanical properties. This study aimed to establish an efficient glomerulus-on-a-chip model for studying glomerular physiology and pathology employing human induced pluripotent stem cells (hiPSCs). To this end, we first successfully differentiated hiPSCs into podocytes and glomerular endothelial cells, the cellular components of GFB. These cells were then co-cultured on a coated porous membrane of a transwell integrated into a 3D millifluidic system designed to replicate key properties of the mechanical and biochemical microenvironment of the human glomerulus. To simulate physiological shear stress, and promote cell adhesion, alignment and maturation, controlled flow conditions were optimized by using a peristaltic pump linked to this 3D millifluidic system. RT-qPCR experiments and immunofluorescence analysis confirmed the proper formation of the GFB, assessing the expression of specific markers, including nephrin and podocin for podocytes, VE-cadherin and vWF for endothelial cells, and laminin and collagen for GBM deposition. Finally, we used this platform to model pathological conditions such as nephrotoxic injury and Alport Syndrome using patient-specific hiPSCs. Studies on filtration properties and selective permeability validated the reliability of these systems to mimic in vitro human glomerulus diseases. This glomerulus-on-a-chip represents a significant step forward in the development of human-relevant models for renal research. It provides a versatile tool for investigating disease mechanisms, enabling personalized medicine approaches, and advancing drug discovery, addressing the growing need for alternatives to animal models.

W1282

ENHANCED ALZHEIMER'S DISEASE MODELING: IPSC-DERIVED CHOLINERGIC NEURONS AND CRISPR-CAS9 APPROACH

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Alzheimer's Disease (AD) has been extensively studied, utilizing models like post-mortem brain samples, cell lines, and animal models. However, it is vital to acknowledge the limitations of these models, as they can impact research outcomes and its translational potential. In this regard, the emergence of iPSC has revolutionized AD research by generating patient-specific neurons for more specific investigations into molecular and cellular mechanisms. Differentiating iPSC into cholinergic neurons, crucial for cognition and affected in AD, remains a challenge, and further research is warranted. Given the above, the aim of this study is to optimize and direct an existing differentiation protocol towards the generation of cholinergic neurons, seeking to develop a specialized cellular model for investigating AD and contributing to a more comprehensive understanding of the initial molecular aspects that underlie this disease. To achieve this goal, our model directs the differentiation towards cholinergic neurons by adding neurotrophins BDNF, GDNF, and NT3 (10 ng/ml) during the final 15 days of differentiation. To model AD, we edited iPSC by introducing a PSEN1 mutation in control lines through CRISPR-CAS9 system. This step allowed us to generate isogenic lines harboring the PSEN1 mutation in both heterozygous and homozygous states, which not only complements our cholinergic neuron differentiation protocol but also offers an opportunity to investigate the impact of this mutation on neural development and function. To assess the effectiveness of our approach, the main analysis was performed using Fiji software and consisted of cell counting. Additionally, neurons were characterized through immunofluorescence staining for choline acetyltransferase (ChoAC) and MAP-2 (neuron marker); which showed a 78.2% efficiency in iPSC differentiation into cholinergic neurons. CRISPR-CAS9 was also performed, and clones were validated through Sanger sequencing. Based on these findings, we conclude that these improvements to our cellular model, combining advanced differentiation techniques with precise genome editing, provide a robust platform for studying the early molecular events of AD and exploring potential therapeutic strategies.

Funding Source: CAPES 88887.816560/2023-00 FAPESP 2013/08028-1 FAPESP 2022/12697-5.

W1284

ESTABLISHING A FUNCTIONAL STANDARD FOR HIPSC-DERIVED CARDIOMYOCYTE ELECTROPHYSIOLOGY USING THE MEA ASSAY

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Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC) are now established as a widely used model system in the safety pharmacology and disease modelling communities. The International Society for Stem Cell Research (ISSCR) recently released 'Standards for Human Stem Cell Use in Research', a document that outlines a set of recommendations that establish the minimum characterization and reporting criteria for working with human stem cells. The overarching goal of these criteria is to standardize practices, and in doing so, improve the quality of results. However, there are still no agreed-upon minimum acceptance criteria for the functional activity of hiPSC cardiomyocyte models. Rather, it is left to the end-user to determine whether the electrophysiological phenotype of their cell model is fit for purpose. Over the last decade, the multielectrode array (MEA) field potential assay has become a popular tool for characterizing the electrical activity of hiPSC-cardiomyocyte batches, studying cardiac disease models, screening for new therapeutics, and evaluating drug-induced cardiotoxicity. The goal of this project is to set the minimum acceptance criteria for a spontaneous beating wild type hiPSC-ventricular cardiomyocyte field potential assay for compound testing and/or disease modeling. Using our own experience with hiPSC-cardiomyocytes in our respective labs in academia and in industry, our work with international consortia developing hiPSC-cardiomyocyte assays (CiPA and JiCSA), combined with published data, we have developed a proposed standard focused on the hiPSC-cardiomyocyte spontaneous beat rate, features of the cardiac waveform (depolarization spike amplitude and field potential duration), and the synchronization of activity in the syncytia. Cell performance data from leading commercial sources of hiPSC-cardiomyocytes with respect to this proposed standard will also be presented for reference.

Funding Source: National Institutes of Health (NIH) Grant R01HD108839 (NGP) and F31HL165818 (DG). American Heart Association Grant 23PRE1021149 (DG).

W1286

ESTABLISHING RELIABLE QC MEASURES FOR IPSC-DERIVED MICROGLIA ENSURING ROBUST AND REPRODUCIBLE PHARMACOLOGY STUDIES

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Microglia, as resident immune cells of the central nervous system (CNS), play pivotal roles in maintaining neural homeostasis, synaptic pruning, and immune responses. Investigating human microglia biology is essential for understanding neurodevelopment, neuroinflammation, and neurodegenerative disorders. Induced pluripotent stem cells (iPSCs) offer a powerful platform for generating human microglia-like cells, which enable the study of drug mechanisms of action, efficacy, and potency, as well as patient-specific microglia to investigate disease molecular mechanisms. Challenges associated with iPSC models for pharmacology studies include substantial genetic variability across different iPSC lines and variability between differentiation

batches within the same iPSC line. These variations can impact microglia behavior, functional properties, and responses to stimuli. Therefore, to have good reproducibility and consistency in iPSC-microglia models, we have stablished rigorous quality control (QC) measures for a widely used iPSC microglia differentiation protocol. In our QC process, we combine qualitative assessments based on morphology, activation capacity and marker expression with quantitative analyses. For the latter we specifically evaluate cell dependency on MCSF—a critical growth factor for microglial survival— to estimate health and purity of the microglia culture and have defined an acceptance threshold. Additionally, comparing IC50 values and efficacy of CSFR inhibition using Plexicon over a large number of successive differentiations has allowed us to identify a normal range of performance. By integrating both the qualitative and quantitative approaches to qualify batches, we have established a QC framework for every microglia batch. Using this framework has led to excellent reproducibility between experiments and hence robust data in downstream pharmacology assays.

W1288

ESTABLISHMENT OF HUMAN IPSC-DERIVED CORTICAL ORGANOIDS TO MODEL H3.3 G34R PEDIATRIC HIGH-GRADE GLIOMA

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Pediatric high-grade glioma (pHGG) are among the most lethal cancers in children. Despite the explosion in our understanding of the etiology of these diseases, median survival remains only 12-15 months. Unlike adult glioma, mutations in histone H3.3 including G34R and K27M have been found to occur frequently, observed in around 50% of patients. Due to this prevalence in pHGG, modeling these mutations in vitro and in vivo is important to understand tumor growth as well as treatment efficacy. The complexity of the human brain poses challenges in developing models and studying brain disorders and cancer. The establishment of human induced pluripotent stem cell (hiPSC)-derived in vitro models represents a unique opportunity to investigate these diseases under tightly controlled and personalized experimental setups. Here we have employed hiPSC-derived three-dimensional cortical organoids to model specific brain tumor subtypes by inducing the expression of the corresponding tumor driver genes. hiPSC-cortical organoids were cultured and electroporated, with a panel of DNA plasmids modeling an array of gain of function and loss of function mutations to model adult and pediatric brain tumors. Utilizing a piggyBac (pb) plasmid expressing H3.3 G34R, we were able to successfully generate transgenic cells in the hiPSC-cortical organoids. Successful transduction of organoids was confirmed via fluorescent imaging. Notably, we often observed focal hyperplasia of tranduced cells, which could overgrow the rest of the organoid. After generating the cortical organoids expressing the H3.3 G34R pb plasmids we sought to perform multiple treatment paradigms such as radiation therapy (RT), a treatment arm of the current standard of care therapy, and ADI-PEG20, an arginine deaminase. From preliminary data, we hypothesize that combining RT and ADI-PEG20 will inhibit tumoral cell growth in culture. Through this project, we have established an in vitro human organoid-based cancer modelling system for human brain tumors. Given the relative lack of patient-derived G34R mutant human tumoroids and cell lines, this approach can provide a valuable, renewable model system for high-

throughput treatment discovery and validation.

Funding Source: CIRM EDUC4-12751.

W1290

ESTRADIOL IN PROMOTING THE REPOPULATION OF TRANSPLANTED HEPATOCYTES

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Hepatocyte transplantation (HTx) holds promise as a therapeutic approach for liver diseases, yet its clinical application has been hindered by the absence of effective conditioning regimens and approved drugs to enhance hepatocyte engraftment and repopulation. In this study, we identified estradiol as the key factor underlying the sex-dependent differences in hepatocyte repopulation observed in Fah-deficient mice. Administration of estradiol post-HTx significantly enhanced the repopulation efficiency of transplanted hepatocytes in both male and female recipients. Mechanistically, estradiol exerts its effects through estrogen receptor alpha (ESR1), which modulates the sterol regulatory element-binding protein 2 (SREBP2)-mediated cholesterol biosynthesis pathway, thereby promoting the proliferation of transplanted hepatocytes. Additionally, we comprehensively evaluated the safety profile of estradiol in the context of HTx, demonstrating that a one-month estradiol treatment had no adverse effects on the liver or estrogen-responsive tissues, including the uterus and mammary glands, in normal mice. These findings establish estradiol as a clinically feasible and safe pharmacological strategy to enhance hepatocyte repopulation, providing a significant step forward in advancing HTx towards clinical implementation.

W1292

EVS FROM GLYCOLYTIC MSCS: A PROMISING THERAPY FOR OSTEOARTHRITIS

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Osteoarthritis (OA) is a joint degenerative disease in which there is cartilage loss and chronic synovial inflammation. Currently, available treatments are solely palliative, offering no regenerative or curative options. Mesenchymal stem/stromal cells (MSCs) and their extracellular vesicles (EVs) have risen as an attractive candidate of choice; however, clinical trials have not reflected the promising pre-clinical data. Therefore, there is a need to improve the therapeutic properties of

MSCs. We have shown that by inducing a glycolytic metabolism in MSCs (MSCglyco) we enchance teir regenerative and anti-inflammatory properties, which also release EVs that reflect these properties, thus making them very appealing for developing new therapies for OA. MSC were treated with oligomycin to induce a glycolytic metabolism. EVs were isolated through ultracentrifugation, quantified by nanoparticle tracking analysis (NTA) and characterized through flow cytometry, western blot, and transmission electron microscopy (TEM). EVs from MSCglyco were added to the in vitro culture media of either chondrocytes or synovial macrophages, both from OA patients. After 24 hours we evaluated EV internalization and OA-associated markers in both target cells. MSCglyco derived EV-treated synovial macrophages phenotypes were further analyzed with single cell RNA sequencing. Finally, we injected EVs from MSCglyco into a collagenase-induced osteoarthritis (CIOA) murine model to assess their regenerative and anti-apoptotic effects in vivo. EVs from MSCglyco showed no significant differences in identity markers compared to those from naive MSC and were internalized by both target cells. Indeed, EVs from MSCglyco promoted a recovery of the healthy chondrocyte phenotype, while also decreasing inflammatory markers in synovial macrophages. Moreover, single cell data revealed an increase in pro-regenerative macrophage populations upon treatment with EVs from MSCglyco. Indeed, our in vivo data reveals cartilage regeneration and a decrease in bone mineralization and chondrocyte apoptosis upon intraarticular injection of MSCglyco derived EVs. Our results show significant evidence of EVs from MSCglyco acting as a multifunctional treatment for OA, targeting both main symptoms, cartilage loss and synovial inflammation.

Funding Source: Research supported by ANID-Chile through Beca Doctorado Nacional folio 21220015; FONDECYT Regular Nº1211353; FONDECYT Iniciación Nº11220549; FONDEF-ID: 21I10194; IMPACT-FB 210024 and TA24I10054.

W1294

EXPLORING AN ANTIRETROVIRAL DRUG DELIVERY RESEARCH TOOL USING HIV-INFECTED BLOOD BRAIN BARRIER COMPONENTS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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The central nervous system (CNS) is one of the primary anatomic reservoirs for HIV latency, where virus can escape from antiretroviral therapy (ART) due to the presence of a blood-brain barrier (BBB), which restricts ART drugs from crossing the barrier and reaching the brain. Residual viral presence in the CNS can cause chronic inflammation and results in HIV-associated neurocognitive disorder (HAND). Obtaining functional human brain vasculature and tissue from healthy donors is nearly impossible, and post-mortem tissue from HIV patients poses a high infectious risk. Humanized animal models engrafted with human cells struggle to fully replicate human brain counterparts. For these reasons, precise recapitulation of the human BBB in vitro is critical to accelerating new ART drug discovery pipelines and treating the CNS-HIV infection. We acknowledge the urgent need for accurate human BBB models based on tissue-relevant, highly-enriched, brain microvascular endothelial cells (BMECs), pericytes, astrocytes, and microglia of consistent quality in order to dissect the mechanisms of cellular reservoirs of HIV in the CNS and to build high-throughput screening (HTS) platforms. To address these challenges, we differentiated

hiPSC to generate highly enriched human BBB components including brain microvascular endothelial cells (BMEC) and pericytes, astrocytes and microglia, respectively. For HIV infection, we engineered GFP-pseudo HIV-1 packaged with a lentiviral GFP reporter and packaging plasmids. In the infectivity test, no significant morphological changes observed. Each BBB components showed high susceptibility to pseudoHIV; BMEC, pericyes, and astrocytes. However, microglia and glutamatergic neurons showed variable and low infectivity. Next, we tested Dolutegravir (DTG), a commercially available antiretroviral therapy (ART) drug, on HIV-infected cells. DTG was highly effective, reducing the viral load by 50% in EPCs, but it was not effective on pericytes and astrocytes. In the present study, we demonstrated the scalable generation of highly enriched hiPSC-derived BBB components and their susceptibility to pseudo-HIV. With enhanced HIV infectivity in microglia, we will mimic HIV infected BBB models in 2D and 3D formats, advancing the development of a comprehensive tool for HIV drug discovery.

W1296

EXPLORING IN VITRO PHENOTYPIC VARIABILITY IN DIGEORGE SYNDROME THROUGH HIPSC MODEL

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DiGeorge syndrome (DGS) is a microdeletion syndrome defined by the deletion of multiple genes, with a highly variable phenotype that does not correlate with deletion size. We hypothesized that various symptoms across organ systems could manifest as in vitro phenotypes. Our specific aims were: 1) to generate human induced pluripotent stem cells (hiPSCs) from blood cells of DGS patients, and 2) to assess whether differences in the in vitro phenotypic profiles of diseased and control hiPSCs and differentiated cell types reflect patient alterations. We studied a family with DGS, where disease severity increased across generations, providing a unique opportunity to explore phenotypic variability. The grandfather (with facial dimorphism) and mother (with vascular ring and hypocalcemia) exhibited milder symptoms, while the progeny presented severe symptoms, including tetralogy of Fallot, pulmonary atresia, and atrial septal defect, despite an identical deletion in all cases. We successfully reprogrammed peripheral blood cells from all family members: DGS patients and healthy controls (grandmother, father). hiPSC clones were characterized for pluripotency, spontaneous differentiation capacity, and genetic background. We investigated cardiovascular aspects of DGS using this hiPSC-based model. Functionally active cardiomyocytes (CMs) were generated from all cell lines, showing no differences in cardiomyocyte marker expression. However, morphological differences in hiPSC-CM clusters were noted in the mother and child compared to controls and the grandfather. Notably, Connexin43 (GJA1) expression was significantly lower in the child's CMs, suggesting that gap junction protein expression may contribute to cardiovascular symptoms. To model vascular symptoms, endothelial cells (ECs) were differentiated from iPSCs. The ECs from symptomatic patients (mother and child) exhibited significant morphological differences from other family members. Bulk RNA sequencing confirmed these cellular differences and revealed affected pathways; therefore, our results can help to explain the development of cardiovascular disorders and symptom exacerbation in DGS.

Funding Source: Hungarian Higher Education Institutional Excellence Programme and NKFIH Fund (RRF-2.3.1-21-2022-00003, TKP2021-EGA-23 and K128444), Intramural Research Program of the NIH, NIDCR, USA, NIH ZIA DE000748, and ZIC DC000086.

W1298

EXPLORING THE HEART-KIDNEY CROSSTALK IN VITRO: THE IMPACT OF KIDNEY DAMAGE ON CARDIAC FUNCTION USING ORGANOIDS

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The crosstalk between heart and kidney is crucial for maintaining organ homeostasis. Both organs have vital functions in the human body and reciprocally influence each other's behavior: acute or chronic damage in one organ often leads to dysfunction of the other. A dual-organ on-chip model to study the cardiorenal axis was previously described, but analysis of their reciprocal interactions in vitro has not been carried out. We combined human induced pluripotent stem cell-derived kidney and cardiac organoids (kOs and cMTs, respectively) in a co-culture setup. The kO and cMT models were first examined separately for proper structure formation and maturation. kOs were subjected to damage by nephrotoxic compounds for 72 hours which affected glomerular and tubular structures. Injury in kOs was evaluated as viability, functionality and gene expression. Within 24 hours, kidney damage was morphologically with changes of nephron structures. Damaged kOs were then co-cultured with cMTs for another 72 hours. This was indirectly detrimental to cMTs, evidenced by decreased viability and altered contraction. Our work suggests that kO and cMT co-culture could capture dual-organ crosstalk. It also highlights the importance of multi-organ systems in disease modeling by providing (patho)physiologically relevant representation of organ interactions. By analyzing the interaction between heart and kidney in this human system, our work advances organoid-based disease modeling and offers new insights into the mechanisms underlying dialogue in organ dysfunction that affect both organs.

Funding Source: This work is supported by the Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW, supported by the Novo Nordisk Foundation grant [NNF21CC0073729]).

W1300

EXPLORING THE MOLECULAR AND FUNCTIONAL EFFECTS OF STIMULATION ON PSC-DERIVED SPINAL INTERNEURONS

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Spinal interneurons (SpINs) are being increasingly recognized for their neuroplastic potential after spinal cord injury (SCI). Transplantation of SpIN progenitors after SCI has demonstrated their ability to spontaneously survive and integrate with injured networks, contributing to improved functional outcome. The extent of this connectivity, however, can be variable, and there is a need for strategies capable of enhancing connectivity. Building off principles of “Hebbian” plasticity, we hypothesize that neural stimulation can be used to drive synaptic integration and connectivity. Here we used an optogenetic human induced pluripotent stem cell (hiPSC) line to differentiate into SpINs and measured the effects of light and electrical stimulation on neurotrophin production, neurite outgrowth and synaptic connectivity. Over a four week period, blue light stimulation (2ms pulses, 20Hz, every 4 days) resulted in an increase in brain-derived neurotrophin production as well as neurite outgrowth, compared to unstimulated and wildtype controls. Optogenetic stimulation of SpINs also resulted in increased overall neural activity and network synchronicity, as measured by multielectrode arrays (MEA). Electrical stimulation (500mV, 5 times, every 180s) over a four-week period revealed enhanced neuronal activity when compared to unstimulated controls (700% increase in activity in stimulated versus unstimulated 240% increase), and network activity increased by 1400% in stimulated compared to unstimulated controls. Patch clamp experiments revealed that light stimulation not only depolarized and evoked firing potentials in these SpINs, but also accelerated the emergence of rebound bursting and network activity when compared to unstimulated SpINs. Future work will focus on unbiasedly characterizing the molecular changes that result from neural stimulation using single cell RNA sequencing and functional in vitro and in vivo assays.

Funding Source: DISC2-14180.

W1302

EXPOSURE TO 2,3,7,8-TETRACHLOROBENZO-P-DIOXIN ALTERED THE DNA METHYLATION PATTERNS OF GERMLINE DEVELOPMENTAL GENES IN HUMAN EMBRYONIC STEM CELLS

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2,3,7,8-tetrachlorobenzo-p-dioxin (TCDD) is an environmental toxin reported to be associated with many systematic diseases including cancers, dementia and reproductive defects. Previous studies revealed that TCDD exposure compromised ovarian function in adult mice. More importantly, TCDD exposure exhibited transgenerational effect on fetal sperm development in rats. Whether early TCDD exposure affected embryonic primordial germ cells (PGC) specification in humans is unclear. In this study, we hypothesized that TCDD exposure induced aberration of DNA methylation in early embryo, leading to dysregulated human PGC development. Human embryonic stem cell (hESC) is biologically equivalent to inner cell mass (ICM), the origin of human PGC. Hence, hESC line VAL3 was cultured with physiological doses of TCDD (10 and 100 pM), followed

by genome-wide DNA methylome profiling by reduced representation bisulfite sequencing. The results showed that nearly 4000 CpG sites were differentially methylated in VAL3 upon TCDD exposure. Interestingly, Gene ontology analysis of the hypermethylated genes demonstrated enrichments of biological processes including “gamete generation”, “spermatid differentiation”, “germ cell development”, “multicellular organism reproduction” and “reproductive process in a multicellular organism”. Among them, Desert Hedgehog (DHH) and Cadherin EGF LAG Seven-Pass G-Type Receptor 2 (CELSR2), were selected for further validation because they have been reported to be important for male gamete development. Gene-specific bisulfite sequencing demonstrated that the average methylation rates of DHH and CELSR2 were significantly increased in VAL3 upon TCDD exposure. The results suggested a potential mechanism that TCDD might pose on germ cell specification during early embryonic development. The established TCDD-treated hESC lines in this study provided a powerful model to validate and study the relationship between TCDD exposure and germ cell specification.

W1304

FUNCTIONAL RESCUE OF NEURONS DERIVED FROM IPSCS OF GGC REPEAT DISEASE PATIENTS USING CYTOSINE BASE EDITOR

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This study aims to establish a CRISPR/Cas9-based treatment for GGC repeat expansion diseases. Specifically, we focused on Neuronal Intranuclear Inclusion Disease (NIID) and Fragile X-associated tremor/ataxia syndrome (FXTAS). The approach involves deriving iPSCs from the blood of NIID or FXTAS patients and developing a CRISPR/Cas9 system to correct the GGC repeat expansions. The corrected iPSCs were differentiated into neurons to restore normal neuronal function, demonstrating the therapeutic potential of this system. Blood samples were collected from NIID and FXTAS patients, PBMCs were isolated, and GGC repeat expansions were confirmed via RT-PCR. iPSCs were established using Sendai virus to introduce Yamanaka factors, and pluripotency, GGC repeat expansions, and methylation were verified. A one-vector CRISPR/Cas9 system was constructed under the CAG promoter. This system included sgRNA, Cas9 (with or without cytosine base editors), and eGFP. Both dCas9 and nCas9 versions were developed, and APOBEC3A/B/H cytosine base editors were incorporated for cytosine-to-thymine conversion. The nCas9-A3A system edited cytosines within the GGC repeats, converting GGC to GGT. This process disrupted the abnormal mRNA structure and normalized cellular function. Corrected iPSCs were successfully differentiated into neurons, and phenotypic changes were assessed using disease-specific markers. This CRISPR/Cas9 system effectively targeted GGC expansions in NIID and FXTAS. The approach has potential applications in other GGC repeat disorders and broader trinucleotide repeat diseases. These include Myotonic dystrophy type 1, Spinocerebellar ataxia, Huntington’s disease, and Friedrich’s ataxia. Furthermore, the system could be applied in vivo for gene therapies in preclinical and clinical trials.

W1306

G3BP1 LOSS ENHANCES AMYLOIDOGENIC mRNA STABILITY AND DRIVES AMYLOID-BETA AGGREGATION IN IRRADIATION-INDUCED SENESCENT HUMAN ASTROCYTES

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Alzheimer's Disease (AD) stands as a prominent age-related neurodegenerative disease, and its connection to amyloid-β (Aβ) accumulation in the aging brain is well-documented. Cellular senescence, marked by irreversible cell cycle arrest, is emerging as a critical factor in AD pathogenesis. In this study, we investigate the role of irradiated primary human astrocytes and human embryonic stem cell-derived astrocytes displaying a senescent-like phenotype (SLP) in Aβ production and aggregation. Our findings reveal that SLP human astrocytes promote Aβ aggregation through enhanced expression of amyloidogenic mRNA transcripts APP and BACE1. Mechanistically, we identify G3BP1, an RNA-binding protein, as a key regulator of post-transcriptional stability for APP and BACE1 mRNAs in astrocytes. Importantly, SLP-irradiated astrocytes exhibit reduced G3BP1 expression, causing APP and BACE1 to be more stable and increasing Aβ production. These results provide insights into potential radiation-induced cognitive impairment and suggest therapeutic avenues targeting G3BP1 to mitigate cognitive decline and delay the onset of AD.

W1308

GENE REPLACEMENT USING WILD-TYPE TNNT2 RESCUES THE PHENOTYPES OF IPSC-CARDIOMYOCYTES DERIVED FROM PEDIATRIC DILATED CARDIOMYOPATHY HARBORING THE TNNT2-R151W MUTATION

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Dilated cardiomyopathy (DCM) is a common cardiomyopathy characterized by ventricular dilatation and systolic dysfunction. The TNNT2-R151W mutation is known to cause DCM in both human and mouse models, demonstrating associations with Ca2+ desensitization and reduced contractility. However, there are no reports of utilizing patient-derived induced pluripotent stem cells (iPSCs) carrying the TNNT2-R151W mutation, looking at functional improvement after gene replacement.

Here, we describe the characteristics of both TNNT2-R151W mutant iPSC-derived cardiomyocytes (R151W-iPSC-CMs) and iPSC-CMs with gene replacement for the mutation in vitro. Two human iPSC lines were generated from blood samples of pediatric DCM patients carrying the TNNT2-R151W mutation, and the TNNT2 overexpression lines derived from these R151W-iPSC lines (TNNT2-OE iPSC) were used to assess Ca2+ handling and sarcomere structures. To investigate contractile dynamics, we fabricated engineered heart tissues (EHTs) using R151W-iPSC-CMs and TNNT2-OE iPSC-CMs. Ca2+ handling analysis revealed that R151W-iPSC-CMs exhibited a significant decrease in maximum fluorescence intensity (amplitude) and a prolongation of time to maximum fluorescence intensity (time to peak), which are characteristic of failing heart tissue. Additionally, Ca2+ reuptake efficacy during relaxation, measured by Ca2+ decay tau, or the time taken for an 80% signal reduction, was also significantly prolonged in R151W-iPSC-CMs. Furthermore, R151W-iPSC-CMs displayed abnormality in sarcomere alignment. Notably, R151W-EHTs displayed diminished contractile force compared to TNNT2-OE-EHTs. These properties were ameliorated in TNNT2-OE iPSC-CMs, indicating that R151W-iPSC-CMs reflect DCM phenotypes in vitro. In conclusion, R151W-iPSC-CMs exhibited Ca2+ desensitization and abnormal sarcomere structure, and impaired contractility. These results suggest that gene therapy with normal TNNT2 may represent a promising therapeutic approach for pediatric DCM patients with TNNT2 mutations.

W1310

GENERATION OF INDUCED PLURIPOTENT STEM CELLS FOR USHER SYNDROME MODEL

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Mutations in the USH2A gene can cause Usher syndrome type 2A, characterised by congenital hearing loss and progressive vision loss due to retinitis pigmentosa. We characterised fibroblast lines derived from patient samples containing USH2A c.949C>A and c.1256 G>T mutations, and healthy controls and generated the induced pluripotent stem cell (iPSC) lines. Fibroblasts were characterised using viability assays and immunocytochemistry; and reprogrammed to pluripotency using Oct4, Sox2, Lin28, Klf4, and L-Myc with episomal vectors. On Day 25, colonies were selected for clonal expansion and USH2A mutations confirmed using Sanger sequencing. iPSC gene expression was measured using quantitative RT-PCR; protein expression was analysed using immunocytochemistry. Fibroblast lines had typical elongated morphology, and normal cells had faster growth than patient cells. All iPSC displayed typical growth characteristics and morphologies of pluripotent stem cell colonies. Pluripotency proteins (OCT4, NANOG, SOX2 and SSEA4) and genes (OCT4, NANOG, SOX2 and KLF4) were expressed similarly in all lines. Trilineage genes (PAX6, DCX, TBXT, AFP, and SOX7) had minimal expression in undifferentiated iPSC and increased expression in embryoid bodies derived from these iPSC. Development and characterisation of iPSC lines from patients with Usher syndrome represents a unique opportunity to study differences in inner ear development from those of healthy controls.

Funding Source: Australian Government Medical Research Future Fund - Stem Cell Therapies Mission Grant, Western Australian Future Health Research and Innovation Fund, Ian Potter Foundation, Perron Charitable Foundation, Channel 7 Telethon Trust.

W1312

GENERATION OF IPSC-DERIVED HUMAN VENOUS ENDOTHELIAL CELLS FOR THE MODELING OF VASCULAR MALFORMATIONS AND DRUG DISCOVERY

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Venous malformations (VMs) represent prevalent vascular anomalies typically attributed to non-inherited somatic mutations within venous endothelial cells (VECs). The lack of robust disease models for VMs impedes drug discovery. Here, we devise a robust protocol for the generation of human induced VECs (iVECs) through manipulation of cell cycle dynamics via the retinoic signaling pathway. We introduce a L914F mutation into the TIE2 gene locus of iPSCs and show that the mutated iVECs form dilated blood vessels after transplantation into mice, thereby recapitulating the phenotypic characteristics observed in VMs. Moreover, utilizing a deep neural network and a high-throughput DRUG-Seq approach, we perform drug screening and demonstrate that Bosutinib effectively rescues the disease phenotype in vitro and in vivo. In summary, by leveraging genome editing and stem cell technology, we generate VM models that enable the development of additional therapeutics.

W1314

GENERATION OF TETRAPLOID CARDIOMYOCYTES BY FUSION OF DIPLOID IPSC LINES DERIVED FROM TWO DIFFERENT INDIVIDUALS

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Human induced pluripotent stem cell (iPSC)-derived cardiomyocytes (iPS-CMs) have great promise for disease modeling, drug development, and regenerative medicine. However, the immaturity of iPS-CMs resembling the human fetal state remains a significant challenge for broader applications. We focused on the polyploidy of human mature cardiomyocytes to address this issue. While over 80% of human cardiomyocytes become tetraploid during maturation, only 10% of iPS-CMs become polyploid using conventional methods. This ploidy difference could limit the maturation of iPS-CMs. To overcome this limitation, we completely changed the differentiation strategy from the conventional scheme by first establishing tetraploid iPSCs (4N-iPSCs) and then differentiating them into cardiomyocytes to replicate the polyploidy of human mature cardiomyocytes. We successfully established 4N-iPSCs by Sendai virus-mediated fusion of diploid iPSCs and then differentiated them into cardiomyocytes using a general protocol. Almost all cardiomyocytes derived from 4N-iPSCs (4N-iPS-CMs) were tetraploid. As we expected, 4N-iPS-CMs exhibited more matured phenotypes, including post-mitotic gene expression signatures, higher mitochondrial content, higher contractile force, and faster upstroke velocity compared to the conventional iPS-CMs derived from diploid iPSCs. These experiments were initially done by fusion of the same diploid iPSC lines. However, if we could generate 4N-iPS-CMs from two different diploid iPSC lines derived from different individuals, we would be able to analyze interactions of two different genetic backgrounds within iPS-CMs. In this study, we established 4N-iPSC lines by fusion of diploid iPSCs derived from a Japanese male (WTC11) and a Caucasian female (HPS0076). We were able to differentiate these hybrid 4N-iPSCs into cardiomyocytes. These

hybrid 4N-iPS-CMs also exhibited organized sarcomere and regular beating signatures. We also conducted RNAseq analysis and found that the gene expression profile of hybrid 4N-iPS-CMs showed intermediate features between those of WTC11 iPS-CMs and HPS0076 iPS-CMs. Therefore, our strategy to generate 4N-iPSCs and 4N-iPS-CMs serves as a versatile platform to study genetic interactions between two different genetic backgrounds in cardiomyocytes.

Funding Source: JSPS, Grant-in-Aid for Scientific Research (B) (24K02028). JCRF, Bayer Scholarship for Cardiovascular Research. SENSHIN Medical Research Foundation Grant. TERUMO Life Science Foundation Research Grant.

W1316

SOLVING CHALLENGING TITINOPATHY CASES VIA MULTI-OMICS AND IPSCS

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Titinopathy, or skeletal muscle dystrophy due to autosomal recessive mutations in Titin (TTN) poses a diagnostic challenge due to significant variability in disease manifestation and incomplete knowledge of the role of different parts of the gene in normal muscle function. Many apparently healthy individuals harbor rare putative deleterious variants in TTN. This confounds interpretation of TTN variants in cardiac and neuromuscular disease patients.
In this study, we investigated six titinopathy patients from four unrelated families with diverse disease manifestation. In each case, a confirmed diagnosis was reached by co-analyzing familial DNA and proband muscle-biopsy based RNA sequencing data. In three out of four families, one of the pathogenic variants is located in metatranscript-only exons. Comparison with in-house and public muscle transcriptomics datasets brought credence to the outlying isoform usage in each family. These patients present commonality in aberrant isoform usage and shared functional changes in muscle transcriptome, highlighting the importance of metatranscript-only exons. However, the regulatory mechanisms underlying the aberrant isoform expression remain unclear. Therefore, we established patient-derived induced pluripotent stem cells (iPSCs) for these metatranscript-exon only titinopathy patients and gender-matched control donors. This provides us opportunity to examine the mechanical abnormalities in skeletal tissue and gain insights into the regulatory processes of titin isoform switching in metatranscript-exon only titinopathy patients.

W1318

HARNESSING SMSER: A HIGH-THROUGHPUT PLATFORM FOR PROGRAMMING MACROPHAGES

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Chimeric antigen receptor macrophages (CAR-M) have demonstrated efficacy in clinical HER2+ solid tumors, yet the development of macrophage-optimized CAR architectures remains

underexplored. A critical challenge lies in identifying design principles for CAR structures that maximize macrophage-specific functions such as tumor infiltration, persistence, and microenvironment remodelling. To address this, we developed a high-throughput platform to systematically evaluate synthetic macrophage-specific engulfment receptors (SMSERs) incorporating diverse domains: macrophage specific signalling peptides, hinge regions, transmembrane (TM) segments, and intracellular domains (ICDs). Using Golden Gate modular cloning, we generated three CAR libraries: single ICD library containing 491 constructs, dual ICD library containing 2,205 variants and triple ICD library containing 1,145 CARs. An in vivo screening platform was established using intra hepatically implanted HepG2 tumors to select CAR-M variants with enhanced tissue infiltration and persistence. Single-cell RNA sequencing analysis of tumor-infiltrating CAR-M further mapped receptor designs to functional phenotypes like cytokine secretion and phagocytosis. To extrapolate design rules, neural networks analyzed screening data to predict optimal CAR configurations. This integrated approach—combining empirical testing with machine learning—reveals structural principles governing CAR-M performance. Our strategy provides a framework for rational design of next-generation CAR-M therapies, enabling personalized receptor architectures tailored to specific immune cell functionalities. This advances the translation of CAR-M from preclinical validation to clinical application in solid tumors.

Funding Source: This research is supported by Health@InnoHK, the Innovation and Technology Commission of the Government of the HKSAR.

W1320

HDAC11-MEDIATED STAT3 ACTIVATION DRIVES INFLAMMATION AND ENDOTHELIAL DYSFUNCTION IN DIABETIC IPS-ECS

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Diabetes mellitus, a chronic disease characterised with low-grade inflammation, increases endothelial dysfunction and microbial infection risk. Bacteria that trigger epigenetic markers make endothelial cells, especially those from diabetics, susceptible. Histone Deacetylase 11 (HDAC11), an epigenetic regulator, is crucial to the inflammatory response and endothelial dysfunction. This work investigates how HDAC11 and STAT3 cause inflammation and endothelial dysfunction, shedding light on diabetes and associated vascular consequences. Using induced pluripotent stem

cell-derived endothelial cells (iPS-ECs) from diabetic donors, this research demonstrates how HDAC11 expression is markedly upregulated upon stimulation with Escherichia coli (E. coli). The activation of HDAC11 was accompanied by a surge in pro-inflammatory cytokines and aggravated endothelial dysfunction, mimicking the chronic inflammatory state observed in diabetes. These findings highlight that HDAC11 plays a central role in amplifying the inflammatory cascade through its interaction with STAT3. Remarkably, when HDAC11 activity was inhibited, STAT3 activation was significantly reduced, leading to a decrease in cytokine production and a marked improvement in endothelial cell function. This study showed that HDAC11 is a key upstream regulator of STAT3, enhancing inflammatory signalling in response to bacterial infection. Targeting HDAC11 may allow early intervention in the inflammatory cascade and mitigate the diabetes-related vascular problems. The discovery of a direct epigenetic relationship between HDAC11 and STAT3 in diabetes is unique. This study shows that HDAC11 modulates inflammation and suggests that inhibiting this enzyme may treat endothelial dysfunction. Restoring endothelial function by inhibiting HDAC11 offers intriguing prospects for restoring vascular health and treating one of the most common and catastrophic diabetes consequences. In conclusion, our study sheds information about diabetes-related endothelial dysfunction and the involvement of HDAC11 in STAT3-mediated inflammation. New therapies targeting epigenetic regulators like HDAC11 may improve diabetic and cardiovascular outcomes by reducing vascular inflammation and dysfunction.

W1322

HIGH-PURITY FUNCTIONAL CORNEAL ENDOTHELIAL CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS VIA A NOVEL WASH-OUT METHOD

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Failure of corneal endothelial function can cause corneal transparency and blindness issues, and currently, corneal transplantation is the only available treatment. Given the shortage of donor corneas, establishing robust methods for generating corneal endothelial-like cells (CECs) from induced pluripotent stem cells (iPSCs) is critical. We developed and utilized a novel method to generate homogenous CEC populations by washing undifferentiated stem cells out of the differentiated populations and demonstrated their therapeutic efficacy and safety in an animal model of corneal endothelial dysfunction (CED). Using clinical GMP-grade human iPSCs derived from cord blood, CEC differentiation was induced with or without the neural crest cell (NCC) stage for 14 to 28 days. The wash-out protocol allows more differentiated CECs to adhere to a VTN-coated surface in the initial stage, whereas the remaining population, with relatively weaker attachment ability, is washed out and removed. We identified robust CEC-specific markers on iPSC-derived CECs with hexagonal morphology. Using a wash-out method, we significantly improved the purity and differentiation efficiency of the CEC population. Single-cell sequencing data showed that iPSC-CECs with wash-out were similar to human primary CECs based on the differentially expressed genes (DEGs) associated with focal adhesion, cell-substrate junctions,

mitochondrial protein complex, metabolic activity, and extracellular vesicles. In vivo, transplantation of iPSC-CECs into a CED rabbit model demonstrated their safety and therapeutic efficacy, with improved corneal transparency. Surviving transplanted cells were observed on the endothelium for up to 16 weeks post-transplantation. We successfully differentiated clinical GMP-grade human iPSC into CEC in vitro. In vitro, results using a panel of iPSC- and CEC-markers proved the efficient and successful induction of CECs through direct differentiation from iPSCs using the novel wash-out method. Notable recovery of corneal clarity in the CED model, without graft rejection, highlights the in vitro and in vivo potential of iPSC-CECs as a powerful source for clinical therapy in patients with CED.

Funding Source: (Project number: 1711174348, RS-2020-KD000148); (21C0723L1-21, Republic of Korea); (RS-2023-00214125); (RS-2023-00302193); (grant number: RS-2024-00438366); (2023IP0069-2, 2024IP0066-1).

W1324

HOMOLOGY-INDEPENDENT TARGETED INSERTION-MEDIATED DERIVATION OF M1-BIASED MACROPHAGES HARBORING MEGF10 AND CD3Z FROM HUMAN PLURIPOTENT STEM CELLS

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Macrophages engineered with chimeric antigen receptors (CAR) are suitable for immunotherapy based on their immunomodulatory activity and ability to infiltrate solid tumours. However, the production and application of genetically edited, highly effective, and mass-produced CAR-modified macrophages (CAR-Ms) are challenging. Here, we used homology-independent targeted insertion (HITI) for site-directed CAR integration into the safe-harbour region of human pluripotent stem cells (hPSCs). This approach, together with a simple differentiation protocol, produced stable and highly effective CAR-Ms without heterogeneity. These engineered cells phagocytosed cancer cells, leading to significant inhibition of cancer-cell proliferation in vitro and in vivo. Furthermore, the engineered CARs, which incorporated a combination of CD3ζ and Megf10 (referred to as FRP5Mζ), markedly enhanced the antitumour effect of CAR-Ms by promoting M1, but not M2, polarisation. FRP5Mζ promoted M1 polarisation via nuclear factor kappa B (NF-κB), ERK, and STAT1 signalling, and concurrently inhibited STAT3 signalling even under M2 conditions. These features of CAR-Ms modulated the tumour microenvironment by activating inflammatory signalling, inducing M1 polarisation of bystander non-CAR macrophages, and enhancing the infiltration of T cells in cancer spheroids. Our findings suggest that CAR-Ms have promise as immunotherapeutics. In conclusion, the guided insertion of CAR containing CD3ζ and Megf10 domains is an effective strategy for the immunotherapy of solid tumours.

Funding Source: This research was supported by the KRIBB Research Initiative Program Grant (No. KGM4562532) and the National Research Council of Science and Technology(NST) grant by the Korea government (MSIT) (No. GTL24021-100).

W1326

HUMAN AIRWAY ORGANOIDS COMBINED WITH LUNG EXTRACELLULAR MATRIX FOR FIBROTIC DISEASE MODELING

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Fibrotic lung diseases, including chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF), encounter substantial challenges in developing effective therapeutic options due to their intricate pathophysiology. Traditional two-dimensional culture systems often fail to recapitulate the intricate architecture and cellular interactions of the human lung. To overcome this limitation, we have established a novel lung fibrotic disease model using human airway organoids cultured in a lung extracellular matrix (LuEM) hydrogel. In this study, we compared the effectiveness of a tissue-specific LuEM hydrogel against the commonly used Matrigel for culturing airway organoids. The LuEM hydrogel preserved the extracellular matrix (ECM) protein composition of native lung tissue. Airway organoids cultured in the LuEM hydrogel exhibited upregulation of genes related to lung development and epithelial cell differentiation, particularly in ciliated cells. When exposed to fibrotic stimuli, organoids in the LuEM hydrogel displayed hallmark features of fibrosis. In contrast to airway organoids cultured in Matrigel, those cultured in LuEM hydrogel demonstrated collagen accumulation and contraction proportional to the degree of fibrosis induction. Moreover, the dramatic increase in gene and protein expression of basal cell and fibrosis markers demonstrates that our organoid model closely mimics the characteristics of lung fibrosis. Our findings demonstrate that the LuEM hydrogel provides an optimal substrate for supporting the growth and differentiation of airway organoids, thereby offering a valuable platform for the study of fibrotic lung diseases. This model holds the potential to deepen our understanding of fibrosis and expedite the development of effective therapeutic strategies.

Funding Source: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government, the Ministry of Science and ICT (MSIT) (No. RS-2021-NR059722) and supported in part by Brain Korea 21(BK21) FOUR program.

W1328

HUMAN PLURIPOTENT STEM CELL-DERIVED NEUROMUSCULAR ORGANOID: A PLATFORM FOR WASTING SYNDROME RESEARCH

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Cachexia, a devastating muscle wasting syndrome in diseases like cancer, significantly worsens patient outcomes. Existing muscle wasting models often lack human relevance, as these are developed in murine cells. We are developing a novel bioengineered 3D human organoid model using human pluripotent stem cells (hPSCs) to replicate healthy and diseased neuromuscular tissue. Once developed, this organoid model, capturing the complexity of the human

neuromuscular interaction, will offer a powerful tool for unraveling cachexia mechanisms and paving the way for personalized therapeutic approaches.

W1330

HYPOIMMUNOGENIC HUMAN MOTOR NEURONS INDUCED FROM IPSCS IN VIVO SUBSTANTIALLY AMELIORATE ALS DISEASE

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Stem cell-based therapy holds great potential for substituting degenerated motor neurons (MNs) in amyotrophic lateral sclerosis (ALS). Missing protocols for advanced differentiation of transplanted cells into MNs, immune rejection, and the lack of suitable ALS models for preclinical trials have slowed the development of effective therapies. Here, we employed multiplex genetic-editing to generate a novel human pluripotent stem cell line containing doxycycline (Dox)-inducible MNs-specific transcription factors and comprehensively modified immunomodulatory genes. We transplanted these cells into the spinal cord of ALS large animal models (SOD1G93A pigs and TIA1P362L rabbits), which faithfully recapitulate pathologies and symptoms observed in ALS patients. The transplanted cells could efficiently differentiate into mature MNs upon Dox treatment in vivo, distribute throughout the spinal cord and motor cortex via extensive migration, survive long-term without the need for immunosuppression. Notably, these MNs integrated into host neural circuits, as evidenced by their long projection of peripheral axons to target muscle and reformation of neuromuscular junctions. As result, pathologies and motor deficits were substantially ameliorated in both animal models.

W1332

IDENTIFICATION AND MITIGATION OF MAJOR CONFOUNDERS IN PSYCHIATRIC DISEASE MODELS: CLONAL, BATCH-TO BATCH, OPERATOR- AND MATERIAL-BASED VARIABILITY

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While iPSC-based models provide a powerful tool for studying psychiatric disorders, even small variabilities related to interclonal homogeneity, robustness of differentiation protocols, and standardization of supplies can compromise reproducibility and data interpretation. Using risk-stratified patient-derived iPSCs and a genome editing-based candidate gene approach, we scrutinized key obstacles impacting iPSC-based modeling of depression-associated neurodevelopmental disorders. Using a cohort of patients and healthy donors, we generated cortical neural progenitors stratified by polygenic risk scores for bipolar disorder (BD1) and assessed neurodevelopmental phenotypes. We also employed a genome editing approach to study GPM6A, a gene linked to depression, schizophrenia, and BD1. Confounders including clonal variability, donor blood mosaicism, and culture conditions were systematically evaluated. In the BD1 model, clonal variability largely impacted reproducibility. Neurodevelopmental phenotypes were further highly dependent on iPSC handling and subtle differences in differentiation dynamics. Furthermore, network activity in GPM6A-deficient cortical neurons was biased by cell density and clone-specific culture composition. After addressing these confounders, only few of the previously reported pathophenotypes related to BD1 and GPM6A could be confirmed. However, we identified several new interesting BD1-associated candidates, including NBPF3, a gene involved in the regulation of vitamin B6 clearance and previously linked to symptom severity in schizophrenia. Our

findings underscore the critical need for improved standardization in iPSC-based models, especially for polygenic disease models and candidate genes with small effect sizes. Patient-derived models require extended cohorts, optimized donor selection, and advanced standardized protocols incorporating genetic and environmental risk factors. In isogenic models, expanded genetic backgrounds and robust culture systems are essential. Both lines of research depend on careful PSC clone selection, a standardized supply of high-end materials, and operator consistency. Such advancements are also vital for enhancing the reliability of iPSC models in therapy development and compound testing.

Funding Source: This project was supported by a grant of the German Research Foundation and Swiss National Science Foundation (grant no. GZ: BR 1337/4-1, project no. 412637376).

W1334

IDENTIFICATION OF CIRCULATING MICRORNA SIGNATURES IN ANTHRACYCLINE-INDUCED CARDIAC INJURY USING IPSC-DERIVED CARDIOMYOCYTES

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Anthracyclines, particularly doxorubicin (DOX), remain cornerstone chemotherapeutic agents for treating various malignancies, but their clinical application is severely limited by dose-dependent cardiotoxicity that can lead to irreversible heart failure. Current cardiac monitoring strategies primarily rely on cardiac troponin T, which only reflects acute cardiomyocyte death but cannot reveal ongoing cardiac degeneration or guide therapeutic interventions. microRNAs are stable in the bloodstream and can negatively regulate mRNA levels, thus they have emerged as biomarkers and therapeutic targets of diseases. We utilized a patient-derived human induced pluripotent stem cell-derived cardiomyocyte model to examine the secretion of microRNAs after DOX treatment. Unlike previous studies which focused on acute toxicity and cell death, we established a chronic DOX treatment model that demonstrated significant DNA damage and dysfunction to mimic cardiac degeneration in patients at later stages of this disorder. MicroRNAs were isolated from culture supernatants and subjected to RNA sequencing. Our analysis identified distinct microRNA signatures associated with DOX-induced cardiac injury. Parallel transcriptomic profiling revealed dysregulation of key pathways involved in DNA damage response, mitochondrial function, and cardiac stress. We next integrated our microRNA and mRNA data to reveal potential regulatory mechanisms underlying DOX-induced cardiotoxicity. This study not only provides novel circulating biomarkers for monitoring anthracycline-induced cardiac damage but also offers potential therapeutic targets for intervention, representing a significant advancement toward personalized cardio-oncology care and improved patient outcomes.

Funding Source: GRF.

W1336

IDENTIFYING PROTECTIVE FACTORS AND UNRAVELING THE MOLECULAR MECHANISMS THAT PRESERVE OCULOMOTOR NEURONS FROM DEGENERATION IN PATIENTS WITH SPINAL MUSCULAR ATROPHY

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Spinal Muscular Atrophy (SMA) is a neuromuscular degenerative disease caused by homozygous mutations or deletion of the survival motor neuron gene 1 (SMN1) gene, resulting in the loss of the ubiquitously expressed SMN1 protein. This leads to progressive and selective degeneration of spinal motor neurons and their innervation to the muscle fibers, causing muscular atrophy, immobility, and death. Although there are three FDA-approved treatments, Nusinersen, Zolgensma, and Risdiplam for restoration of SMN loss, they are not curative. Therefore, it is crucial to identify new therapeutic targets for effectively treating SMA. Interestingly, oculomotor neurons, responsible for eye movements, remain unaffected in SMA patients, implying a unique intrinsic mechanism to protect them from degeneration in response to the loss of SMN1. Previous studies revealed preferential expression of factors in oculomotor neurons of SMA mice but not in spinal motor neurons, suggesting their protective role. To investigate if similar factors are expressed in human ocular motor neurons, we utilized the expression of PHOX2A as a critical determinant of these neurons to generate a knock-in of the tdTomato reporter into its endogenous locus, enabling the enrichment of ocular motor neurons from induced pluripotent stem cells derived from healthy individuals- and SMA patients' urine samples for transcriptomic profiling analysis. This approach holds promise for identifying protective factors in oculomotor neurons that confer resistance to SMA and may serve as new therapeutic targets to enhance the efficacy of SMA treatments.

Funding Source: Research Impact Fund R7018-23F IRB: UW 20-329; UW11-190 General Research Fund 17114619 Seed fund for collaborative research 2023-2024.

W1338

IN SILICO AND IN VITRO IDENTIFICATION OF ANTI-MICROBIAL PEPTIDES IN SYNERGY WITH MESENCHYMAL STROMAL CELLS AGAINST UROPATHOGENIC ESCHERICHIA COLI (UPEC)

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The increasing prevalence of infectious diseases and the limited effectiveness of conventional therapies highlight the urgent need for novel approaches to combat bacterial infections, including urosepsis. Prolonged antibiotic use fosters antimicrobial resistance, posing a global health threat. Mesenchymal stem/stromal cells (MSCs) offer promising therapeutic potential due to their regenerative, immunomodulatory, anti-inflammatory, and antimicrobial properties by secreting

antimicrobial peptides (AMPs). This study explored the synergistic effect of MSCs and AMPs in combating pathigenesis by UPEC. We shortlisted six AMPs for in silico analysis targeting HisC, a key virulence factor of CFT073, followed by in vitro validation. The AMPs were docked with the HisC receptor (PDB ID: 1fg7) using the HDOCK web server. Molecular dynamics (MD) simulations were performed on the peptide-HisC complexes using gmx_qk software to evaluate key parameters: RMSD, RMSF, and binding free energy (BFE). The peptide with the lowest negative BFE was tested in vitro against CFT073 according to CLSI guidelines. A checkerboard analysis assessed the synergistic effect of the selected peptide and MSCs secretome. Biofilm formation and membrane disruption were evaluated by crystal violet staining and scanning electron microscopy (SEM). The Cecropin-LL-37 derivative (C-L) exhibited the lowest BFE (-1125.88 kJ/mol). MD simulation revealed RMSD and RMSF values of the Apomer and HisC-C-L complex (0.393 ± 0.086; 0.537 ± 0.354) and (0.140 ± 0.157; 0.197 ± 0.092). The antibacterial assay demonstrated a minimum inhibitory concentration (MIC) of C-L at 60 µg/mL. MSCs secretome also showed significant antibacterial activity against CFT073. Checkerboard analysis revealed that the combination both synergistically inhibited bacterial growth more effectively than each alone. This was further validated by significantly reduced biofilm formation and membrane disruption in the combination group. In conclusion, MSCs secretome and C-L peptide demonstrated significant antibacterial activity against CFT073 in vitro. Their synergistic effect will be further validated in a uroseptic preclinical mouse model.

W1340

IN VITRO HUMAN NEUROVASCULAR UNIT MODEL USING CELLS DIFFERENTIATED FROM A SINGLE IPSC LINEAGE FOR METABOLIC AND NEURODEGENERATIVE DISEASES RESEARCH

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The neurovascular unit (NVU) is the functional interface between the vascular system and the brain, coordinating communication among neurons, glial cells, and blood vessels. It is vital for brain homeostasis, and its dysfunction is linked to metabolic and neurodegenerative diseases such as diabetes, obesity, and Alzheimer's disease. Most NVU studies rely on animal models or mixed human-animal systems, which can limit its application in human diseases. To address this, we developed a human NVU model comprising endothelial cells (iEC), astrocytes (iAST), and neurons (iNeu), all differentiated from the same iPSC line. Human iPSCs were differentiated into iEC, iAST, and iNeu, characterized for functionality, and optimized for culture conditions using tailored media and cellular densities. The three cell types were co-cultured in a transwell system to mimic the human NVU (hNVU). The model was validated via permeability assays, TEER measurements, and fatty acid transport tests. The hNVU exhibited high TEER values (>2000 Ω·cm²) and low permeability (< 4% FITC-Dextran paracellular transport). Cells expressed key proteins and transporters, such as MFSD2A, P-gp, FABP5, FABP7, GLUT1, and CD36. Additionally, iECs

internalized and transported fluorescent fatty acid analogs (BODIPY FL C16 and LysoPC-TopFluor). The model's applicability for metabolic and neurodegenerative research was evaluated by exposing iECs to hyperglycemia (15 mM glucose + 100 µg/mL AGE-BSA) or 200 nM oligomeric human β-amyloid (1-40) for 48 hours. Both treatments disrupted tight junction proteins (ZO-1 and occludin) and altered transporter expression (GLUT1 and MFSD2A) with β-amyloid clustering and aggregation. In conclusion, the hiPSC-derived NVU model closely mimics in vivo conditions, offering a human-specific platform for studying metabolic and neurodegenerative diseases. Using a single iPSC line for all cell types enhances physiological relevance and enables a personalized approach.

Funding Source: FAPESP (2018/12605-8; 2021/14327-8); CNPq (INCT Model3D 406258/2022-8; INCT Regenera 465656/2014-5; 311026/2022-2); CAPES (Fin. Code 001).

W1342

INDUCTION OF ALPHA-SYNUCLEIN PATHOLOGY IN HUMAN IPSC-DERIVED NEUROSPHEROIDS TO MODEL SYNUCLEINOPATHIES

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Synucleinopathies are a group of neurodegenerative disorders characterized by the abnormal accumulation of misfolded alpha-synuclein (αSyn) protein in the brain. While significant insights have been gained so far into the aggregation, prion-like transmission, and toxicity of αSyn fibrils, most of these findings stem from animal models and traditional 2D in vitro cultures. To enable the study of αSyn pathology in a more human brain-like environment, we evaluated different strategies for inducing αSyn pathology in human iPSC-derived neurospheroids. Hereto, five month-old human iPSC-derived neurospheroids, composed of both neurons and astrocytes, were exposed to three conditions: (i) AAV-mediated overexpression of wild-type αSyn, (ii) addition of pre-formed αSyn fibrils (PFFs), or (iii) a combination of both, and were analysed by immunocytochemistry after 1 week, 1, 2 and 3 months. Immunostainings for αSyn, phosphorylated αSyn (p-αSyn) and thioflavin

S were performed to evaluate αSyn overexpression, fibril uptake and the formation of pathological inclusions. Immunostaining for αSyn antigen confirmed successful αSyn overexpression in the AAV-transduced group and demonstrated fibril uptake by the outer cell layers of the neurospheroids in the PFF-treated groups. Staining for p-αSyn, a marker of pathological αSyn accumulation, revealed induction of αSyn pathology in the PFF-treated conditions, with accelerated spread and accumulation observed when PFFs were combined with αSyn overexpression. Thioflavin S staining to detect β-sheet-rich structures characteristic of pathological inclusions further confirmed these findings. In conclusion, the addition of PFFs to five month-old human iPSC-derived neurospheroids, with or without AAV-mediated αSyn overexpression, proved to be the most effective in inducing widespread pathological changes, resembling those seen in human synucleinopathies. Further transcriptomic and proteomic analyses will now have to confirm that neurospheroids treated with PFFs represent a promising and physiologically relevant platform for studying αSyn pathology and potential therapies in a human brain-like context.

Funding Source: Fonds Wetenschappelijk Onderzoek (FWO).

W1344

INNOVATIVE FLUORESCENT DYE FOR RNA BINDING IN ADULT MESENCHYMAL STEM CELLS

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The objective of this study was to evaluate the cytotoxicity of newly developed fluorescent dyes with specific binding properties to RNA. The nucleic acid-binding dye was tested for its specificity toward RNA and/or DNA. Differences in structure, charge, and hydrophilicity were anticipated to influence cellular permeability and toxicity. A three-step protocol was implemented to assess the novel dye. Mesenchymal stem cells from the apical papilla were isolated and cultured until reaching 80% confluence. The cells were then trypsinized using 0.05% trypsin-EDTA (Gibco) for 5 minutes at 37°C. Subsequently, the cells were divided into two groups: one seeded in a 48-well plate and the other in a 25 cm² plastic flask. The dye was administered to the cells in the 48-well plate, which were immediately observed using the InCell Analyzer 6000 (GE Healthcare). In the flask group, the dye was added 24 hours prior to flow cytometry analysis. Cellular penetration was monitored and recorded with the InCell Analyzer 6000 at 30-minute intervals over a six-hour period. Apoptotic and viable cells were differentiated using the Annexin V kit for flow cytometry on the Navios system (Bio-Rad). High-throughput fluorescent cellular analysis visualized apoptotic cells with an FITC-conjugated anti-annexin V antibody, while nuclei were counterstained with DAPI. Results indicated that the tested dye exhibited minimal cytotoxicity and demonstrated specific binding to RNA, particularly within the nucleus. High-throughput fluorescent cellular analysis revealed a strong signal from the nuclei of living cells that were not in an apoptotic state. The development of new, specific dyes is crucial for advancing our understanding of cellular processes. Currently, only one RNA-specific binding dye is commercially available. Creating novel, non-toxic dyes capable of distinguishing between RNA and DNA or binding specific protein regions will provide valuable tools for studying transmembrane transport, RNA translation, protein folding, and interactions.

Funding Source: Grant - Bulgarian Science Fund (Sofia, Bulgaria, BG) GRANT NUMBER: КП-06-Н73/12.

W1346

INTESTINAL ORGANOID MODEL TO EXPLORE NOVEL LATE-STAGE THERAPEUTIC STRATEGIES FOR RESTORING CHOLERA-INDUCED INTESTINAL DAMAGE

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Cholera, caused by Vibrio cholerae, leads to severe diarrhea due to cholera toxin (CT), which disrupts intestinal barrier function. While CT's mechanisms are primarily associated with ion secretion, an effective late-stage therapy is urgently needed. The impact of CT on intestinal stem cells (ISCs) and their niche remains poorly understood. In this study, we use adult stem cells-derived intestinal organoids to investigate how CT affects not only ISC niche but also BMI1+ cells, a subset of quiescent intestinal stem cells, over 72 hours. Our results show a significant initial increase in BMI1 expression, followed by a substantial decline, suggesting a potential rescue effect ultimately followed by stem cell exhaustion. Additionally, lysozyme production remains consistently reduced, indicating impaired local antimicrobial response. CT exposure alters ISC markers (such as LGR5 and SOX9) at gene and protein levels, reduces stem cell proliferation, and disrupts tight junctions, leading to compromised intestinal barrier integrity. We also observe a mislocalization and mismatch of tight junction proteins, including claudin-2, β-catenin and zonulin-1, resulting in organoid shrinkage and opening. Based on these findings, we propose a dual-mechanism therapy combining calcium channel inhibitors and zonulin-1 modulators, dissolved in the organoid medium. This combination aims to prevent the cystic phenotype, reduce ion release, and restore tight junction integrity, addressing the cascade of barrier dysfunction. The dual-action therapy could provide a promising alternative to restore intestinal barrier function and enhance cholera treatment in resource-limited settings.

W1348

INVESTIGATING GLP-1 RECEPTOR AGONISTS AS A NOVEL THERAPEUTIC APPROACH FOR PARKINSON'S DISEASE USING PATIENTS IPSCS-DERIVED MIDBRAIN ORGANOIDS

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Parkinson's disease (PD), the second most common neurodegenerative disorder, is a multifactorial disease involving interplay of genetics, aging, and environmental factors. Given that most drugs entering clinical trials fail to reach patients or effectively mitigate PD, there is an urgent need to explore novel therapeutic strategies using advanced patient-specific model systems. Very recent observatory studies revealed a correlation between glucagon-like peptide-1 (GLP-1) receptor agonists, commonly known as "anti-obesity medication", and the slowing of PD symptoms progression in both animal models and humans. These findings raised numerous questions about the cellular and metabolic mechanisms by which GLP-1 receptor agonists alleviate PD symptoms. To investigate this, we developed midbrain organoids derived from PD patients. By harnessing PD patient-specific induced pluripotent stem cells (iPSCs) from the unique PD mutations, LRRK2 and GBA1, we generate midbrain organoids that mimic the substantia nigra, the brain region most affected in PD. As an initial step, we observed that the brain organoids express alpha-synuclein (α-Syn) in vitro and exhibit differential survival of dopaminergic neurons, which are hallmarks of PD. We evaluated the effects of GLP-1 receptor agonists on key PD phenotypes, including dopaminergic neurons survival, and α-Syn accumulation. Moreover, we preformed transcriptomic and metabolic profiling to identify molecular alterations associated with GLP-1 receptor agonist treatment. We are currently analyzing the results to explore the effects on PD- and metabolic-related pathways. This research tackles a timely challenge by employing patient-specific iPSCs-derived midbrain organoids model to study PD and explore potential treatments. Our findings highlight the therapeutic potential of GLP-1 receptor agonists in modulating PD-related phenotypes.

W1350

INVESTIGATING THE BIOLOGICAL PROPERTIES OF CIRCULATING HEMATOPOIETIC STEM/PROGENITOR CELLS IN PEDIATRIC SUBJECTS AS NOVEL SOURCE FOR GENE THERAPY

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Autologous Hematopoietic Stem/Progenitor Cells (HSPCs) gene therapy (GT) foresees HSPC collection and ex vivo gene correction before re-infusion in patients to rescue a disease pathophysiology. However, HSPC harvest, through bone marrow (BM) aspirates or leukapheresis after mobilization (MPB), remains technically challenging, particularly in very young patients. Interestingly, we found that pediatric individuals (Ped) during the first years of life showed increased number of physiologically circulating (cHSPC) in the peripheral blood (PB) and might be exploited as target for GT. Nevertheless, pediatric cHSPC biology remains unexplored to this day. We phenotypically, functionally and transcriptionally profiled cHSPC from pediatric healthy donors (HD) in comparison with other clinically-relevant sources including cord blood (CB), BM and MPB. Through multi-parametric immunophenotyping, applied on a cohort of over 100 Ped HD , we observed comparable HSPC composition among cHSPC, CB and MPB with enrichment of primitive HSPC subsets. Furthermore, single sorted primitive Ped cHSPC showed a high differentiation efficiency and multi-lineage hematopoietic production. To gain insight on the molecular features of cHSPC, we generated a Ped HSPC single-cell RNAseq ATLAS exploiting in-house and published datasets of 65,417 HSPC from PB, BM, CB and MPB. Notably, cHSPC were characterized by a lower cycling activity (~ 90% G0/G1 cells), and were enriched in primitive cells in line with phenotypic characterization. We also identified a cluster classified as long-term HSC, expressing gene signatures associated with stemness properties comparably to the other sources. Ped cHSPC can also be efficiently transduced with lentiviral vectors and expanded ex vivo using UM171. Preliminary results suggest that transduced and expanded Ped cHSPC retain multi-lineage differentiation potential in vitro. Ongoing in vivo experiments in mice will allow to estimate the BM-homing and reconstitution properties of pediatric cHSPC as a novel source and cell target for ex vivo and in vivo Gene Therapy strategies.

Poster Session 1 (EVEN)
5:00 PM – 6:00 PM

TRACK: GLOBAL STAKEHOLDER INITIATIVES

W1354

HUMAN REPRODUCTIVE CELL GENOME EDITING IN SOUTH KOREA: AN ANALYSIS OF REGULATORY GAPS AND ETHICAL IMPLICATIONS

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This study examines the normative, legal, and ethical frameworks surrounding human reproductive cell genome manipulation in South Korea. It reviews key legislation about the Constitution and major laws directly impacting human embryo research such as the Bioethics and Safety Act (BSA), the Pharmaceutical Affairs Act, and the Advanced Regenerative Bio Act while considering recent changes. Additionally, it examines the roles of regulatory bodies involved in embryo research approval processes and oversight including the Ministry of Health and Welfare, the National Bioethics Committee, and Institutional Review Boards. CRISPR-based genome editing research which was first practically utilized in 2012 has been subject to numerous legal restrictions for nearly two decades. This study critiques the irrationality of these restrictions and note that although South Korean law effectively prohibits human reproductive cell gene therapy, regulatory environments allow research due to legal loopholes. This raises concerns about whether the balance between scientific progress and human rights protection is adequately maintained. To address these issues, the study advocates for governance involving proper experts from the administrative and legislative branches, incorporating perspectives on fetal and embryonic rights, human dignity, and the 'right to science' as outlined by international human rights norms. Ultimately, it proposes that South Korea's human reproductive cell genome editing research should be supported by legal and ethical safeguards while continuously considering therapeutic, clinical possibilities, and scientific advancements.

W1356

DON'T CALL ME "IPSC1". ADDRESSING STEM CELL IDENTITY AND PROVENANCE IN AUSTRALIAN STEM CELL RESEARCH

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Best practices in stem cell research start with confidence in the pluripotent stem cell line used in your experiments. Registration ensures that your line has a unique and persistent identifier linking information about how the line was made with the data and publications generated from that line, and forms part of the ISCCR Standards for Human Stem Cell Use in Research (2023). We asked whether registration was now routine, or whether using lab-derived names impacted on the integrity of published stem cell research. Surveying Pubmed for stem cell research published in 2024, we identified 152 Australian stem cell lines described in 59 publications. We examined the names used to describe the lines, and assessed whether registration assisted readers to unambiguously identify which lines were used in the study. We found that 36.2% of Australian lines are named by gene phenotype (mutation/ modification/ disease state), 17.1% by generic terms ("iPSC", "Control", "Clone 3"), or are not clearly named at all (17.7%). Reassuringly, 49.4% of new Australian lines published in 2024 were registered with hPSCreg, however reuse of previously published registered lines was rarely accompanied by the registered cell line identifier, meaning (in 78% of cases) users had to read cited papers to find the hPSCreg ID. Disturbingly, lab naming conventions were dynamic, and lines names changed frequently. When unregistered, this meant the provenance of the line was unclear- unable to be tracked unequivocally to parent cell or the original publication/ provider in over 70% of cases. Registration is the most reliable way to remove ambiguities about an iPSC line. The Australian Stem Cell Registry will launch in 2025 to assist Australian researchers assign lines with a digital identifier using the hPSCreg nomenclature. Our aim is to provide a user-friendly local resource to facilitate transparency and visibility of Australian

Stem Cell research in the global sphere.

Funding Source: The authors gratefully acknowledge funding from Phenomics Australia, the National Collaborative Research Infrastructure Strategy (NCRIS), and the Medical Research

W1358

PERSPECTIVES OF WOMEN ASKED TO DONATE FETAL TISSUE FOR RESEARCH

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Human fetal tissue (HFT) has played a pivotal role in many areas of biomedical research. Vaccines developed using HFT have saved millions of lives worldwide, and HFT is the only way to study developmental processes that are not fully recapitulated in animal, cell-based, or organoid model systems. Since its inception, HFT research has been a topic of ethical debate and regulation due to its link to abortion, with the most recent policy change in 2019 requiring additional ethics review for all federally-funded HFT research in the United States (subsequently reversed in 2021). To date, the ethical debate surrounding HFT donation for research has proceeded without the benefit of empirical data to guide policy decisions. Intending to fill this gap, we share results from an empirical study investigating the perspectives of pregnant women seeking abortion towards HFT donation. We conducted qualitative interviews with a sample of 51 women who chose, or declined, to donate HFT. Results revealed that none of the interviewees felt pressured to donate, and no one objected to being asked to participate. The majority of those who chose to donate HFT felt invested in the research and motivated to learn results. Participant feedback about the consent process, however, suggested that current policy requirements governing informed consent for HFT donation—intended to protect the decisional autonomy of potential donors—may in fact serve to undermine it. We conclude with recommendations, crafted in close collaboration with partnering clinics, for short-term changes to local recruitment procedures and long-term policy amendments that could improve the experience of potential participants recruited to donate HFT for biomedical research.

Funding Source: University of Washington Royalty Research Fund.

Thursday, 12 June 2025

Poster Session 2 (ODD)
4:00 PM – 5:00 PM

TRACK: DISEASE MODELING AND DRUG DISCOVERY (DMDD)

T1001

INVESTIGATING THE IMPACT OF STRESS AND BLOOD-BRAIN BARRIER DYSFUNCTION ON ALZHEIMER'S DISEASE PATHOLOGY USING 2D AND 3D MODELS
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Despite approximately 95% of Alzheimer's Disease cases being sporadic, there is limited understanding of how genetic and environmental factors come together to drive disease development. Here, we explore how two key risk factors - stress and blood-brain barrier dysfunction (BBBD) - independently and jointly influence known AD pathways and potentially drive pathology. Previous research in AD patients and mouse models has shown that elevated stress hormones and increased BBBD both precede hallmark AD pathology, such as the accumulation of amyloid-β plaques, hyperphosphorylated tau tangles, and neurodegeneration. However, human studies are limited in their ability to examine these mechanisms at the cellular and molecular levels, and transgenic mouse models predominantly reflect the rarer familial form of AD and only partially replicate the complexity of the disease. To address this gap, we employ advanced stem cell-derived 3D brain organoid models to explore the potential causal relationship between stress, BBBD, and AD progression at both the tissue and cellular levels. In our disease model, we add cortisol to mature organoid culture to recaptulate chronic stress and supply prolonged exposure to serum albumin, a blood-borne protein that transverses the blood-brain barrier when it is dysfunctional, to model BBBD. Preliminary results from these brain organoids indicate that serum albumin affects AD-related biomarkers, including elevated Aβ, increased tau phosphorylation, and decreased synaptogenesis. These results are supported by 2D culture of neurons and glial cells, where initial data suggests that stress modulates neuronal vulnerability, with an increased effect observed in cells pretreated with cortisol before serum albumin exposure. These findings indicate that stress and BBBD may combine to drive AD pathology. By leveraging cutting-edge stem cell-derived 3D disease models, this research provides valuable insights into the cellular and tissue-level mechanisms that underlie AD. These findings have important implications for neurodegenerative disease modeling, early AD detection, and the identification of potential therapeutic targets.

T1003

INVESTIGATING THE ROLE OF THE EXTRACELLULAR VESICLES ENRICHED PROTEINS FOR SARS-COV-2 PATHOGENESIS IN HUMAN RESPIRATORY ORGANOIDS
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Huang, Jingjing, *The University of Hong Kong, Hong Kong*
Zhou, Jie, *The University of Hong Kong, Hong Kong*

Background Extracellular vesicles (EVs) are membrane-derived nanoparticles released from cells that are critical in cell communication by transporting various bioactive components. The interplay between EVs and SARS-CoV-2 has become an appealing research topic during the COVID-19 pandemic. However, most studies on this topic have been conducted in traditional or engineered cell lines such as HEK293, 293T overexpressing ACE2, and Vero-E6. Interestingly, studies conducted in different cell lines have produced contradictory findings regarding the effect of EVs on SARS-CoV-2 infection. Similarly, circulating EVs were demonstrated to be a contributor or a mitigator to disease deterioration in COVID-19 clinical studies. Overall, it remains unclear regarding the role of EV during SARS-CoV-2 infection in human respiratory epithelial cells, the primary target of the virus. We have established the first respiratory organoid culture system that enables us to rebuild and expand the human respiratory epithelium in culture plates with excellent efficiency and stability. With these robust and biologically active respiratory organoids, we aim to investigate the role of EVs in SARS-CoV-2 pathogenesis, identify novel host factors mediated through EVs, and elucidate the mechanism(s) underlying the biological effects in our respiratory organoids model. Results We conducted an LC-MS proteomics analysis on EVs derived from SARS-CoV-2-infected or mock-infected airway organoids. The proteomics analysis identified several upregulated EV-associated proteins in EVs from SARS-CoV-2 infected airway organoids. Among these upregulated proteins, we have identified some EV-enriched candidate proteins affecting SARS-CoV-2 replication. In conclusion, we investigated the role of EVs and EV-related host factors in SARS-CoV-2 pathogenesis within the human upper respiratory tract using human respiratory organoids.

T1005

INVESTIGATION ON MECHANISM OF AMPK ANTIVIRAL FUNCTION IN EV-A71 INFECTION
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Hand, foot, and mouth disease (HFMD) is a common viral illness mostly seen in infants and children under the age of five. Enterovirus A71 (EV-A71) is one of the causative agents of HFMD. While most cases of EV-A71 infection are mild and self-limiting, some can progress to severe neurological complications, including aseptic meningitis, brainstem encephalitis and acute flaccid paralysis, which makes EV-A71 infection a serious public health problem. The progression of EV-A71 infection is typically characterized by an initial fever, followed by the development of painful oral ulcers, a rash on the hands and feet, and in severe cases, neurological symptoms. The precise molecular mechanisms underlying the pathogenesis of EV-A71 are not fully understood, but several factors have been implicated in disease severity. Although several EV-A71 vaccines have been developed and are currently available in China and other countries, additional research is still needed to further enhance our understanding of EV-A71 virology and to develop more effective therapeutics and vaccines. AMP-activated protein kinase (AMPK) is a key energy sensor that regulates energy homeostasis in response to the changes in cellular environment. It is a serine/threonine kinase that is activated by an increase in the intracellular AMP/ATP ratio, which indicates cellular energy depletion. Research findings highlight the potential of targeting AMPK as a therapeutic strategy for various diseases, including cancer, metabolic disorders, cardiovascular disease, and neurodegenerative diseases. In addition to its metabolic functions, AMPK has been shown to play an important role in viral infection in recent studies. Our preliminary study has shown that EV-A71 may induce AMPK activation during infection, which may be a strategy of host immune response or requirement of viral infection. The AMPK agonists AICAR and metformin showed significant effects on the suppression of EV-A71 replication. However, the underlying

mechanism is still unknown. Enterocytes derived from human pluripotent stem cells will be hired to investigate if AMPK activation participates in EV-A71 infection. Our research findings will be a new antiviral strategy used in developing new drugs and vaccines for HFMD.

T1007

IPSC-DERIVED TUBULOIDS: A HIGH-CONTENT SCREENING PLATFORM FOR UMOD VARIANTS IN KIDNEY DISEASE

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The rise of widespread genetic sequencing has linked thousands of missense variants and variants of uncertain significance (VUS) with kidney disorders. Determining the functional impact of these variants in a physiologically relevant human model remains a significant challenge, often requiring labor-intensive, approaches for individual genes and VUS. To address this limitation, we established an iPSC-derived epithelial human kidney organoid (tubuloid) platform to facilitate high-throughput functional analysis of VUS. Tubuloids, made from iPSC-derived human kidney organoids, model the 3D structure and expression profiles of the distal nephron, loop of Henle, and proximal tubule, providing an ideal model to study a variety of VUS. UMOD is a protein coding gene predominantly expressed in the thick ascending limb of the loop of Henle. Mutations in UMOD are linked to autosomal dominant tubulointerstitial kidney disease (ADTKD-UMOD), characterized by progressive renal dysfunction and fibrosis. The pathogenicity of novel UMOD variants is challenging to predict and the mechanisms of ADTKD-UMOD remain incompletely understood. To address this, we employed lentiviral overexpression of UMOD variants including wildtype, benign, pathogenic, and novel clinical VUS in iPSC-derived tubuloids. High-content imaging, immunofluorescence, and biochemical assays were utilised to assess protein localisation, secretion, intracellular aggregation, and stress responses associated with variant expression. Wildtype and benign UMOD variants localised to the basolateral membrane of tubuloids, while known pathogenic variants and some VUS were characterised by altered protein stability and mislocalisation. Our findings provide evidence of pathogenicity for novel UMOD VUS and correlate mislocalisation patterns and associated cellular stress responses with disease severity., This tubuloid-based system offers a scalable functional genomics platform for genetic disorders, with potential to improve diagnostic outcomes for patients and foster the development of precision medicine approaches.

Funding Source: Australia Medical Research Future Fund Australian Functional Genomics Network.

T1009

LABEL-FREE FUNCTIONAL ANALYSIS FOR THE SCREENING OF IPSC-DERIVED NEURAL ORGANOID RESPONSE TO NEUROACTIVE COMPOUNDS

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In vitro neuronal models are valuable tools for studying brain function, neural diseases, and toxicology. Recent advances in induced pluripotent stem cell (iPSC) technology have allowed researchers to produce 3D models of neural tissue, termed neural organoids, that better recapitulate the cellular diversity and spatial architecture of in vivo tissue. Here, a toxicology screening workflow to monitor the differentiation of neural organoids and their response to neuroactive compounds is described. Neural organoids were both generated via commercially available kits and purchased as a finished product for this study. To generate neural organoids, iPSCs were first plated and monitored using a whole-vessel imaging system (the Omni). Colony area, diameter, and coverage increased over a three-day period, at which point the colonies reached an optimal size for passaging and subsequent embryoid body formation. Following neural induction using STEMCELL Technologies' Dorsal Forebrain Organoid Differentiation Kit (Catalog #08620), organoids in suspension culture were imaged for 50+ days using the Omni, and organoid size and count were monitored, illustrating the progression of the differentiation process. For electrophysiological measurements, pre-formed Human iPSC-Derived Midbrain Organoids (Catalog #200-0792 and Catalog # 200-0793) from STEMCELL Technologies were cultured using the STEMdiff TM Neural Organoid Maintenance Kit (Catalog #100-0120). The midbrain organoids were plated onto CytoView 6 MEA plates and, on Day 125 post-differentiation, dosed with neuroactive compounds or a DMSO control. Baseline and post-dose (1 hour after drug addition) recordings were taken using the Maestro Pro and showed robust changes in midbrain organoid activity patterns in response to the potassium channel blocker 4-aminopyrirdine and in response to the mitochondrial complex I inhibitor rotenone. Dosing with 4-aminopyrirdine increased the mean firing rate and network burst frequency of midbrain organoids, while dosing with rotenone led to a decrease in both metrics. In total, this study shows that the Omni and Maestro Pro systems are valuable tools for studying the differentiation of neural organoids and their subsequent use in toxicological screening applications.

T1011

LARGE-SCALE GENERATION OF INK AND CAR INK CELLS FROM CD34+ HEMATOPOIETIC STEM AND PROGENITOR CELLS FOR ADOPTIVE IMMUNOTHERAPY

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Chimeric antigen receptor (CAR) natural killer (CAR NK) cells, leveraging safety and not requiring HLA match in adoptive infusion, have emerged as promising alternative cells to CAR-T cells for immunotherapies. High and multiple doses of CAR NK cell infusions are essential to maintain therapeutic efficacy in clinical trials. This requires efficient methods for generating large-scale CAR

NK cells and significantly reducing CAR engineering costs. In this study, we develop a three-step strategy to generate high yields of induced NK (iNK) and CAR iNK cells from human umbilical cord blood CD34+ hematopoietic stem and progenitor cells (CD34+ HSPCs). Starting from a single umbilical cord blood CD34+ HSPC, our reliable method efficiently produces 14-83 million mature iNK cells or 7-32 million CAR iNK cells with high expression levels of CD16 and CAR and zero T cell contaminations. Introducing CAR expression elements at the HSPC level reduces the quantities of CAR pseudoviruses to 1/140,000 - 1/600,000 compared to engineering CARs in mature NK cells. The iNK and CAR iNK cells, including fresh cells and thawed cells from cryopreserved conditions, demonstrate remarkable tumoricidal activities against various human cancer cells and significantly prolong the survival of human tumor-bearing animals. The high yields of CAR NK cells and negligible costs of CAR engineering of our method support the broad applications of CAR NK cells for treating cancer patients.

T1013

LIVER HEPATOBILIARY PROGENITORS-DERIVED ORGANOIDS CULTURED IN DECELLULARIZED EXTRACELLULAR HYDROGEL FOR BILIARY ATRESIA MODELING

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Biliary atresia (BA) is a severe infantile biliary system disease characterized by the obstruction and damage of the bile ducts, resulting in cholestasis, which may cause the liver to deteriorate progressively and eventually lead to liver failure. There is a multifactorial cause of BA, while the precise pathogenesis is still unknown. Clinical research has been conducted to investigate environmental and host factors, including contamination toxins, virus infection, genetic mutation, and aberrant immune responses. The liver extracellular matrix (ECM) contains growth factors and bioactive molecules to regulate diverse cellular functions, which play a key role in liver development and disease diagnosis and treatment. The Ross River virus (RRV)-induced animal BA model, mimicking most types of extrahepatic BA in children, is used for studying BA development and liver fibrosis processing. Mice infected with RRV were used to study the variation of ECM protein expression in BA and mimic liver fibrosis processing in humans. The symptoms of cholestasis appear between the fourth- and eleventh-day post-infection, and 14-day-old mice are used to mimic the state of BA in infants and understand fibrosis in this phase. A stark contrast is seen between the healthy control group and BA mice, with the latter showing yellow and dark surface livers with yellow spots. WT and BA mouse livers were harvested for preparing ECM and ECM hydrogels. Both WT and BA liver ECM were analyzed using a proteomic method. 1768 protein segments were detected and identified, and 1080 of them are upregulated and 688 of them are downregulated. The top 10 upregulated and downregulated protein segments in BA were demonstrated. The C5/C5b a factor of the complement system was detected in the BA mouse and showed a higher expression compared to the controlled liver tissue sections. ECM was further digested to form WT and BA liver ECM-derived hydrogel for liver hepatobiliary progenitors-derived organoid culture. It is seen that some remaining ECM factors could inhibit organoid growth and proliferation. The liver ECM of mice with/without BA can be used to identify potential niche factors that impact disease initiation/progression of BA. The ECM derived from decellularized tissues can be used as a tissue engineering scaffold for tissue repair and regeneration.

Funding Source: The authors thank all the patients who participated in this study. This work is supported by Theme-based Research Scheme 2021 (T12-712/21-R); Health and Medical Research Fund (08192376).

T1015

LONG-TERM CORRECTION OF HEMOPHILIA A THROUGH AAVS1-TARGETED INTEGRATION OF A FUNCTIONALLY ENHANCED FVIII GENE USING CAS9 NICKASE IN PATIENT-DERIVED IPSCS

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Hemophilia A (HA) is a genetic disorder resulting from mutations in the gene encoding coagulation factor VIII (FVIII). Genome editing in conjunction with patient-derived induced pluripotent stem cells (iPSCs) is a promising cell therapy strategy, as it replaces dysfunctional proteins resulting from genetic mutations with normal proteins. However, the low expression level and short half-life of FVIII still remain major challenges in the efficacy of these approaches in HA. Here, we constructed a functionally enhanced FVIII variant, F309S/E1984V-mutated B domain-deleted (BDD)-FVIII (FE-FVIII), with increased activity and stability. We inserted FE-FVIII with a human elongation factor-1 alpha (EF1α) promoter into the AAVS1 locus of HA patient-derived iPSCs via CRISPR/Cas9 (D10A) nickase to ensure expression in any cell type. FE-FVIII was expressed not only in undifferentiated FE-FVIII-inserted (FE-KI) iPSCs but also in endothelial cells (ECs) differentiated from them in vitro. Compared with mice transplanted with wild-type BDD-FVIII-containing ECs, immunocompetent HA mice intravenously transplanted with FE-KI ECs presented a 2.12-fold increase in FVIII activity in the blood and an approximately 20% greater survival rate after hemorrhagic tail injury. For sustained efficacy, FE-KI ECs were subcutaneously transplanted into immunodeficient HA mice, resulting in amelioration of the hemophilia phenotype for more than 3 months. This strategy may provide a universal therapeutic approach for treating HA.

Funding Source: This work was supported by National Research Foundation of Korea (NRF) grants funded by the Korean government (MSIT) (2022R1A2C2091165 and 2022R1A2C1091800) and the Korean Fund for Regenerative Medicine (KFRM) (RS-2024-00332790).

T1017

MECHANICAL TENSILE LOADING AND STRESSING ALTER ECM DEPOSITION OF HUMAN MENISCUS TISSUE RESIDENT PROGENITOR CELLS

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The meniscus is a crucial cartilage tissue bearing the majority of the mechanical load in the knee joint. Adult human meniscus stem/progenitor cells (hMeSPCs) are the tissue resident progenitor cells of meniscus tissue, and are shown to be sensitive to mechanical stimulations. Our previous study has revealed how the biomimetic mild cyclic tensile loading (0.5 Hz, 10% strain, 1h/day) activates hMeSPCs in 3D hydrogel cultures in the perspective of stem cell activation, ECM accumulation and remodelling. However, the influence of fast and intense tensile loading on hMeSPCs is yet to be revealed. In previous study, 20% of tensile stretch was applied to mimic intense mechanical stress on chondrocytes which shown an overload effect of the cells. By introducing servomotor and conveyor pulleys to in-house built bioreactor, we are able to implement considerable frequent stretch up to 4 Hz with a maximum 34% of hydrogel extension in length. We then seeded the hMeSPCs (n=8 biological donors) into GelMA hydrogel and introduce a fixed frequency of 1 Hz and 20% of stretching to the 3D cultured hMeSPCs, which is a biomimetic situation of joint meniscus load in fast walk scenario (120 step/min). The hMeSPCs were loaded (1 Hz, 1h/day, 10% strain and 20 strain, respectively) for 14 days. The cell-hydrogel cultures were then collected and analysed by histology of Safarinin O staining. We quantified the positive staining area of Safarinin O staining in hydrogel of each group (Ctrl: no loading; 10%: 1 Hz, 10% Strain; 20%: 1 Hz, 20% strain; n≥6 cell-hydrogel cultures/group), and observed increased cartilage-like ECM deposition in the hydrogel after frequent stretching in both 10% (15.52±9.41%, $P < 0.05$) and 20% stretch (13.89±7.99%, $P < 0.01$) compared with no stretching control (6.96±1.81%). Meanwhile, the porosity is not significantly changed by different loading parameters (Negative control: 40.33±8.18%, 10%: 43.62±11.17%, 20%: 44.24±9.04%). Next, we will apply flow cytometry and transcriptome analysis to further investigate the influence of intense stretch on hMeSPCs in the aspect of cell viability and underlying mechanisms. In summary, the in vitro findings provided better understanding of the mechanobiology of hMeSPCs, consolidating the knowledge for further cell-based therapy in meniscus-related diseases.

Funding Source: National Key Research and Development Program [2019YFA0111900, to YJ], MOST, China; General Research Fund (GRF, 14104022, to YJ) RGC, UGC of Hong Kong; the Center for Neuromusculoskeletal Restorative Medicine [CNRM at InnoHK, to RST, YJ].

T1019

METABOIC DYSFUNCTION-ASSOCIATED STEATOHEPATITIS MODELING WITH HUMAN LIVER MICROENVIRONMENT-INCORPORATED ORGANOIDS

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The prevalence of steatohepatitis is on the rise annually, with metabolic dysfunction-associated steatohepatitis (MASH) emerging as a significant contributor to global liver cirrhosis and cancer. Despite its growing impact on public health, there exists only one FDA-approved drug designed to

treat MASH. To understand disease pathology and screen potential therapeutic candidates, the development of an in vitro human liver-mimetic steatohepatitis organoid model is crucial. Conventional fatty liver organoid models for therapeutic candidate screening are constrained by several limitations, such as the absence of non-parenchymal cells (NPCs), the absence of a liver-specific extracellular matrix (ECM), and insufficient understanding of mechanisms related to MASH progression. Herein, we established stem cell-derived advanced steatohepatitis organoid models through co-culture of endothelial cells, hepatic stellate cells, and Kupffer cells combined with liver-mimicking hydrogel. We also developed an organoid chamber chip for efficient fatty acid accumulation inside the organoid, and simultaneously, allows for on-chip drug screening. Compared to existing steatosis organoid models, which are typically cultured in Matrigel under static well-plate condition, our advanced steatohepatitis organoid model exhibited a phenotype closely resembling the characteristics observed in actual MASH patients. Our study highlights the development of the first human liver microenvironment-incorporated steatohepatitis organoid model for drug screening, contributing to the understanding of drug mechanisms related to the progression of MASH.

Funding Source: This research was supported by the ABC-based Regenerative BioTherapeutics (ABC project) grant funded by the Korea government (the Ministry of Science and ICT, the Ministry of Health and Welfare) (RS-2024-00432653) and by Brain Korea 21.

T1021

METABOLIC REPROGRAMMING IN ALZHEIMER'S DISEASE DISRUPTS NEURONAL RESILIENCE THROUGH DYSREGULATION OF ALTERNATIVE SPLICING

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Aging is the primary risk factor for Alzheimer's disease (AD), a devastating neurodegenerative disorder marked by progressive neuronal loss. Healthy neurons typically possess intrinsic mechanisms that confer resilience to external stressors, enabling survival over decades. Our previous studies using induced neurons (iNs) directly converted from patient fibroblasts, revealed that metabolic reprogramming in AD disrupts these mechanisms, leading to compromised neuronal resilience. As iNs retain transcriptional and metabolic features of aging and AD, they serve as an ideal model to study human neuron-specific mechanisms of age-related neurodegeneration. In this study, we used iNs from sporadic AD patients and age- and sex-matched controls to identify druggable metabolic pathways underlying the loss of neuronal resilience in AD. UHPLC-MS metabolomics and genetically encoded metabolic sensors revealed a diversion of citrate from the mitochondrial TCA cycle toward nuclear acetyl-CoA production via ATP-citrate lyase (ACLY). This metabolic shift caused hyperacetylation of RNA-binding proteins (RBPs), disrupting RNA splicing. To further explore the impact of ACLY-mediated acetyl-CoA generation on RNA splicing, we employed a novel single-cell long-read RNA sequencing approach. This innovate technique previously demonstrated that human neurons express distinct RNA isoform patterns critical to their function and identity. These patterns, which are unique to the human brain and absent in animal models, partly explain the differing susceptibility of humans and mice to neurodegeneration,

emphasizing the value of studying human cells to understand neurodegenerative diseases. In AD iNs, we identified splicing changes linked to cell death and neuronal dysfunction. Crucially, ACLY inhibition that reduced nuclear acetyl-CoA levels, also restored a healthy neuronal isoform landscape, and reversed splicing alterations specifically driven by hyperacetylated RBPs. These findings reveal a metabolic mechanism driving splicing defects in AD and highlight targeting acetyl-CoA dynamics as a promising therapeutic strategy.

Funding Source: BirghtFocus Foundation A20222024F, Theodor Koerner Fonds, L'Oreal Austria/OeUK/OwAW.

T1023

MICE WITH 16P11.2 DELETION AND DUPLICATION SHOW ALTERATIONS IN BIOLOGICAL PROCESSES ASSOCIATED WITH WHITE MATTER

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Copy number variants (CNVs) of the chromosomal locus 16p11.2 has been predisposed individuals to neurodevelopmental diseases, including autism spectrum disorder, intellectual disability, epilepsy/seizures, dysmorphic features, congenital anomalies, macrocephaly, and microcephaly. Intracortical myelin is thought to play a significant role in the development of neural circuits and functional networks, with consistent evidence of typical network connectivity in children with neurodevelopmental disorders. Emerging imaging studies suggest abnormal white matter microstructure which can explain some of the associated clinical phenotypes including cognitive decline and developmental delay. In this study, we seek to discover myelin-related alternations in the corpus callosum using 16p11.2 alternation mouse model. We found, compared with controls, the myelin expression level alternation in the 16p11.2 duplication and deletion mouse model and these myelin changes will not improve with the mouse ageing. We further identified the cell number of mature and immature oligodendrocytes. We found the cell number was not changed in 16p11.2 CNVs mouse model. However, the myelin thickness changed in the area of the corpus callosum. These findings suggest that the myelin microstructure change is associated with the alternation of the 16p11.2 locus which may be the reason for neurodevelopmental disorders caused by 16p11.2 CNVs.

T1027

MINIATURE ENGINEERED HEART TISSUES FROM HUMAN IPSC-CARDIOMYOCYTES ON A HYPOXIA ON-A-CHIP PLATFORM

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Ischemic heart disease (IHD) is the leading cause of death globally. In the disease, the blood flow to the myocardium is reduced or blocked, leading to oxygen deprivation and tissue damage. The primary treatment is reperfusion to restore blood flow and antiarrhythmic medication to prevent arrhythmias. Reperfusion, although necessary for recovery, can lead to worsening of the tissue injury. Previous animal experiments have provided promising results for medical interventions during reperfusion to reduce arrythmias or improve tissue regeneration, but they have failed in human clinical trials, indicating the need for human based models. Understanding the pathophysiology on a cellular lever, e.g., what mechanisms cause the arrythmias, promotes the development of more effective treatments. Human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) have been used to study IHD, but the experiments have been conducted mostly with 2D cell models. To better mimic physiological environments, in-vitro disease modelling and preclinical drug testing are being shifted from 2D to more complex 3D systems. Here, we present a novel 3D hypoxia platform, combining engineered heart tissue (EHT) model with a system enabling precise control over oxygen concentrations on the chip. The base of the platform is an OxyGenie mini-incubator (BioGenium Microsystems, Finland) combined with field stimulation electrodes and an EHT-insert. Miniature EHT s are constructed on the EHT-insert lid by embedding 300 000 hiPSC-CMs within fibrinogen. The hypoxia-on-a-chip platform allows electrical stimulation on the tissue, and a way to analyze cardiac functionality real time during hypoxic periods, including assessment of the beating rate, emerging arrythmias and contractile force. Here we show that with our hypoxia-on-a-chip platform, iPSC-CMs and EHT technology can be effectively utilized to study cardiac tissue function under hypoxia. The miniature EHTs start beating spontaneously within the first week of culture and respond to the electrical stimulation. The chip design enables advanced imaging and precise control over temperature and oxygen concentration for real-time monitoring of the oxygen dynamics. Our preliminary results show strong potential for use in IHD modeling.

T1029

MODELING CDKL5 DEFICIENCY DISORDER USING CEREBRAL ORGANOIDS REVEALS HUMAN-ENRICHED CELLULAR DEFICITS

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Pathogenic variants in the CDKL5 genes result in CDKL5 deficiency disorder (CDD), an X-linked neurodevelopmental disorder. Patients with CDD exhibit a wide range of symptoms, including early-onset epilepsy, global developmental delay, intellectual disability, autistic features, visual impairment, and motor impairment. The CDKL5 gene is located at position 22 on the X chromosome and encodes the CDKL5 protein, a member of the serine/threonine kinase family. Currently, the molecular mechanisms underlying the pathophysiology of CDD remain unclear and there is no cure for patients with CDD. Notably, genetic knockout of CDKL5 in mice fails to reproduce the seizure phenotypes observed in human patients, emphasizing the urgent need for disease-relevant human models of CDD to elucidate the functional roles of CDKL5. To comprehensively study the molecular functions of CDKL5 and expedite therapeutic development, it is crucial to identify its direct substrates and dissect its function in clinically relevant cell types and models. Three-dimensional brain organoids generated from patient-derived induced pluripotent

stem cells (iPSCs) serve as a robust model for investigating the underlying mechanisms of CDD with a human background. By employing single-cell RNA sequencing and immunostaining techniques, this study uncovered previously unrecognized proliferation deficits among major neural progenitor stem cells in CDD organoids. Given that CDKL5 is a serine/threonine kinase, the study explored several potential downstream substrates, including the microtubule-binding protein EB2. The findings of this study propose a novel mechanism underlying CDD pathology and offer crucial insights into the exploration of novel treatment strategies for CDD.

Funding Source: Lo Kwee-Seong Biomedical Research Fund (J.I), Faculty Innovation Award (FIA2020/A/04) from the Faculty of Medicine, CUHK (J.I.), Hong Kong RGC Research Matching Grant Scheme (J.I.) and Hong Kong PhD Fellowship (PF20-43681; Y.Z.).

T1031

MODELING HEREDITARY SENSORY AND AUTONOMIC NEUROPATHY TYPE IV (HSAN IV) USING HUMAN DRG ORGANOIDS DERIVED FROM PATIENT INDUCED PLURIPOTENT STEM CELLS

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Hereditary Sensory and Autonomic Neuropathy Type IV (HSAN IV) is a rare autosomal genetic disorder characterized by Congenital Insensitivity to Pain with Anhidrosis. It is caused by mutations in the Neurotrophic Receptor Tyrosine Kinase 1 (NTRK1) gene. While previous animal studies have revealed the significance of NTRK1 in nociceptive neuron formation in the dorsal root ganglia (DRG), its role in congenital sensory neuropathy and the underlying disease mechanisms in the human context are less explored. In this study, we successfully established human DRG organoids and remodelled HSAN IV disease using induced pluripotent stem cells (iPSCs) lines derived from an HSAN IV patient's urine. The patient carries homozygous mutations, including a G deletion in one allele at exon 7 leading to early stop code, and a G to A alteration in the other allele at exon 16 that converts glutamic acid to lysine. To eliminate the genetic variations, we also generated isogenic control by correcting the patient's mutation using CRISPR-based gene editing. By analysing different developmental stages of DRG organoids, we found that DRG organoids derived from HSAN IV patients underwent a lineage switching between sensory neurons and glial cells without affecting the neural crest stem cell population. During early neurogenesis, a marked reduction of sensory neurons expressing ISLET+ and BRN3A+ cells was detected. Additionally, DRG organoids derived from the patient exhibited few mature sensory markers, TRKA, TRKC, TRPV, and CGRP, and defective axonal outgrowth and extension Notably, gliogenesis was

initiated prematurely, with a significant upregulation of FABP7. These findings suggest that NTRK1 mutations disrupt the balance of neuronal and glial differentiation in human DRG during development, which may contribute to sensory neuropathy in adults. Future studies will focus on uncovering the molecular mechanisms mediated by NTRK1 and identifying therapeutic targets to restore proper neuronal-glial differentiation signals in human DRG.

T1033

MODELING RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA (RDEB) USING PATIENT-SPECIFIC IPSC-DERIVED SKIN ORGANOIDS AND CRISPR/CAS9 GENE EDITING

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Recessive Dystrophic Epidermolysis Bullosa (RDEB) is a severe genetic skin disorder caused by mutations in the COL7A1 gene, leading to truncated type VII collagen (C7) at the epidermal–dermal junction and resulting in extensive blistering, large non-healing wounds, and increased squamous cell carcinoma risk. In this study, patient-derived induced pluripotent stem cells (iPSCs) harboring the codon 1573 C→T (R525Ter) mutation, which introduces a premature stop codon at exon 12, were used alongside wild-type iPSCs (SC81103). Both were differentiated into fibroblasts (FB) and keratinocytes (KC), then combined with extracellular matrix and seeded onto polydimethylsiloxane (PDMS) devices to form a 3D microtissue-based skin organoid. Differentiation was confirmed by immunofluorescence staining of COL1A1/Vimentin (FB) and KRT5 (KC) and by real-time PCR of lineage markers (FB: COL1A1, COL3A1; KC: KRT5, KRT14, TP63). Further characterization using embryoid bodies (EBs) and single-cell assays supported the transition toward KC identity. These organoids recapitulated key molecular and structural features of healthy and RDEB skin, serving as a versatile in vitro model for disease mechanism studies and therapeutic testing. Future work will employ CRISPR/Cas9 and adenine base editing to correct the COL7A1 mutation and rescue functional C7 expression, thereby highlighting a potential path toward definitive treatments for RDEB.

Funding Source: NSTC112-2636-M-006-010,NSTC112-2321-B-006-012, NSTC113-2320-B-006-047, NSTC113-2321-B-006-012.

T1035

MODELLING GENETIC PARKINSON'S DISEASE IN A MULTIPLEXED MOSAIC ORGANOID SYSTEM

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Parkinson's disease (PD) is a common neurodegenerative disorder affecting millions of people worldwide. The hallmark of PD is the loss of dopaminergic (DA) neurons in the substantia nigra pars compacta region of the ventral midbrain (VM) and inclusion of Lewy bodies. PD presents as both idiopathic and genetic cases, complicating its study. Current pre-clinical models, while insightful, face limitations that highlight the need for more human-relevant models. Brain organoids have revolutionized disease modeling, offering a humanized 3D system that mimics brain regions such as the VM. VM organoids, though promising, encounter challenges in reproducibility and scalability, hindering the study of inter-individual genetic variations. In this study we develop a model where multiple individuals are represented, creating a multi-donor VM organoid "mosaic VM organoid". Following this we aim to create a mosaic organoid where multiple donors affected by genetic PD would be represented in a single organoid, reducing the variability and enabling patient-specific pathology screening. Utilizing genetic demultiplexing, we can observe at single-cell level effects on development and stress responses of individual cell lines with different genetic backgrounds. Successful generation of mosaic brain organoids would take disease modeling and drug screening to a new level, ultimately leading development of personalized therapies.

T1037

MODULAR IN VITRO PLATFORM FOR MIMICKING THE MECHANICAL FORCES INDUCED ON TISSUES IN HEALTH AND DISEASE

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Most tissues in the human body are constantly subjected to mechanical stress, which is crucial for proper functioning (e.g., forces in the heart) and plays a significant role when tissues are damaged (e.g., in traumatic brain injury). While mechano-physiological forces are essential for tissue functionality, replicating these forces in vitro remains a major challenge— particularly when aiming to apply external forces without direct cell contact while preserving cell viability and functionality over time. Existing methods have sought to address this challenge, but none have fully achieved dynamic, non-contact force application with adjustable parameters. This research presents a modular platform capable of inducing external forces on cells without direct physical interaction. The platform provides precise control over force magnitude, duration, and applicability across various cell types, enabling to induce physiological forces to mimic both healthy and disease conditions. Two case studies are highlighted: Healthy conditions (Heart-load): The platform successfully mimicked pressure-volume (PV) loops in vitro, demonstrating a strong correlation with in vivo data; Disease conditions (Traumatic brain injury): The same platform was used to simulate mechanical injuries, providing new insights into neuronal responses to trauma. This versatile platform is not limited to a specific tissue type, offering a powerful tool for simulating in vivo mechanical forces in various biological contexts and extracting unique insights that are unattainable with existing methodologies.

T1039

MOLECULAR MECHANISMS OF STRESS RESILIENCE: NOVEL THERAPEUTIC STRATEGIES FOR DELAYING AGING AND NEUROPROTECTION

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Aging is characterized by a gradual decline in physiological functions, with notable changes in the central nervous system accompanied by hyperactivation of hypothalamus-pituitary-adrenal (HPA) axis. Stress resilience, the ability to adapt to and recover from stress, is influenced by both environmental and genetic factors, which can reduce the risk of stress-induced disorders such as Alzheimer's disease (AD) and depression. Consequently, resilience has emerged as a critical factor in alleviating HPA axis dysregulation, which mitigates the aging process. However, stress resilience is a complex and multifaceted trait influenced by subjective experience, genetic predisposition, and environmental interactions, making it difficult to assess human stress resilience. Therefore, in this study, we exposed mice with Chronic Unpredictable Mild Stress (CUMS) model which usually impairs cognitive and mood regulation in mice. We then identified resilient mice maintaining behavior similar to the control group in the tail suspension test and Y-maze test, while susceptible group exhibited both cognitive deficits and depression-like behaviors. Also, we performed total RNA sequencing and Assay for Transposase-Accessible Chromatin using sequencing (ATAC) sequencing to identify genes associated with stress resilience and further evaluated their ani-aging effects. We further assessed the neuroprotective effects of these genes in an aging animal model. This study highlights the molecular mechanisms underlying resilience and suggests that targeting resilience pathways may offer novel therapeutic strategies for delaying aging and maintaining neural health.

T1041

MOTOR NEURON-SPECIFIC DLC1-I1 DEFICIENCY LEADS TO NEUROMUSCULAR DEFECTS IN SPINAL MUSCULAR ATROPHY

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Spinal muscular atrophy (SMA) is a genetic neuromuscular disease caused by the loss of the ubiquitously expressed survival motor neuron 1 (SMN1) protein, resulting in progressive degeneration of spinal motor neurons and muscular atrophy. The selective impact of global SMN

deficiency on motor neuron integrity remains elusive. Here, we show that the Rho GTPase-activating protein DLC1 isoform 1 (DLC1-i1) is expressed in human MNs and crucial for axon outgrowth and survival. Reduced DLC1-i1 expression contributes to impaired neuromuscular junction (NMJ) formation and increased cell death in neuromuscular organoids differentiated from SMA patients' urine-derived induced pluripotent stem cells. Conversely, overexpression of DLC1-i1 reverse these phenotypes by enhancing motor axon regeneration. Furthermore, DLC1-i1 expression is significantly reduced in the lumbar spinal cord of SMA mice, and postnatal knockout of Dlc1 in the neuronal cells recapitulates the neuromuscular defects observed in SMA mice. Gene therapy with DLC1-i1 proved more effective than SMN1 in extending lifespan and improving locomotor function in SMA mice, which can be enhanced by co-administration of DLC1-i1+SMN1. Mechanistically, DLC1-i1 not only enhances SMN2 exon 7 inclusion and SMN protein levels by binding with hnRNPC to both exon and intron 7, but also promotes motor axon outgrowth by interacting with mRNAs encoding mitochondria ATP production. In summary, our findings reveal that deficiency of motor neuron-specific DLC1-i1 is a key contributor of NMJ defects in SMA, and restoration of DLC1-i1 expression represents a more effective therapeutic approach for SMA.

Funding Source: Research Grants Council and University Council of Hong Kong (GRF_17114619, GRF_17114817) Li Po Shan/Dr Vincent Lui Endowment Fund for Motor Neurone Disease Research Impact Fund (R7018-23F).

T1043

MPOX VIRUS OPG175 NEGATIVELY REGULATES VIRAL REPLICATION VIA CONTROLLING WNT SIGNALING

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Mpox is an infectious disease caused by the Mpox virus (MPXV). In 2022, a mpox outbreak has occurred, leading the World Health Organization (WHO) to declare a Public Health Emergency of International Concern (PHEIC) in July of that year. The 2022 outbreak-causing MPXV is classified as clade IIb, and is phylogenetically distinct from the endemic MPXV strains, MPXV clade Ia or IIa. MPXV clade IIb is also different from the endemic strains in terms of fatality rate, symptoms, and epidemiological characteristics. However, there has been insufficient molecular biological research on the differences between MPXV clades. In this study, we conducted functional analysis of MPXV genes which are specifically expressed in MPXV-infected cells to elucidate the characteristics of MPXV. Human keratinocytes or human induced pluripotent stem (iPS) cell-derived colon organoids were infected with three MPXV strains, MPXV clade Ia, MPXV clade IIa, and MPXV clade IIb. After infection, RNA was extracted from the cells and RNA-seq analysis was performed. Among the

approximately 170 MPXV genes conserved between the three clades, we identified the MPXV gene which is highly expressed in MPXV clade IIb-infected cells, and then performed functional analysis of that gene. We found that the OPG175 gene was highly expressed in MPXV clade IIb-infected keratinocytes and colon organoids. Although the replication efficiency of MPXV clade IIb was lower than that of MPXV clade Ia and IIa, suppression of OPG175 expression significantly upregulated the replication efficiency of MPXV clade IIb. Conversely, we found OPG175 overexpression to enhance the expression of Wnt signaling-related genes and activation of Wnt signaling to decrease the replication efficiency of MPXV. Therefore, high OPG175 expression in MPXV clade IIb-infected cells likely inhibits MPXV replication via activation of Wnt signaling.

Funding Source: This research was supported by the iPS Cell Research Fund, JSPS Core-to-Core Program (A. Advanced Research Networks), and the Japan Agency for Medical Research and Development (AMED) (JP21gm1610005, JP23fk0108583, JP23jf0126002).

T1045

MULTIOMICS MAPPING AND CHARACTERIZATION OF CELLULAR SENESCENCE IN AGING HUMAN SKELETAL MUSCLE UNCOVERS A NOVEL SENOTHERAPEUTIC FOR SARCOPENIA

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Cellular senescence is recognized as a hallmark of organismal aging but how it drives aging particularly in human tissues is not fully understood, partly due to the complex heterogeneous nature of senescence. Here in this study, we leverage single-nucleus multiomics to profile senescence in mononucleated cells of human skeletal muscle and provide the first senescence atlas. We demonstrate the intra- and inter-populational transcriptomic and epigenomic heterogeneity and dynamics of senescence in the cells. We also identify commonalities and variations in senescence-associated secretory phenotypes (SASPs) among the cells and elucidate the function of SASPs in mediating cellular interactions and niche deregulation. Furthermore, we identify targetable SASP factors and demonstrate the possibility of using Maraviroc as a pharmacological senotherapeutic for treating age-associated sarcopenia in muscles. Lastly, we define transcription factors that govern senescence state and SASP induction in aging muscle and elucidate the key function and the underlying mechanism of JUNB in regulating SASP activation in senescent cells. Altogether, our findings demonstrate the prevalence and function of cellular senescence in skeletal muscle and identify a novel pharmacological intervention for sarcopenia.

Funding Source: National Key Research and Development Program of China General Research Fund, Theme-based Research Scheme Collaborative Research Fund Health and Medical Research Fund National Natural Science Foundation of China Health@InnoHK program.

T1047

MUTANT HUNTINGTIN DISRUPTS GLOBAL DNA METHYLATION IN HUMAN IPSC-DERIVED CEREBRAL ORGANOIDS

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Huntington's disease (HD) is a genetic neurodegenerative disorder, caused by a CAG repeat expansion (>39) in the poly-glutamine (polyQ) tract of the Huntingtin (HTT) gene. Despite its relatively late onset, HD has been shown to cause abnormal neurodevelopment during embryogenesis and early childhood. Moreover, DNA methylation was found to play an important role as an epigenetic mechanism in HD among adults. Therefore, we sought to explore early neurodevelopmental alterations in DNA methylation using cerebral organoids from juvenile forms (72Q and 180Q) of isogenic HD pluripotent cells. Additionally, to differentiate between gain- and loss-of-function phenotypes in HD, we generated HTT-KO organoids for comparison. HD, but not HTT-KO organoids, displayed widespread DNA hypomethylation, in a CAG-repeat length dependant manner. Bulk RNA-seq revealed neuronal differentiation-related pathways shared between the KO and HD systems, supporting a loss-of-function, as well as pathways uniquely altered in the 72Q-iPSCs organoids, with the latter being associated with methylation changes in the relevant promoters. We further found a highly significant enrichment for the DNMT3B motif among the differentially methylated positions. Using multiple methods, we demonstrate polyQ-length-dependent interactions between DNMT3B and HTT, suggesting the sequestering of DNMT3B by mutated HTT. Our findings reveal neurodevelopmental and epigenetic defects during early neurogenesis in HD models, and highlight DNA methylation as a central pathway.

T1049

NERVE GROWTH FACTOR-DERIVED PEPTIDES ACCELERATE OSTEOGENESIS OF HUMAN BONE MARROW MESENCHYMAL STEM CELLS AND PROMOTE BONE FORMATION

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As a skeletal neurotrophin, nerve growth factor (NGF) participates in the maintenance of skeletal pain, and NGF signal pathway involves in bone formation and fracture healing. Bone loss occurs as a result of traumas and injuries, and poor bone healing leads to functionally debilitating conditions of patients. Human bone marrow mesenchymal stem cells (BMSC) contribute to the homeostasis of the skeletal system under the regulation of different cytokine profiles. NGF can

promote BMSC survival during BMSC transplantation therapy. The NGF-derived peptides were investigated here because of the advantages of low immunogenicity, high bioactivity, and specific binding to tropomyosin receptor kinase A (TrkA, high-affinity receptor for NGF) in our earlier study. In this study, we used artificial intelligence-based approaches to generate NGF-derived peptides. Two peptide candidates were screened by molecular docking simulation and surface plasmon resonance (SPR) experiments. Human primary BMSCs were isolated from hip femoral heads obtained from patients who underwent total hip arthroplasty. To study the biological effects of the peptide candidates, hBMSCs were cultured in an osteogenic medium with NGF or NGF-derived peptides for 4 weeks. Alizarin red and von Kossa staining intensity revealed that NGF-derived peptides significantly enhanced the calcification of hBMSC compared to the control group. We then established an immortalized hBMSC-derived single-cell clone. RNA sequence analysis in this cell line demonstrated that inflammation- and angiogenesis-related signalings were induced by NGF-derived peptides. At last, the peptide candidates were delivered into the osseous cavities in rat models with bone defects to verify their function on bone formation in vivo, and fast bone healing was observed in the treatment groups at week 1 and week 4.

Funding Source: 1. National Key Research and Development Program, the Ministry of Science and Technology (MOST, China), (2019YFA0111900 to Yangzi Jiang) 2. Center for Neuromusculoskeletal Restorative Medicine (CNRM, Health@InnoHK to Yangzi Jiang).

T1051

NEURAL PLASTICITY CHANGES INDUCED BY SEROTONERGIC PSYCHEDELIC COMPOUNDS IN CORTICAL ORGANOIDS

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There is a pressing need to develop novel and effective treatments for depression and anxiety disorders, which impact a significant portion of the global population. Neurotherapeutic psychedelic compounds offer a promising solution due to their rapid and enduring effects on synaptic plasticity and behavior. While their clinical efficacy is under investigation, the precise mechanisms by which acute treatments lead to long-term structural and functional changes remain poorly understood. For instance, the balance between excitation and inhibition (E/I) plays a critical role in shaping cortical circuitry and is implicated in numerous neuropsychiatric and neurodevelopmental disorders. However, the spatio-temporal mechanisms by which excitatory and inhibitory activity regulate circuit refinement across developmental windows are not well characterized. Emerging evidence suggests that compounds such as 5-MeO-DMT can alter the expression of over 900 proteins, including those involved in cytoskeletal dynamics, neural network formation, and neuroimmune communication. To address these gaps, my research will investigate the molecular impact of serotonergic psychedelic compounds on the synaptic development of cortical organoids derived from human induced pluripotent stem cells (iPSCs). Specifically, I will examine the biochemical pathways that regulate E/I balance and synaptic development across different stages of organoid maturation. To achieve this, I will employ state-of-the-art techniques, including optogenetics combined with multi-electrode array (MEA) recordings, to monitor immediate and long-term molecular and physiological changes. Additionally, transcriptomic analysis and immunofluorescence imaging will be used to evaluate neurite growth and synaptic connectivity. This research aims to identify critical periods during which neural circuits are most susceptible to

disruptions in E/I balance and determine how these vulnerabilities differ across neurodevelopmental stages, such as axon guidance and synaptic formation.

Funding Source: The Human Frontier Science Program (HFSP), The Brain and Behavior Research Foundation (BBRF), The California Institute for Regenerative Medicine (CIRM).

T1053

NEUROMUSCULAR ORGANOIDS AS A HUMAN IN VITRO MODEL FOR SARCOPENIA

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Sarcopenia, the progressive loss of skeletal muscle mass and function with aging, affects millions worldwide and poses a growing burden on aging societies. Despite its prevalence, effective treatments remain elusive. Notably, the failure of clinical trials targeting myostatin highlights the limitation of animal models for drug development, underscoring the urgent need for human-based models. Neuromuscular organoids (NMOs) have emerged as promising human in vitro platform that recapitulate the motor unit, comprising both neural and muscle compartments. Here, we present an optimized NMO model with enhanced motor neuron differentiation and myofiber maturation, demonstrated by increased expression of markers including ChAT, OLIG2, MYH2, and MYH7. Our protocol also resulted in larger muscle tissue size, increased number of neuromuscular junctions, and critically, synchronous contractions of skeletal muscle tissue, distinct from the sporadic and localized twitching commonly observed in previous models. To investigate sarcopenia, we successfully induced age-associated phenotypes by long-term culture of the optimized NMOs. At day 200, NMOs exhibited hallmark features of sarcopenia, such as centralized nuclei in muscle fibers, reduced neurite density within the muscle compartment, and degradation of laminin surrounding muscle fibers. Gene expression analysis revealed a significant upregulation of inflammatory cytokines and a downregulation of oxidative phosphorylation (OXPHOS) genes, indicative of mitochondrial dysfunction. Additionally, increased DNA damage, elevated expression of senescence marker P16, and senescence-associated β-galactosidase activity were observed. These findings establish our optimized NMO system as a robust human model for sarcopenia, offering a valuable platform for studying disease mechanisms and identifying potential therapeutic targets.

T1055

NOVEL PATHOGENESIS OF DIAMOND-BLACKFAN ANEMIA EXPLORED THROUGH REGULATION OF PROTEOSTASIS

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Diamond-Blackfan anemia (DBA) is a congenital bone marrow failure syndrome characterized by a significant decrease in red blood cells and physical abnormalities. DBA results from heterozygous mutations in ribosomal protein (RP) genes, with over 20 causative genetic mutations. However, approximately 40% of the patients do not exhibit these mutations, suggesting the presence of other causative genes or mechanisms. Our previous report confirmed that DBA causative genes are regulated post-translationally in human induced pluripotent stem cells (hiPSCs). We generated and compared hiPSCs derived from DBA patients. RPL5/11 DBA-hiPSCs showed defective differentiation potential in the early mesoderm. Furthermore, although the protein levels of RPs in DBA-hiPSCs were comparable to those in healthy hiPSCs, they decreased during mesodermal differentiation. By knockdown of RPL5 during the differentiation, differentiation defects observed in patient-derived clones were recapitulated. Global protein expression analysis identified ECM-related genes were commonly downregulated during mesodermal differentiation in both patient and knockdown clone-derived cells. These findings suggest that DBA genes undergo different quantity control between hiPSCs and differentiated cells, and the dysregulation of protein levels may occur during developmental processes. This study would propose a novel mechanism for the onset of DBA, and lead to a better understanding of common principles underlying mutation-independent DBA pathogenesis.

Funding Source: AMED-PRIME (21gm6410003h0001 (M.I.)).

T1057

ORGANOID-BASED NEUTRALIZATION ASSAYS REVEALED A DISTINCTIVE PROFILE OF SARS-COV-2 ANTIBODIES AND RECAPITULATED THE REAL-WORLD EFFICACY

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The efficacy of VIR-7831, a class 3 anti-SARS-CoV-2 monoclonal antibody (mAb), was verified in multiple clinical trials; yet cell line-based neutralization assays underestimated its potency, leading to the erroneous withdrawal from clinical use. Thus, there is an unmet demand for a biologically relevant neutralization assay to provide a correlate of in vivo protection. We sought to develop organoid-based neutralization assays to measure the potency of mAbs against coronaviruses, based on the high biological relevance of nasal organoids and the robust organoid culture system. Due to the biologically relevant low ACE2 expression, nasal organoid-based neutralization assays adequately recapitulated the real-world effectiveness of VIR-7831. Most class 3 SARS-CoV-2 mAbs, especially those not blocking RBD-ACE2 binding, exhibited a higher potency in the organoids than in the cell lines. Moreover, due to the high TMPRSS2 expression in nasal organoids, reminiscent of native human respiratory epithelial cells, organoid-based neutralization assays reproduced the in vivo protection of S2 mAbs, which was not manifested in cell lines. Collectively, the robust organoid culture system and biologically relevant expression profile of ACE2 and TMPRSS2 grant nasal organoids to present a unique and optimal correlate of in vivo protection of neutralizing mAbs. The organoid-based neutralization assays, superior to the conventional cell-line-based neutralization assays, can recapitulate and predict the real-world

efficacy of mAbs.

T1059

OXYTOCIN PROMOTES ELONGATION OF HAIR PEG-LIKE SPROUTING IN HAIR FOLLICLE ORGANOIDS

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Oxytocin (OXT) is a neuropeptide hormone termed “love hormone” produced and released during childbirth and lactation. It is also produced in response to skin stimulation (e.g., during hugging and massaging), music therapy, and interaction with pet dogs. The effects of OXT on various organs; however, its specific effects on hair follicles remain unclear. Therefore, in this study, we aimed to investigate the hair growth-promoting effects of OXT. Dermal papilla cells control hair growth by providing growth/regression signals to the hair follicles. Gene expression analysis revealed that OXT significantly upregulated expression levels of hair growth-promoting factors, including vascular endothelial growth factor A, in dermal papilla cells. Hair growth-promoting effects of OXT were further tested using an in vitro drug screening model. Recently, we developed a hair follicle organoid (HFO) culture system where hair follicles regenerate efficiently in vitro (approximately 100%; T. Kageyama et al. Science Advances, 8, 42, eadd4603, 2022). Hair growth-promoting drug minoxidil increased the hair peg-like sprout length in HFOs (T. Kageyama et al. Scientific Reports, 13, 4847, 2023). Here, OXT also promoted the hair peg-like sprout growth in HFOs (T. Kageyama et al. Scientific Reports, 13, 15587, 2023). As OXT is an oligopeptide and a relatively large molecule (Mw = 1007), smaller molecular weight alternatives are needed to activate the OXT pathway via topical administration to the skin. Therefore, we examined the effects of OXT receptor activators and agonists as OXT alternatives on hair growth. Cinnamic acid (OXT receptor activator) and LIT001 (OXT receptor agonist) promoted hair sprout elongation in HFOs (T. Kageyama et al. Scientific Reports, 14, 4709, 2024 and T. Kageyama et al. Scientific Reports, 14, 23935, 2024). Collectively, our promising results can be beneficial for the development of novel hair growth products focused on OXT signaling.

T1061

PATIENT-DERIVED CELLULAR PLATFORM IN MODELLING INFLAMMATORY PROCESS OF LUPUS NEPHRITIS

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Lupus nephritis (LN) is a prevalent and life-threatening manifestation, affecting 30-60% systemic lupus erythematosus (SLE) patients worldwide. Pathogenesis of LN remains elusive and patients often present poor treatment responses, urging for a need in understanding LN progression for therapeutics development. Previous studies in SLE mouse models have indicated the pathogenic roles of macrophages in LN, yet the translational potential of these findings was limited as mice fail to recapitulate genetic heterogeneity in human. To overcome this hurdle, we have generated an LN model from patient-derived expanded potential pluripotent stem cells (EPSCs) for mechanistic study. Specifically, peripheral blood mononuclear cells (PBMCs) from healthy individuals and SLE patients were reprogrammed into EPSCs, then differentiated into macrophages and kidney organoids for phenotypic and functional characterization. Irrespective of disease status, EPSC-derived macrophages were phenotypically and functionally comparable to PBMC-derived macrophages in terms of the ability to phagocytose apoptotic cells and releasing inflammatory cytokines upon bacterial ligand stimulation. In addition, all EPSCs followed a concordant differentiation trajectory into kidney organoids, suggesting that there is unlikely any kidney developmental defect in SLE patients. To mimic SLE-like microenvironment, macrophages or kidney organoids were exposed to various pathogenic factors for characterization. Transcriptomic analysis revealed unique enrichment in genes related to protein translation and cell activation in patient EPSC-macrophages upon exposure to SLE patient serum. On the other hand, enrichment of genes related to kidney diseases and fibrosis were noticed in immune complex (IC) stimulated kidney organoids. Further work will be done to delineate the interplay between kidney cells and macrophages in the presence of SLE pathogenic factors. Ultimately, this platform will be useful for drug screening to identify therapeutics specific for managing kidney inflammation in LN patients.

Funding Source: This project is funded by Health@InnoHK under Innovation and Technology Commission, Hong Kong.

T1063

PD-L1 OVEREXPRESSING MESENCHYMAL STEM CELLS AND THEIR DERIVED EXTRACELLULAR VESICLES IN THE TREATMENT OF TYPE 1 DIABETES

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Type 1 diabetes (T1D) is an autoimmune disease characterized by the destruction of pancreatic β cells. Programmed death ligand-1 (PD-L1) is critical in maintaining peripheral tolerance and immunological homeostasis. This study investigates the protective effects of mesenchymal stem cells (MSCs) overexpressing PD-L1 and their derived extracellular vesicles (PEVs) in murine models of T1D. PD-L1-engineered MSCs (PD-L1-MSCs) were generated by infecting human bone marrow-derived MSCs with a lentiviral vector encoding the human PD-L1 gene. The immunosuppressive properties and impact on islet cell death of PD-L1 MSCs and PEVs were evaluated in vitro by coculturing them with peripheral blood mononuclear cells (PBMCs) and murine islets. In vivo, 8-week-old female NOD mice were given one infusion of MSCs (n=27), PD-L1-MSCs (n=18), MSC-EVs (n=20), PEVs (n=20), or PBS (control, n=20), respectively. Blood glucose levels were monitored weekly for 25 wks. In separated groups of mice, the pancreas, pancreatic lymph nodes, and spleen were collected 3 weeks post-treatment to assess immune cell infiltration, T cell profiling, and function via H&E staining and flow cytometry. Statistical differences were analyzed using one-way ANOVA with post-hoc correction. In vitro, PD-L1-MSCs and PEVs effectively suppressed T cell proliferation and increased T regulatory cells (Tregs, $p < 0.01$ vs. control), highlighting their immunomodulatory potential. Islet viability was significantly improved

when cocultured with PD-L1-MSCs (viability: 75.3±5.3%) or PEVs (91.3±1.9%), vs. controls (59.9±6.0%, $p< 0.05$ vs control in each group). In vivo, PD-L1-MSCs or PEVs significantly reduced blood glucose and delayed T1D onset in NOD mice (CTR vs. PD-L1-MSC, $p< 0.05$; CTR vs. PEV, $p< 0.01$, Logrank test). H&E staining showed a dramatic decrease in immune cell infiltration in the islets. In PLN, PD-L1 MSCs or PEVs decreased CD8+ T cell number ($p< 0.03$ vs control), and increased CD8+ T cell exhaustion and Tregs. These findings suggest that PD-L1-overepressing MSCs enhance their immunosuppressive and protective effects on pancreatic islets, partly by enhancing Tregs and CD8+ T cell exhaustion. This underscores the therapeutic potential of PD-L1-MSCs and PEVs in modulating immune responses in T1D and reveals the underlying cellular mechanisms.

Funding Source: This grant is funded by the NIDDK.

T1065

PHARMACEUTICAL RESEARCH OF RESPIRATORY SYNCYTIAL VIRUS USING HUMAN IPS-DERIVED RESPIRATORY ORGANOIDS

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Respiratory syncytial virus (RSV) infection is a seasonal respiratory infection that mainly infects younger children, leading to lower respiratory tract disease with dyspnea in severe cases. Despite the fact there are many patients, our understanding of RSV infection pathophysiology remains limited, and thus the development of antiviral drugs is still insufficient. To resolve this issue, the development of RSV infection models is necessary. Although cell lines such as HEp-2 cells, which are highly susceptible to RSV, have been widely used in RSV research, it is difficult to precisely evaluate host response of human respiratory tract. In this study, we investigated whether our human iPS cell-derived respiratory organoids, which contain not only respiratory epithelial cells but also immune cells, fibroblasts, and vascular endothelial cells, have the potential to recapitulate

host responses in the human respiratory tract during RSV infection and to be applied in drug discovery. Firstly, respiratory organoids were infected with RSV and analyzed at 4 days post infection. The high expression level of viral genome and viral protein was observed in the infected respiratory organoids. Histological analysis showed that RSV infection induced respiratory epithelial layer destruction and collagen accumulation. In addition, the concentration of pro-inflammatory cytokines in culture supernatants of respiratory organoids was increased by RSV infection. These results suggest that respiratory organoids can be used to analyze host responses in RSV infection, including inflammatory responses. Next, the efficacy of monoclonal antibodies and ribavirin against RSV was investigated using respiratory organoids. Nirsevimab, palivizumab, suptavumab, and clesrovimab significantly inhibited RSV infection at low concentrations (< 10 ng/mL), whereas low-concentration ribavirin (< 10 μM) did not. These results suggested that respiratory organoids could not only mimic pathological conditions of RSV infection but also evaluate the antiviral effect of monoclonal antibodies and compounds against RSV.

Funding Source: This research was supported by the iPS Cell Research Fund, the Mitsubishi Foundation, Japan Society for the Promotion of Science (JSPS), and Japan Agency for Medical Research and Development (AMED).

T1067

PNPLA3 POLYMORPHISM AS A POSSIBLE DETERMINANT OF BILE ACIDS DYSREGULATION AND MASLD PROGRESSION

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Metabolic dysfunction-associated steatotic liver disease (MASLD) includes a spectrum of conditions that can progress in severity, potentially leading to fibrosis. Genetic factors, such as the PNPLA3 gene polymorphism (SNP), have been implicated in this progression. For MASLD progression studies, traditional biochemical tests often yield inconclusive results, while liver biopsy remains the gold standard, despite being invasive. This study aims to address these limitations by integrating omics-based techniques and induced pluripotent stem cells (iPSC) as an in vitro model to elucidate cellular, molecular and genetic mechanisms underlying MASLD severity. Patients were recruited from the Clementino Fraga Filho University Hospital (UFRJ) (CAAE: 16079319.0.0000.5257). Laboratory analyses included biochemical and coagulation profiles, while fibrosis severity was assessed via liver biopsy. Plasma samples were analyzed using bileomic to characterize bile acid profiles. PNPLA3 polymorphism (SNP) was determined through Sanger sequencing. The cohort consisted of 8 patients (2 male, 6 female) aged 47–77 years. Histopathological analysis revealed that four patients had absent or mild fibrosis, while the remaining exhibited advanced fibrosis. No significant differences in laboratory analyses were

observed between these groups. Genetic analysis identified four patients with PNPLA3 SNP (three heterozygous, one homozygous). Advanced fibrosis was observed in one heterozygous and one homozygous patient, suggesting that PNPLA3 SNP may influence disease progression in the future. Bileomic analysis revealed a distinct toward bile acid profiles based on PNPLA3 SNP status, with SNP carriers exhibiting lower levels of the primary bile acid CDCA and its taurine conjugates, such as taurine conjugated CA. We highlight the potential of PNPLA3 SNP as a determinant of bile acid dysregulation and disease severity. Currently, iPSC from SNP and non-SNP patients are being generated. Future work includes developing iPSC-derived hepatocyte model to investigate the functional impact of genetic and clinical background. This research advances precision medicine by providing insights into MASLD pathophysiology and highlighting the utility of omics and iPSC for patient-specific approaches to diagnosis and treatment.

Funding Source: CNPq, FAPERJ, CAPES, INCT-REGENERA, MINISTÉRIO DA SAÚDE.

T1069

PRECISION-CUT LUNG SLICE CULTURE FOR VALIDATING PRO-FIBROTIC AND PRO-RESOLVING PROTEINS IN HUMAN LUNG FIBROSIS

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Recent revolutionary advancements in transgenic mice and sequencing technologies allow us to extend our knowledge of lung diseases, including pulmonary fibrosis. However, considering the fundamental differences between human and mouse lungs, the validation of findings in humans is essential. Precision-cut lung slices (PCLS) culture, an ex vivo tissue culture technique, offers a significant advantage in preserving the spatial composition of the extracellular matrix, making it more useful for tracking epithelial-mesenchymal interactions in fibrosis. We recently developed and applied single-cell RNA sequencing, spatial transcriptomics, and a machine-learning-based fiber quantification algorithm to a mouse model of pulmonary fibrosis, identifying fibroblast subtypes with pro-fibrotic and pro-reparative roles. We also found that Pi16 and Serpine2, expressed in these fibroblasts, are important candidates. Here, we performed the validation of these proteins in a human PCLS model in an unbiased manner. Both conventional and machine-learning-based analyses showed that the roles of these proteins in human lungs are similar to those in mouse lungs. In the era of hypothesis-driven science, these approaches are becoming increasingly important.

T1071

PRIMARY EPITHELIAL LUNG ORGANOIDS DERIVED FROM THE HUMAN AMNIOTIC FLUID AS PERSONALISED MODEL FOR CONGENITAL DIAPHRAGMATIC HERNIA

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Congenital Diaphragmatic Hernia (CDH) is a congenital malformation characterized by an incomplete diaphragm closure, with subsequent herniation of abdominal organs into the chest cavity. This causes a mechanical compression exerted on the fetal airways, that results in an underdevelopment of fetal lungs and pulmonary vasculature with consequent hypertension. Fetuses with severe CDH undergo FETO, an in utero procedure where an inflatable balloon is inserted into the fetal trachea, causing the retention of lung secretions in order to ameliorate lung hypoplasia. Despite advances in prenatal diagnosis and management of CDH cases, predicting disease severity, response to treatment and patients' clinical outcomes remains a challenge, therefore limiting parental counselling. More reliable predictive parameters are therefore needed to obtain personalized modeling and improve CDH outcomes. In this context, primary organoids are able to recapitulate in vitro some pathophysiological features of patients' tissues, allowing for disease modelling and drug screening. Relevantly, our group has recently identified in the amniotic fluid (AF) epithelial precursors able to originate AF-derived organoids (AFO) resembling different fetal organs (kidney, lung and intestine). AFO have the potential to model fetal diseased organs without the ethical and legal limitations of accessing fetal tissues. In this project we derived fetal

lung organoids from 41 CDH AF, collected during scheduled amniocentesis and FETO procedures. Our data suggests that, when derived from CDH samples, lung AFO reflect in vitro some features of the disease. In detail scRNA sequencing analysis reveals altered cellular composition in CDH lung AFO, when compared to age-matched controls. Likewise, upon differentiation, we observed alterations in surfactant production and ciliation. These differences align to what has been observed in CDH animal models and human tissue specimens. In conclusion, the use of lung AFO would allow disease modelling in a patient-specific manner and help underpin the molecular mechanism underlying lung hypoplasia in CDH patients.

Funding Source: This work was supported by the Academy of Medical Science, UCL Therapeutic Acceleration Support, GOSH-CC, Kidney Research UK, CDH-UK, NIHR GOSH BRC and Rosetrees Trust. GD is supported by an EMBO Scientific Exchange Grant.

T1073

PROGERIN-DRIVEN CELLULAR AGING AND NEURODEGENERATION IN DOPAMINERGIC NEURONS: AN IPSC-BASED MODEL FOR PARKINSON'S DISEASE RESEARCH

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Parkinson's disease is a neurodegenerative disorder characterized by a progressive loss of midbrain dopaminergic neurons (mDAn). Aging, among others, is considered as a potential degenerative factor. It has been shown that progerin, a truncated form of Lamin A, accelerates cellular aging and is implicated in pathological conditions like Hutchinson-Gilford Progeria Syndrome. This study investigates the impact of progerin overexpression on mDAn derived from human-induced pluripotent stem cells (hiPSCs), focusing on aging hallmarks and neurodegenerative phenotypes. An inducible Tet-ON GFP-T2A-progerin construct was used to overexpress progerin in hiPSC-derived mDAn alongside an isogenic control. Neurons were treated with doxycycline (DOX) during the maturation phase. Immunofluorescence (IF) was employed to assess markers of cellular aging (H3K9me3, HP1γ, γH2AX, p53BP1) and neurodegeneration (TH, α-synuclein). Quantifications were performed using fluorescence microscopy. The proportion of TH+ and α-synuclein+ cells was analyzed alongside DNA damage foci and chromatin remodeling markers. Progerin overexpression induced significant aging hallmarks at day 30 of differentiation, with an increased levels of H3K9me3 and HP1γ ($p< 0.05$) and elevated DNA damage foci as indicated by γH2AX and p53BP1 ($p< 0.01$). Neurodegenerative phenotypes were evident, with a reduction in TH+ neurons ($p< 0.001$) and an increase in α-synuclein+ cells ($p< 0.0001$). The proportion of α-synuclein+ cells within the TH+ population also increased significantly ($p< 0.0001$). Notably, the GFP+ DOX - induced mDAn (TH+) revealed to be the ones with increased levels of α-synuclein, thus correlating progerin overexpression with neurodegeneration. These results highlight the dual impact of progerin on aging and neurodegeneration. This model provides a robust in vitro platform to study the interplay between aging and neurodegeneration, offering valuable insights into the mechanisms underlying Parkinson's disease and related disorders. Furthermore, it serves as a promising tool for identifying and testing potential therapeutic interventions targeting age-related neuronal dysfunction.

T1075

RAPID ANTIVIRAL SCREENING IN TROPHOBLAST CELLS AGAINST EMERGING VIRUSES

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Trophoblast stem cell and their progenitor cells are critical for pregnancy establishment and maintenance, yet their vulnerability to viral infections has been inadequately explored. This study examines the interactions between the Influenza virus and human trophoblast cells, highlighting the cells' significant susceptibility to the virus. We demonstrate that trophoblasts facilitate Influenza virus entry and support extensive viral replication, suggesting their potential as an effective model for viral infection studies. By utilizing this model, we performed rapid drug screening, identifying compounds within 24 hours post-infection that are safe and effective against Influenza, COVID-19, and Monkeypox. These findings not only shed light on trophoblasts' interaction with viral pathogens but also introduce a promising avenue for accelerating the development of antiviral agents. Our research opens new opportunities for addressing the global health challenge posed by emerging diseases like Monkeypox, highlighting the importance of rapid screening platforms in the fight against viral infections.

Funding Source: Funding support from Health@InnoHK, Innovation and Technology Commission.

T1077

RARE DISEASE MODELLING USING HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTE MODELS IN 3D FN-SILK SCAFFOLDS

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The rare disease group of Muscular Dystrophy is incurable genetic diseases where the dystrophin gene has been compromised which can range from mild to severe forms. Muscular Dystrophy mainly manifests itself during early childhood and causes progressive muscle deterioration which leads to a high risk of death due to eventual respiratory or cardiac failure. Disease modelling and drug development currently rely on animal models which fail to accurately recapitulate the human disease mechanism. To address the need for a more accurate patient-derived cell model, a human induced pluripotent stem cell (hiPSC)-approach has been investigated with the goal of generating cardiomyocyte organoids with retained disease phenotype. To better represent in vivo environments, the novel FN-silk scaffold comprising a recombinant spider silk protein

functionalized with a cell adhesion motif from fibronectin was used to form 3D culturing systems. Cell-integration and cultivation compatibility of both healthy wild-type hiPSC and disease carrying isogenic hiPSC lines has been reproducibly confirmed in various 3D FN-silk formats such as fibers and free-floating networks, complemented with extracellular matrix proteins. hiPSCs integrated in FN-silk retained pluripotency and proliferative growth which have been confirmed with comparative live/dead viability assays and pluripotency marker staining using immunocytochemistry. Furthermore, hiPSC-derived cardiomyocyte progenitors in 3D FN-silk were generated with a cardiomyocyte differentiation kit. Cardiac markers and beating frequency were used for evaluation of differentiation parameters such as maturity. Although further optimization of cultivation and differentiation parameters is needed, this proof of concept study represents a significant first step for hiPSC-derived rare disease modelling while exploring alternative 3D hiPSC-culturing systems.

T1079

RNA THERAPEUTICS FOR EARLY-ONSET FAMILIAL ALZHEIMER'S DISEASE USING HUMAN STEM CELL MODELS

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Alzheimer's disease (AD) is a devastating neurodegenerative disorder, with early-onset familial forms strongly linked to APP gene dosage. Genomic duplication of the APP locus causes autosomal dominant early-onset AD, while individuals with Down syndrome (trisomy 21), who carry three copies of APP, consistently develop progressive AD with hallmark neuropathology. Despite the clear role of APP in disease pathogenesis, therapeutic strategies to normalize APP expression and mitigate its downstream effects remain limited. To address this gap, we tested antisense oligonucleotides (ASOs) targeting APP in human iPSC-derived cortical neurons from APP duplication and Trisomy 21 models. These ASOs effectively reduced APP protein levels, restoring endolysosomal and autophagy function—key processes disrupted in AD. Using ultrasensitive single-aggregate imaging, we demonstrated a significant reduction in intracellular and extracellular amyloid-β aggregates, a hallmark of AD pathology. These findings reveal the potential of APP-targeting ASOs as a novel therapeutic approach for AD caused by APP gene duplication, including monogenic and Down syndrome-associated AD. By leveraging human stem cell models, our work advances the field of RNA therapeutics, providing new insights into targeting genetic drivers of AD to prevent or slow disease progression.

T1081

SAFETY AND EFFICACY OF MESENCHYMAL STROMAL CELLS MITOCHONDRIA TRANSPLANTATION AS A CELL-FREE THERAPY FOR OSTEOARTHRITIS

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In osteoarthritis (OA), inflammatory responses from synovial fibroblasts and macrophages, along with mitochondrial dysfunction in chondrocytes, lead to oxidative stress and disrupt extracellular matrix homeostasis, accelerating cartilage degradation. Recent studies suggest that mesenchymal stromal cells (MSCs) can transfer functional mitochondria to damaged cells in response to stress, which may contribute to their therapeutic effects. Based on this, we hypothesize that direct mitochondrial transplantation could serve as a novel, cell-free therapy for OA, restoring both cellular and mitochondrial homeostasis to mitigate cartilage degeneration. In this study, mitochondria were isolated from Umbilical Cord MSCs (Mito-MSC) and characterized for their morphology, functionality, and ability to be internalized by articular cells. Transcriptional changes following mitochondrial uptake were assessed in chondrocytes via Affymetrix analysis. Additionally, the therapeutic efficacy, biodistribution, and immunogenicity of Mito-MSC were evaluated in vivo using a collagenase-induced OA (CIOA) mouse model via intra-articular injection. Our results show that Mito-MSC maintain functional integrity and are efficiently internalized by chondrocytes, synovial macrophages, and fibroblasts. Transcriptomic analysis revealed the upregulation of stress-related genes, including those involved in DNA repair and antiviral responses. Finally, Mito-MSC transplantation led to significant reductions in joint mineralization and improvements in OA-related histological signs, with the lower dose providing the most effective therapeutic response. Moreover, Mito-MSC persisted in the knee joint for up to 24 hours post-injection, without inducing an inflammatory immune response in CIOA mice. Collectively, our results reveal that mitochondria derived from MSC are transferred to key articular cells and retained in the joint without generating an inflammatory response. This supports the potential of MSC-derived mitochondria as a novel, cell-free therapy for OA, capable of restoring mitochondrial function and reducing cartilage degradation. This approach could offer an effective strategy for mitigating OA progression, with broader implications for treating diseases with mitochondrial dysfunction.

Funding Source: ANID-Fondecyt Regular PLC N°1211353; Fondecyt Iniciación AMV N°11220549; Fondecyt Regular N° 1201420 FF; IMPACT, #FB210024, FONDEF ID21I10194 PLC, PRI-MitoMir project and the project METAB-OA (ANR-20-CE18-0014).

T1083

SINGLE-CELL RNA SEQUENCING OF FELINE MAMMARY TUMOR ORGANOIDS REVEALS DIFFERENT GENE PROFILES AND PATHWAYS BETWEEN BASAL AND LUMINAL TYPES

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Feline mammary tumor (FMT) is known as a highly malignant and intractable disease. Due to the scarcity of appropriate experimental models, the availability of efficient treatment options is few. Previously, we had established the FMT organoid culture method derived from patient cats and

evaluated its usefulness as a feline patient-derived culture model. In this study, FMT organoid was mainly classified with the “Basal” or “Luminal” type. We performed single-cell RNA sequencing (scRNA seq.) using two different FMT organoid lines to evaluate the genetic components in various organoid-derived cell phenotypes. The gene expression identified in the clusters was compared between basal and luminal organoids. They were classified into 13 clusters in basal or luminal-type organoids. In the basal cell cluster, expression of KRT5 and KRT17 (basal cell markers) and FABP5 (fatty acid metabolism marker) were increased, while in the luminal-type organoid, the expression of KRT19 and KRT8 (luminal cell markers) were upregulated. In KEGG analysis, the cell adhesion-related signals were activated in basal compared with luminal-type organoids. We for the first time demonstrated that the markers related to morphology in each FMT organoid were expressed, and there are differences in the pathway between basal and luminal organoids.

T1085

SINGLE-CELL TRANSCRIPTOMIC ROADMAP TO HUMAN INDUCED HEPATOCYTE-LIKE CELLS REVEALS A LIVER DEVELOPMENTAL TRAJECTORY DURING LINEAGE REPROGRAMMING

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Hepatocytes are crucial for drug screening, disease modeling, and clinical trans-plantation, yet generating functional hepatocytes in vitro is challenging due to the difficulty of establishing their authentic gene regulatory networks (GRNs). We have previously developed a two-step lineage reprogramming strategy to generate func-tionally competent human induced hepatocytes (hiHeps), providing an effective model for studying the establishment of hepatocyte-specific GRNs. In this study, we utilized high-throughput single-cell RNA sequencing (scRNA-seq) to explore the cell-fate transition and the establishment of hepatocyte-specific GRNs involved in the two-step reprogramming process. Our findings revealed that the reprogramming process mimics the natural trajectory of liver development, exhibiting similar tran-scriptional waves of developmental genes. CD24 and DLK1 were identified as sur-face markers enriching two distinct hepatic progenitor populations respectively. Li-pid metabolism emerged as a key enhancer of hiHeps maturation. Furthermore, tran-scription factors HNF4A and HHEX were identified as pivotal gatekeepers directing cell fate decisions between hepatocytes and intestinal cells. Collectively, this study provides valuable insights into the establishment of hepatocyte-specific GRNs during hiHeps induction at single-cell resolution, facilitating more efficient production of functional hepatocytes for therapeutic applications.

Funding Source: This work was supported by the National Natural Science Foundation of China. (32300675, 32400598), Sichuan Science and Technology Program (2022YFS0615, 2025ZNSFSC1019), Luzhou Science and Technology Program of China (2023SYF136).

T1087

SMA PATIENT-SPECIFIC IPSC-DERIVED MOTOR NEURONS EXERT ELECTROPHYSIOLOGICAL, MOLECULAR, AND METABOLIC ABNORMALITIES

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Spinal muscular atrophy (SMA) is a genetic disorder characterized by motor neuron degeneration, leading to muscle weakness and atrophy due to SMN protein deficiency in infants. The discovery of the genetic cause of SMA has led to the development of several treatment options; however, thus far there is no complete cure for SMA. Since the in vitro evaluation of human spinal motor neurons is not possible without the use of postmortem tissue, induced pluripotent stem cells (iPSCs) have emerged as a promising avenue to understand the pathophysiology of SMA and represent a radical tool in the quest for effective SMA treatments. In this study, we aimed to generate iPSCs from PBMCs of SMA patients and healthy controls using the Sendai viral vector system, differentiate these iPSCs into motor neurons, and establish a disease model to determine the effect and participation of ion channels and metabolic processes. During differentiation, electrophysiological activities were evaluated via patch clamp, and motor neuron and ion channel-specific gene expressions were examined via qPCR in SMA and control motor neurons. We have found that SMA motor neurons were less developed and smaller in size and axonal length, and ChAT, HB9, and synaptophysin gene expressions were significantly reduced compared to control motor neurons, indicating impaired maturation and synaptic connectivity. Our patch clamp and qPCR data showed a lack of voltage-gated sodium current and reduced voltage-gated potassium current in SMA patients. Voltage-gated calcium, calcium-activated potassium, and hyperpolarization-activated cyclic nucleotide-gated channels also showed reduced activity in SMA. Additionally, in SMA iPSCs, ROS levels were measured to be three times higher than in control iPSCs, indicating mitochondrial dysfunction and overall metabolic complications. Similarly, SMA motor neurons displayed elevated hydrogen peroxide levels. In summary, we were able to generate the first-ever iPSCs from SMA patients in Turkey and to report the molecular, biochemical, electrophysiological, and metabolic differences between SMA and control motor neurons. iPSCs' ability to model SMA and facilitate the development of targeted therapies underscores their significance in advancing our understanding and management of this debilitating condition.

Funding Source: This work was funded by the Scientific and Technological Research Institution of Turkey (TUBITAK) 1004 project.

T1089

SMALL EXTRACELLULAR VESICLES FROM METABOLICALLY REPROGRAMMED MESENCHYMAL STEM/STROMAL CELL AS A POTENTIAL IMMUNOSUPPRESSIVE MECHANISM FOR INFLAMMATORY AND AUTOIMMUNE DISEASES

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Inflammatory and autoimmune diseases significantly impact patients' health and quality of life, and their treatment remains a clinical challenge. Mesenchymal stem cells (MSCs), with diverse biological functions, represent a promising therapeutic approach due to their immunoregulatory effects. The immunomodulatory activity of MSCs is primarily mediated by paracrine factors. However, small extracellular vesicles (sEVs) have been identified as key players in mediating MSCs' biological functions. Our studies on human umbilical cord MSCs (UC-MSCs) demonstrated that metabolic reprogramming to glycolysis significantly enhances their immunoregulatory capacity, particularly in modulating proinflammatory T cells (Th1, Th17) and inducing regulatory T cells (Tregs). In this study, we evaluated the immunosuppressive properties of sEVs derived from glycolytic and non-glycolytic UC-MSCs both in vitro and in vivo. sEVs-MSCglyco and sEVs-MSCnon-glyco were isolated and characterized using NTA and FACS. Their immunosuppressive activity was assessed first on PBMCs. Additionally, sEV internalization by T cells was evaluated using RTqPCR. We also analyzed the effect of sEVs on memory T cells by FACS, and the production of IL-10, measured by ELISA. Finally, we investigated the immunosuppressive activity of sEVs in vivo using mouse models of delayed-type hypersensitivity (DTH) and collagen-induced arthritis (CIA). sEVs-MSCglyco significantly reduced T cell proliferation, decreased Th1 cell populations, and induced Treg cells in vitro. Additionally, sEVs were internalized by memory T cells, leading to a reduction in Th1 and Th17 cell populations without affecting Tregs, alongside an increase in IL-10 production. In vivo, sEVs-MSCglyco significantly reduced the inflammatory response in the DTH model by decreasing proinflammatory T cell populations. In the CIA model, infusion of sEVs-MSCglyco reduced disease incidence and progression compared to the control group, correlating with a significant decrease in Th1 and Th17 cells in lymph nodes and peripheral blood. Glycolytic MSC-derived sEVs effectively modulate activated T cells, enhancing immunoregulatory capabilities both in vitro and in vivo, underscoring their potential as a therapeutic tool for treating inflammatory and autoimmune diseases.

Funding Source: Supported by the ANID from Chile through the grants FONDECYT Regular N°1211353; FONDEF ID: 21110194; and IMPACT FB 210024; Fondecyt Inicio: 11220549; Beca

Doctorado Nacional Folio:21210735 and beca FAI Universidad de los Andes.

T1091

STROKE-HEART SYNDROME ON-A-CHIP

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Cardiac autonomic nervous system regulates the heart function through innervation. In Stroke-Heart Syndrome (SHS) an acute stroke leads to elevated serum levels of cardiac markers and arrhythmias, impacting heart function, leading to cardiovascular complications that are the second leading cause of post-stroke mortality. Completely human cell-based in vitro models studying functional neuron-cardiomyocyte connections with central nervous system (CNS), peripheral nervous system (PNS) and cardiac tissue are missing from the field. Our aim is to model SHS in vitro by combining three cell types; CNS type neurons (CNs), PNS type neurons (PNs) and cardiomyocytes (CMs) all derived from human induced pluripotent stem cells (hiPSC), in compartmentalized microfluidic devices, so called 3D3C chips. The structure of 3D3C chip enables the culturing of each cell type separately while allowing axonal growth through microtunnels to the adjacent cardiac compartment. Integrated in-house produced microelectrode arrays (MEA) enable the electrophysiological functionality measurements. By modifying the oxygen conditions in different compartments of the chip, such as subjecting CNs to oxygen deprivation, local stroke-like simulations can be generated for studying aspects of SHS on-a-chip. Here, hiPSC-derived CNs, PNs and CMs were successfully cocultured in the 3D3C chip up to three weeks. The physical axonal interactions were investigated with microscopy and immunocytochemistry and the functionality of the cells with MEAs. The axonal elongations between CNs to PNs and PNs to CMs were detectable, indicating successful innervation. All cell types developed cell-specific electrophysiological functionality. Oxygen deprivation was induced chemically solely to CN compartment and cell response measured morphologically, metabolically and functionally. Stroke-like conditions altered the function of all cell types, mimicking SHS on-a-chip. As a proof of concept, the compartmentalized microfluidic chip with integrated MEA allowed formation of physiologically relevant and functional connections with neurons and cardiomyocytes. This advanced, completely human cell-based cardiac innervation on-a-chip presents a powerful platform not only for the study of SHS but any disease where brain-heart axis is essential.

T1093

TARGETING STAT-1 GOF IN INBORN ERRORS OF IMMUNITY: A PATIENT-DERIVED EPSC PLATFORM FOR DRUG DISCOVERY

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Inborn Errors of Immunity (IEI), with increased susceptibility to infections, autoimmune diseases, and malignancies but challenging to model and manage, is starving for deeper molecular underpinnings and novel therapeutic approaches. Signal transducer and activator of transcription-1 (STAT-1) gain of function (GoF) was previously identified as a crucial pathogenic feature in IEI. Based on our patient-derived expanded potential stem cells (EPSC) platform modeling to STAT-1 GoF, we identified some potential candidates, which not only inhibit STAT-1 function independently but also synergize with existing clinical treatments. Our findings suggest a promising avenue for clinical applications, offering new insights for personalized therapeutic strategies in IEI management

Funding Source: Funding support from Health@InnoHK, Innovation and Technology Commission.

T1095

THE ROLE OF CRISP3 IN REGULATING ENDOMETRIAL RECEPTIVITY IN REPEATED IMPLANTATION FAILURE

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Repeated implantation failure (RIF) is a clinical scenario characterized by failure to achieve a positive pregnancy test after the transfer of a sufficient number of viable euploid embryos. Some RIF patients had a defective endometrial receptivity during the implantation window as compared to those with normal fertility. Identifying key factors influencing receptivity is essential for understanding the etiology of RIF and increasing the clinical pregnancy rates. The aim of this study was to identify key factors affecting endometrial receptivity with the use of human expanded potential stem cell derived trophoblastic spheroids as embryo surrogate. Analysis on published datasets of single-cell RNA sequencing of human endometrial tissues revealed that cysteine rich secretory protein 3 (CRISP3) was one of the upregulated genes in luminal epithelial cells during the secretory phase as compared to the proliferative phase. CRISP3 was also found to be one of the down-regulated genes in the luminal epithelial cells of patients with RIF at 7 days after luteinizing hormone surge (LH+7). Its expression was significantly higher in receptive endometrial epithelial cell (EEC) line (RL95-2) when compared to non-receptive EEC lines (AN3CA and HEC-1B). The expression levels of CRISP3 in primary EEC were further compared between endometrial

tissues collected from receptive phase on LH+7 day (n=13) and pre-receptive phase on LH+2 day (n=6). It was found that significantly higher levels of CRISP3 was detected in EEC on LH+7 day. The functional role of endometrial CRISP3 was studied by the knockdown approach, followed by the attachment assay of the trophoblastic spheroid. It was shown that CRISP3 knockdown by siRNA in RL95-2 cells significantly reduced the attachment rate of the trophoblastic spheroids. The results of this study will provide some possible clues for understanding the mechanisms underlying RIF and identifying key factors for improving endometrial receptivity.

T1097

THE VILLAGE IN A DISH MODEL: A PLATFORM FOR INVESTIGATING IPSC POPULATION DYNAMICS AND DNA DAMAGE RESPONSE

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DNA damage response (DDR) is a critical process involved in cancer, aging, and neurodegenerative diseases. Understanding how genetic variations influence individual responses to DNA damage is essential for developing personalized prevention and treatment strategies. Although, the use of induced pluripotent stem cells (iPSCs) for in vitro population genetics has gained popularity, traditional approaches, where iPSC lines are cultured in mono-culture, are time-consuming, expensive, and prone to technical variability. To address these challenges, the "village-in-a-dish" strategy has emerged. In this strategy, iPSCs from multiple donors representing the “normal” population, are cultured and stimulated together in a single dish. This platform increases throughput, reduces variability, and lowers costs, making it particularly useful for mimicking population studies in vitro, which require high donor numbers. Here, we established a pipeline to generate both small (5–10 iPSC lines) and large (up to 85 iPSC lines) villages, minimizing growth rate variability and enabling monitoring of cluster composition. For the first time, using cytoplasmic dyes, we demonstrated that iPSC clusters can form from both single and multiple donors. Additionally, we confirmed that the village platform does not affect the growth rates of the iPSC lines. We are now exploring how DDR, induced by X-ray and cisplatin, varies across different donors. After testing X-ray exposure on four iPSC lines (in mono-culture), we performed bulk RNA sequencing at multiple time points to capture variability and identify the optimal time point for scaling up towards the use of the village in a dish setup.

T1099

THERAPEUTIC POTENTIAL OF EXTRACELLULAR VESICLES IN UV-INDUCED PHOTOAGING

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Aging significantly contributes to the development of many degenerative diseases, with the most visible effects appearing on the skin, particularly in the form of photoaging caused by ultraviolet (UV) light exposure. This complex process results from the synergistic action of multiple mechanisms. The lack of effective therapeutic options poses a major challenge for current research. Our previous studies have demonstrated that human dental pulp stem cell-derived small extracellular vesicles (hDPSC-sEV) protect submandibular gland ductal cells from X-ray-induced long-term progression of senescence. Here, we aim to ascertain whether hDPSC-sEV exerts a therapeutic effect on photoaging. Senescence of human dermal fibroblasts (hHDFs) was induced in vitro using ultraviolet radiation B (UVB). The EV-treated group exhibited enhanced cell proliferation in a concentration-dependent manner. Furthermore, EVs markedly improved the migration and wound healing capabilities of the cells. Notably, EVs reduced the expression of the senescence marker SA-β-Gal and facilitated the repair of DNA damage. In conclusion, hDPSC-sEVs have the potential to enhance the physiological function of photoaged cells and delay the aging process, making them promising candidates for therapeutic applications.

T1101

THERAPEUTIC PROOF OF CONCEPT STUDY FOR THE USE OF ANTISENSE OLIGONUCLEOTIDES IN KLEEFSTRA SYNDROME

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Kleefstra syndrome is a rare, neurodevelopmental disorder characterized by intellectual disability, childhood hypotonia, craniofacial anomalies, and psychiatric regression. Genetically, Kleefstra syndrome arises from either a heterozygous deletion in the 9q34.3 chromosomal region (including the EHMT1 gene) or an intragenic pathogenic variant in EHMT1, in either case leading to haploinsufficiency of EHMT1. EHMT1 encodes a lysine methyltransferase protein, critically tasked with methylating histone 3 to regulate gene expression. The c.2712+1866G>A is a newly identified pathogenic variant in Kleefstra syndrome, an intronic splice variant in EHMT1 that activates a cryptic acceptor site. Using human-derived pluripotent stem cells as a model, we have demonstrated that this variant leads to the expression of an alternate transcript, which contains a retained portion of an intron, and reduced EHMT1 protein on western blot. Antisense oligonucleotides (ASOs) are a powerful emerging class of drugs that can target disease at the level of pre-mRNA. We have designed ASOs that sterically block the cryptic acceptor site, in order to restrict access to the spliceosome and encourage skipping of the cryptic exon. Given the heterozygous nature of this disorder, a steric-blocking ASO approach aims to promote functional EHMT1 protein from the pathogenic allele. This study utilizes human-derived stem cells as a model to demonstrate a novel use for ASOs in this rare disorder.

Funding Source: Canadian Institutes of Health Research (CIHR) Doctoral Scholarship.

T1103

TRANSCRIPTOMIC ANALYSIS AND BIOENGINEERED TISSUE MODEL OF DYSTROPHINOPATHY WITH PATIENT-DERIVED IPSC-CARDIOMYOCYTES

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Mutations in the DMD gene are responsible for a range of rare diseases known as dystrophinopathies, that negatively impact cardiac and skeletal muscle function. The lack of available cures has devastating consequences for afflicted patients and their families, and reflects a need for new human-specific preclinical models to study disease mechanisms and develop effective treatments. To help address this unmet need, this study established induced pluripotent stem cell (iPSC) lines from 8 patients with DMD-related dystrophinopathies, including Duchenne Muscular Dystrophy (DMD), Becker Muscular Dystrophy (BMD), and X-Linked Dilated Cardiomyopathy (XLDCM). DMD, the most common and severe form, is characterized by early onset progressive skeletal muscle weakness and subsequent cardiomyopathy. The less common BMD presents with milder skeletal muscle weakness but can have severe cardiomyopathy. XLDCM, the rarest form, is distinguished by early onset severe cardiomyopathy without initial skeletal muscle weakness. IPSCs from 1 DMD, 2 BMD, and 1 XLDCM patients were differentiated into cardiomyocytes (iPSC-CMs), and their transcriptomic differences were analysed using bulk RNA sequencing, comparing to healthy and isogenic control data. Bioengineered human ventricular cardiac tissue strips (hvCTS) were also created to evaluate contractility. The findings revealed enrichment in KEGG pathways and multiple biological processes, including cardiomyopathies, cardiomyocyte structure organization, signal transduction, metabolism, cell adhesion, cell-matrix interaction, and repair response. Notably, DMD-derived iPSC-CMs showed significantly more dysregulated gene expressions, mirroring DMD's severe clinical phenotype. The study further indicated that a reduction in contractile force of patient-derived hvCTS could be linked to the dysregulated gene expressions and pathways, potentially impairing biological functions like cardiac contractility. This study provides vital insights into the cellular and molecular mechanisms underlying dystrophinopathy and underscores the potential of patient-derived iPSCs, and the derived cardiac cells and tissues, as a model for studying these conditions and accelerating the

development of new therapies including cardiac gene therapy for clinical trials.

Funding Source: Start-Up Research Grant and Seed Fund for Basic Research (201711159006) by HKU General Research Fund (17123122) by RGC, UGC Innovation and Technology Fund (PRP/062/21FX) by ITC, HKSAR.

T1105

TRANSFORMING THE STORAGE AND SHIPMENT OF TISSUES AND IN VITRO CELL MODELS USING ATELERIX'S HYDROGEL TECHNOLOGY

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Traditional methods for transporting cells and in vitro models rely on expensive and hazardous cryopreservation techniques, which are prone to failure during shipment delays. Atelerix's innovative technology addresses these challenges by using alginate hydrogels to encapsulate cellular material, offering protection from biochemical and physical damage during storage and transport. This approach eliminates the need for dry ice, requires no specialised logistics, and presents a more sustainable solution, reducing carbon footprint and avoiding toxic chemicals. Atelerix's product, WellReady™, enables the storage and shipment of cells, organoids, and microtissues in multi-well plates at room temperature for up to seven days. Post-preservation, in vitro hepatocyte models retain viability, marker expression, and active drug-metabolising enzymes. iPSC-derived cortical neurons maintain their morphology and axonal networks, while iPSC-derived cardiomyocyte models exhibit full metabolic recovery and contractile forces. WellReady™ is particularly effective for preserving primary cells, which have a short shelf-life and are vulnerable to cryopreservation-induced stress. High recoverable viability has been demonstrated in various human cell types, including adipose-derived mesenchymal stromal cells, dermal fibroblasts, dermal keratinocyte epithelial cells, and airway epithelial cells. Alongside cell models, fresh primary tissue is essential for advanced screening techniques, diagnosis, drug development, and patient-derived model generation. However, tissue quality declines rapidly after retrieval, and the distance between collection and processing sites can lead to extended distribution times and diminished tissue quality. Atelerix's TissueReady™ provides a novel solution for storing and shipping fresh cancer tissue at room temperature for up to five days, preserving viability, cancer cell phenotype, histological integrity, and RNA quality. Maintaining the quality of patient-derived tissues for physiologically-relevant model generation is becoming increasingly important in drug discovery with the replacement of animal models.

T1107

TRIM28 LINKS TRANSPOSABLE ELEMENT ACTIVATION TO NEURODEVELOPMENTAL DISORDERS: EVIDENCE FROM IPSC-BASED MODELS

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Perturbations in early brain development can lead to neurodevelopmental disorders (NDDs), often with unidentified genetic causes. Their research has been hindered by limited access to human brain tissue and incomplete recapitulation of patient phenotypes in animal models. In this project, we use state-of-the-art iPSC-derived models coupled to cutting-edge gene editing and sequencing techniques to study mutations in TRIM28. TRIM28 is an epigenetic co-repressor protein that silences transposable elements (TEs), repetitive mobile genetic sequences, to protect genome integrity. Perturbations of this pathway in mice result in aberrant neurodevelopment and an inflammatory response in the adult brain. However, to date, a link between TRIM28 and brain disease in humans has not been shown. Our aim is to prove that mutations affecting TRIM28 can cause neurodevelopmental phenotypes and to understand how TRIM28 impaired function and transposable element dysregulation impair neurodevelopment and lead to disease. In this study, we describe two patients with a neurodevelopmental phenotype that carry de novo heterozygous missense mutations in TRIM28. We generated CRISPR-edited induced iPSC lines carrying the two mutations and differentiated them to unguided neural organoids. Combining bulk and single-nuclei RNA-seq and CUT&RUN histone mark profiling, we found that TRIM28 variants lead to loss of H3K9me3 over TEs resulting in transcriptional activation of the elements and nearby protein-coding genes. We saw that these effects are mirrored when silencing TRIM28 using a CRISPRi approach, which suggests a loss-of-function effect of the patients' mutations. These results show that dysregulation of the TRIM28-mediated epigenetic mechanism and downstream aberrant TE and gene expression can be related to neurodevelopmental phenotypes. Our findings highlight the critical importance of regulating TEs during human brain development and will ultimately lead to the description of a novel monogenic neurodevelopmental disorder caused by mutations in TRIM28.

T1109

UNRAVELING THE ROLE OF HOXB5 IN HEMATOPOIETIC STEM CELL SELF-RENEWAL AND DIFFERENTIATION

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Hematopoietic stem cells (HSCs) are crucial for maintaining the blood system through their unique abilities of self-renewal and multilineage differentiation. Our lab previously identified the transcription factor Hoxb5 as a key marker defining long-term HSCs (LT-HSCs), but its precise function in regulating stem cell behavior remains elusive. Here, we investigate the molecular mechanisms by which Hoxb5 governs LT-HSC maintenance and differentiation. Using bulk and single-cell ATAC-sequencing on rare adult bone marrow Hoxb5+ LT-HSCs and Hoxb5- short-term HSCs (ST-HSCs), we reveal distinct chromatin accessibility patterns associated with stemness and lineage commitment. Our data show that Hoxb5+ LT-HSCs exhibit restrictive chromatin states, with reduced accessibility at lymphoid-related genes, while differentiation involves the opening of genomic regions enriched for Gata and Runx transcription factor motifs. Additionally, we have developed a novel V5-tagged, AID2-degradable Hoxb5 mouse model, enabling precise identification of Hoxb5 genomic targets and assessment of its necessity in LT-HSC function. Our

preliminary data highlight specific loci involved in early lineage commitment that become accessible as Hoxb5+ LT-HSCs transition toward differentiation. By combining chromatin landscape analysis with functional validation of Hoxb5 targets, this study provides new insights into the regulatory networks sustaining LT-HSCs. This research has the potential to significantly advance our understanding of hematopoietic stem cell biology and may inform future therapeutic strategies for hematological disorders.

Funding Source: This research was supported by NIH grant TL1DK139565 and the Stanford Graduate Smith Fellowship.

T1111

UNVEILING TISSUE-SPECIFIC TRANSCRIPTIONAL ADAPTATIONS IN IPSC-DERIVED FIBROBLASTS VIA CO-CULTURE SYSTEMS

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Induced pluripotent stem cell-derived fibroblasts (iFBs) are increasingly used in autologous disease models, including cardiac 3D systems, due to their accessibility and compatibility. However, whether iFBs can adapt to tissue-specific characteristics within these models is underexplored, raising questions about their ability to fully recapitulate the specialised properties of native fibroblasts. Fibroblasts are highly heterogeneous, with distinct subtypes exhibiting tissue-specific functions critical for organ integrity and cellular interactions. For example, dermal and cardiac fibroblasts perform unique roles, and primary skin and lung models show improved performance when cultured with tissue-specific fibroblasts. In this study, we developed iFBs using a modified differentiation protocol and examined their capacity to acquire tissue-specific molecular phenotypes upon co-culture with primary cells. Early-stage iFBs were co-cultured with keratinocytes (ectoderm), cardiomyocytes (mesoderm), and bronchial epithelial cells (endoderm) to assess whether interactions could induce tissue-specific transcriptional changes across all three germ layers. Transcriptomic analyses revealed that the molecular profiles of iFBs shifted context-dependently, suggesting they exhibit functional plasticity and highlighting both their potential and limitations in mimicking native fibroblast behaviour. Additionally, targeted experiments with iFBs indirectly co-cultured with keratinocytes, cardiomyocytes, and bronchial epithelial cells showed that paracrine signalling could induce tissue-specific molecular and morphological changes. However, these adaptations were lost when co-culture was removed, indicating the phenotype is not fully stable without continued interaction. While autologous systems are a key goal for in vitro disease modelling, variability in iPSC differentiation and maturity can influence the performance of multi-cellular models relative to human physiology. Our findings emphasise the importance of validating iFBs' ability to integrate into more complex tissue models. This study demonstrates that iFBs can adopt tissue-specific transcriptional profiles, bringing them closer to their primary counterparts.

T1113

USING DIRECTED DIFFERENCIATION TO ESTABLISH A NOVEL NEURAL MODEL OF KABUKI SYNDROME

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Kabuki Syndrome (KS) is an orphan disease caused by a genetic disorder that leads to abnormal growth and development affecting 1 in every 32,000 new births. It is often characterized by facial features that resemble traditional Japanese Kabuki theater masks. KS can lead to craniofacial abnormalities, hearing loss, problems with vision, congenital heart defects, feeding difficulties, immune dysfunction, poor growth, skeletal and dermatoglyphic abnormalities, and difficulties with cognition and/or development. There are two known genetic mutations that result in KS and they are considered sporadic. Type 1 is a result of a genetic mutation on the KMT2D gene located on chromosome 12. This gene is responsible for providing instructions for making an enzyme that modifies histone H3K4. This genetic mutation is known in approximately 75% of KS patients. The second less common mutation is the KMD6A mutant, which is found on the X chromosome. KMD6A is a histone demethylase which plays a crucial role in gene regulation during development. The KMD6A mutant has only been found in 144 individuals around the world as of 2024. Due to the high mortality rate and rarity of the disease, there are limitations on the ability to study KS with physiologically relevant models resulting in a lack of cure or established therapy for KS. Importantly, insights into understanding and treating KS can provide guides for treating other diseases whose etiology rests on abnormal epigenetic regulation. With parental consent, we obtained discarded skin fibroblasts from a Type 1 KS baby undergoing gastrostomy tube placement to generate induced pluripotent stem cells (iPSCs). We accomplished this using standard Yamanaka factors delivered via Sendai Virus. When validation is completed by performing ICC for SC markers and Embryoid Body formation, we will use these hiPSCs, differentiated into an array of neural cells, to create 2D and 3D models of the human brain. By validating and optimizing the lab's established protocols for generating cortical interneurons (CINs) and cerebral organoids (CODs), we aim to provide a model that might shed light on the physiological, compositional, or circuitry deficiencies in KS and provide a model for identifying or designing therapies that can target and preempt these problems in utero, potentially improving quality of life.

Funding Source: SDSU CIRM.

T1115

USING MITOCHONDRIA-RICH, PATIENT-DERIVED HIPSC-CARDIOMYOCYTES TO STUDY DOXORUBICIN-INDUCED CARDIOTOXICITY

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Doxorubicin (DOX) is effective against cancer, but can damage the mitochondria and cause DOX-induced cardiotoxicity (DCT). The identification of treatments is hampered by the lack of suitable human models and the need to balance cardioprotection and cancer control. Conventional human induced pluripotent stem cell derived-cardiomyocytes (hiPSC-CMs) have sparse mitochondria and do not recapitulate the cardioprotective effect of FDA-approved treatment, dexrazoxane. Here we established a patient-derived and mitochondria-rich hiPSC-CM model which was protected by dexrazoxane. Using these hiPSC-CMs and a mice model, we found that ICG-001, an inhibitor of Wnt/β-catenin signalling and dynamin-related protein 1 (DRP1), suppressed DCT in vitro and in vivo, similar to dexrazoxane. Unlike dexrazoxane, ICG-001 was cytotoxic to cancer cells. Mechanistically, ICG-001 inhibited DRP1 and protected the mitochondria in CMs, but repressed Wnt signalling and killed cancer cells. In summary, our mitochondria-rich, patient-derived, mitochondria-rich CM model is critical for the identification of new treatment against DCT. Using this and a mouse model, we showed that ICG-001 can protect the heart and suppress cancer by targeting different proteins/pathways, and is therefore potentially superior to conventional treatment with dexrazoxane.

T1117

UTILIZING IPSC MODELS TO INVESTIGATE MAPT MUTATION-INDUCED TAU AGGREGATION AND THERAPEUTIC STRATEGIES

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Tauopathies, including Alzheimer's disease and frontotemporal dementia, are neurodegenerative disorders characterized by the intracellular accumulation of Tau protein aggregates. These aggregates propagate between interconnected neurons through prion-like mechanisms, emphasizing the pivotal role of Tau spreading in disease progression. The Tau protein harbors amyloidogenic motifs, such as VQIVYK within its microtubule-binding domain, which are critical for Tau aggregation and seeding. Despite the significance of these processes, therapeutic strategies targeting Tau pathology remain limited. In this study, we employed CRISPR/Cas9 gene-editing technology to generate isogenic induced pluripotent stem cell (iPSC) lines with MAPT mutations (N296Δ, P301L, P301S, and P332S) located within or near the R2R3 interface. To model Tau pathology, these isogenic iPSCs were differentiated into neuronal cells (hiPSC-iN) using an NGN2-based protocol. By week six, these neurons exhibited maturation and stress-induced Tau aggregation following thapsigargin treatment. Future investigations will evaluate whether MAPT mutations exacerbate Tau aggregation in hiPSC-iN and assess the efficacy of VQIVYK inhibitors in mitigating Tau seed formation and aggregation. This study highlights the utility of isogenic iPSC models and hiPSC-derived neurons as robust platforms for elucidating the molecular mechanisms underlying Tau pathology and for advancing therapeutic approaches targeting Tau aggregation and propagation.

Funding Source: NSTC 112-2923-B-A49 -004 -MY3.

T1119

VASCULARIZED BRAIN ORGANOID-ON-CHIP WITH FUNCTIONAL VASCULATURE AND BLOOD-BRAIN BARRIER CHARACTERISTICS

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Brain organoids represent a transformative tool for studying human brain development, offering a physiologically relevant in vitro model to explore complex cellular features and functionality. However, existing models are hindered by limitations, including the inability to replicate all features of the human brain, absence of critical cell types like vascular cells, and significant variability in organoid size and cellular composition between batches. Incorporating vasculature within brain organoids is pivotal for advancing these models by mitigating necrotic cores, promoting nutrient and oxygen exchange, and supporting enhanced maturation. To address these challenges, we developed a protocol integrating microfluidic chip technology with brain organoid culture to construct a vascularized and standardized brain in vitro model. Our approach enables the formation of an on-chip vascular network that effectively penetrates into the organoid, establishing a physiologically relevant interface. Immunocytochemistry (ICC) and sequencing analyses confirm that vascularization not only supports cellular diversity but also significantly enhances the maturation of brain organoids, with gene expression profiles aligned with advanced developmental stages. This method ensures reproducibility across experimental settings and offers a robust platform for scalable drug screening and precision medicine applications. These findings underscore the transformative potential of vascularized brain organoids for advancing neuroscience research and therapeutic discovery.

Funding Source: Hong Kong Research Grants Council; HKUST Center for Aging Science.

TRACK: PLURIPOTENCY AND DEVELOPMENT (PD)

Poster Session 2 (ODD)
4:00 PM – 5:00 PM

T1121

A PROTEOMICS STRATEGY TO IDENTIFY ELUSIVE STEMNESS PROTEINS

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Identification of stemness proteins is a cornerstone of stem cell biology. Several attempts have been made to search for stemness genes using transcriptomics with controversial results. We applied a proteomics approach to identify stemness proteins based on label-free quantitative mass spectrometry. Our results bridge previous transcriptomics approaches and show the significant relevance of DNA repair and helicase activity of stemness proteins located not only in the nucleus, but also in the mitochondrion and cilium. Synteny analysis revealed potential stemness hot spots in the G-bands D2.2 of chromosome 4 and D2 and A3.3 of chromosome 17. Our results provide a different set of stemness markers than previously published work that relied on transcriptome analysis. Although our stemness markers differed from transcriptomics approaches, we found similar functions and chromosome hot spots. We can see our stemness proteins as a bridge connecting the stemness genes found in the previous transcriptomics studies. There is an apparent paradox in the fact that different cell lineages with stem cell properties do not share a meaningful set of genes. We conclude that different stem cell populations use different regulatory networks to achieve their stem state, suggesting that stemness is not a universal property. Nevertheless, the proteomic measurements show that there are some common proteins involved in DNA repair and transcriptional activities that are shared by all stem cell lineages analyzed. Thus, although high-throughput techniques may not be able to reveal a universal stemness simply due to the possible non-existence of such a universal network, the convergence of our functional proteomics results with the previous transcriptomics results reveals the existence of a functional and synteny stemness signature. Thus, our approach sheds new light on the stemness search problem, where the proteomics measurements provide a synergetic view to the transcriptomics approaches.

Funding Source: M.J.A.-B. was supported by Ministerio de Ciencia e Innovación, Spain Grant No. PID2023-152752OB-I00/AEI/10.13039/501100011033.

T1123

KOREA NATIONAL STEM CELL BANK: HUMAN PLURIPOTENT STEM CELL BANKING PROGRESS

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The Korea National Stem Cell Bank has been banking pluripotent stem cell (PSC) lines since 2012. Quality-controlled and ethically sourced cell lines have been developed for distribution to scientists

and clinicians. As of 2024, among the 45 deposited lines, 4 human embryonic stem cell (hESC) lines and 16 induced pluripotent stem cell (iPSC) lines from healthy donors have been distributed. Ten fluorescence-tagged iPSC lines have also been developed to monitor fate commitment into the three germ layers—ectoderm, mesoderm, and endoderm. Furthermore, 8 iPSC lines derived from patients with specific diseases, including Down syndrome, Rett syndrome, and limb-girdle muscular dystrophy, as well as 7 gene-edited iPSC lines, have been newly established and deposited in the banking system. To ensure the quality of these cell lines, eighteen different quality tests have been conducted to assess identity, sterility, consistency, stability, and safety. Regarding genetic stability, we have been collecting SNPchip, whole-exome sequencing (WES), methylation sequencing (Methyl-seq), and RNA sequencing (RNA-seq) data, which are publicly accessible. PSCs provided by Korea National Stem Cell Bank are certified under the international standard of quality management systems, ISO 9001:2015. Based on these quality assessments, we provide high-quality cell lines to support research in cell and gene therapy.

T1125

YBX1 DELETION DRIVES MURINE EMBRYONIC STEM CELLS INTO A STABLE 2-CELL-LIKE TOTIPOTENT STATE

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Embryonic stem cells (ESCs) are capable of developing into the three germ layers and germ cells, but cannot generate trophectoderm or extraembryonic lineages, which are recognized as pluripotent. However, ~1% of ESCs, referred to as 2-cell-like cells (2CLCs), resemble 2-cell-stage blastomeres and maintain a totipotent-like capacity to produce both lineages. Whether and how pluripotent ES cells can be reprogrammed into a stable totipotent state remain formidable challenges in current researches. In this study, we discovered that deletion of the Ybx1 gene effectively reprograms mESCs into stable totipotent like state, which can be maintained over long-term culture in vitro. Ybx1-deficient mESCs exhibit key characteristics of 2-cell embryos, including expression of totipotent markers and similar transcriptomic profiles, such as the Zscan4 cluster transcripts and endogenous retrovirus (ERVs) MERVL. In vivo chimera formation assays further revealed that Ybx1-null mESCs possess both embryonic and extraembryonic developmental potentials at the single-cell level. Finally, we demonstrated that Ybx1 functions as a transcriptional repressor of Zscan4, a factor essential for totipotency establishment. Thus, our study identifies Ybx1 as a novel regulator of stable 2CLC totipotent state establishment, representing a component of a previously unrecognized 2C-like transcriptional network, thereby opening up new avenues for future clinical applications and therapeutic strategies.

Funding Source: National Key Research and Development Program of China (2023YFC2705700,2024YFC2707002,2024YFC2707001), National Natural Science Foundation of China (82271890).

T1127

ACE2 REGULATES HUMAN TROPHOBLAST STEM CELL PROLIFERATION AND DIFFERENTIATION

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Trophoblast development is essential for a successful pregnancy, as these cells form the outer layer of the blastocyst and facilitate the embryo's attachment to the uterus, leading to placenta formation. The placenta plays a critical role in supplying nutrients and oxygen to the fetus. Cytotrophoblasts differentiate into two main types: Extravillous trophoblasts (EVTs) and syncytiotrophoblasts (STBs), which are crucial for releasing hormone and remodelling blood vessels to support fetal development. Abnormal trophoblast development can result in serious pregnancy complications, including preeclampsia, intrauterine growth restriction, and preterm labor. Angiotensin-converting enzyme 2 (ACE2) is an enzyme that regulates blood pressure and has protective effects against cardiovascular and renal diseases. It is part of the Renin-Angiotensin System (RAS), which manages blood pressure and fluid balance. In pregnancy, ACE2 is expressed in the placenta and plays a vital role in regulating blood flow to the fetus. During the COVID-19 pandemic, ACE2 gained attention as the entry point for the virus in various cells. In this study, we utilized an in vitro placental model derived from human Extended Potential Stem Cells (hEPSCs) to investigate ACE2's function in trophoblast development. We find that ACE2 functions in trophoblast development at both pre- implantation and post-implantation stages. Further understanding ACE2's role in placental biology could provide insights into managing pregnancy complications and infectious diseases.

Funding Source: This project is supported by Health@InnoHK, Innovation Technology Commission, HKSAR.

T1129

ENGINEERING THERMOSTABLE FLUORESCENT LIGHT-UP RNA APTAMER/FLUOROGEN PAIRS FOR CELLULAR IMAGING AND FUTURE PERSPECTIVES ON CELL PHYSIOLOGY AND INTRACELLULAR BIOMARKER SENSING

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Fluorescent light-up aptamer/fluorogen pairs are crucial in tracking RNA within cells, although they face challenges in terms of thermostability and fluorescence intensity. Current in vitro selection techniques struggle to replicate the intricate intracellular environments, which results in limitations in biomolecule functionality in vivo. Inspired by the microenvironment-dependent RNA folding observed within cells and droplets that mimic organelles, we developed a system using microscale heated water droplets to imitate intracellular conditions. This system successfully replicates the intracellular RNA folding landscape. We combined this system with microfluidic droplet sorting to evolve RNA aptamers. Through this method, we engineered an RNA aptamer with enhanced fluorescence activity by exploring the chemical fitness landscape under conditions that mimic biological environments. We named our improved RNA aptamer eBroccoli, which demonstrated increased fluorescence intensity and thermal stability, both in vitro and in vivo in bacterial and mammalian cells. In the future, tracking of RNA in cells in this way could be used investigate cell physiology. Expanding upon this technology offers opportunities for non-destructive intracellular biomarker sensing, that could be used to characterize cell differentiation.

Funding Source: HKU Grants Council GRF [No. 17303123, 17306221, 17317322, 17163416, 17127515, 17125920], HKU Grants Council Theme-based Research Scheme [T12-201/19-R], Health@InnoHK program of the ITC and HKU Seed Funding [No. 102009959].

T1131

NOVEL RNA REPROGRAMMING OF BLOOD-DERIVED CELLS

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Induced pluripotent stem cell (iPSC) therapies hold significant promise for regenerative medicine, particularly for treating degenerative diseases and serving as a renewable source for allogeneic cell therapies. Efficient and footprint-free iPSC generation systems, like RNA-based reprogramming, are preferential due to their transient nature and eliminating the need for residual factor clearance. However, broader application of RNA reprogramming has been impeded by difficulties in efficiently delivering RNA to starting materials like blood cells. To that aim, the Solupore platform offers a solution using physicochemical processes to temporarily permeabilize cell membranes, ensuring effective cargo delivery while maintaining cell health for non-viral delivery. This non-invasive approach has shown superior cell viability when compared to traditional electroporation, and successful delivery to various cell types. In this study, the Solupore was utilized to reprogram CD34+, T cells, and PBMCs with both messenger RNA (mRNA) and self-replicating RNA (srRNA). Repeated transfections resulted in high cell viability and recovery, and successful iPSC generation. iPSC clones exhibiting typical morphology were cultured for at least five passages before undergoing characterization. Pluripotency was verified using flow cytometry and immunocytochemistry. Embryoid bodies were formed to assess tri-lineage differentiation potential. Select clones were then further modified using the Solupore for CRISPR-Cas9 delivery. This pioneering study demonstrates the successful generation and modification of iPSCs from blood cells using RNA delivery, thereby broadening the range of starting cells, reprogramming techniques, and delivery methods, and underscoring its potential in cell therapy applications.

T1133

THE ROLE OF THE RHOA/ROCK PATHWAY IN THE DYNAMIC MIGRATION OF ENTERIC NEURAL CREST-DERIVED CELLS

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Enteric neural crest-derived cells (ENCCs) migrate long distances to the developing gastrointestinal tract to form enteric ganglia. Abnormal migration of ENCCs in the gut may result in congenital gut motility/peristalsis disorders such as Hirschsprung's disease. However, the molecular mechanisms governing ENCC migration remain elusive. In this study, the role of RhoA, a key molecule of the RhoA/ROCK signalling pathway which is an important signal transduction system for cell migration, in ENCC migration was investigated. Specifically, small interfering RNAs (siRNAs) were employed to knock down the RhoA expression, which resulted in significant alterations of the migration behaviour of ENCCs in vitro. To visualize the dynamic change of intracellular RhoA activities during migration, time-lapse live cell imaging combined with a Förster resonance energy transfer (FRET)-based biosensor was employed. It was found that active RhoA molecules were dynamically redistributed inside ENCCs, differentially accumulating at the rear end and the leading edges of migrating ENCCs which suggested the involvement of the RhoA in ENCC migration. Next, bulk RNA sequencing was performed on ENCCs following RhoA knockdown. The expression of other molecules related to cell migration and tight junctions was altered. These suggested that the RhoA/ROCK signalling might cross talk with other pathways to regulate ENCC

migration. Particularly, Igfbp5 demonstrated a decrease in expression after supressing RhoA expression. siRNAs were employed to lower the Igfbp5 expression. The wound healing assay showed a delay in filling up the wound gap with Igfbp5-suppressed ENCCs. Besides, the live cell tracking demonstrated the loss of directionality of ENCCs 24 hours after suppression under a GDNF gradient. qPCR results also showed the decrease expression in migration-related genes. In summary, this study indicated the intricate interplay of different signalling pathways during ENCC migration. Results found that RhoA was recruited during ENCC migration, and RhoA may be involved in regulating ENCC migration through Igfbp5, which may be one of the downstream targets of the RhoA/ROCK pathway mediating the directional migration. These results provided information for potential therapeutic targets for congenital gut motility disorders.

Funding Source: The work was supported by the General Research Fund from the Research Grants Council of Hong Kong Special Administrative Region, China (Ref. no.: CUHK14120522).

T1135

CHARACTERIZATION OF CELL-BASED THERAPEUTIC PRODUCTS DERIVED FROM TP53-MUTATED HUMAN IPS CELLS FROM A PERSPECTIVE OF GENOMIC INSTABILITY

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Human pluripotent stem cells (hPSCs) have infinite self-renewal capacity and can differentiate to many cell types in vitro, thus making them an appropriate choice as a raw material for cell-based therapeutic products (CTPs). In order to ensure the safety of CTPs, assessment and appropriate management of tumorigenicity of the final product are important issues. Genomic instability is a potential hazard related to the risk of tumorigenicity because it is expected to increase the probability of karyotypic abnormalities or genomic mutations and to thereby increase the probability of cellular transformation. In particular, CTPs derived from human induced pluripotent stem cells (hiPSC) are predicted to accumulate genetic mutations and increase genomic instability because they require long-term culture for somatic cell reprograming, cloning of cell lines, cell banking, maintenance and expansion, and cell differentiation into desired cell type. Currently, it is recommended that hiPSC-derived CTPs be checked for karyotypic abnormalities and SNV/Indel or structural abnormalities in cancer-related genes. However, the safety of cells with genomic mutations, which are seen in vitro, has not been concluded. In this study, we generated three TP53 mutant hiPSC lines by homozygous mutation of TP53 high frequency mutations (R175H, R248Q, and R273H) by genome editing and analyzed the effects of TP53 mutations on genomic instability and tumorigenicity of hiPSC derived CTPs. The results of this study showed that (1) TP53 mutants have an increased proliferation rate compared to the parental line, (2) TP53 mutants show resistance to cell differentiation, (3) analysis by exome sequencing shows no increase in SNVs common to TP53 mutations, and (4) in vivo tumorigenicity study of cardiomyocytes derived from TP53 mutated hiPSCs, immature teratomas were observed in the TP53 mutant groups. These results are important findings showing the association between TP53 mutations and genomic instability/tumorigenicity, and are expected to lead to the establishment of scientific evidence-

based genomic instability evaluation criteria.

Funding Source: This work was partly supported by the Japan Agency for Medical Research and Development (AMED) under grant number 24bk0104157j0203.

T1137

EFFECTS OF HEPARAN SULFATE ON THE DIFFERENTIATION OF HESCs INTO PANCREATIC BETA-CELLS IN VITRO

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Although pancreatic β-cells derived from human pluripotent stem cells (hPSCs) hold promise for the therapy of diabetes mellitus, their clinical application is limited by low differentiation efficiency and insufficient functionality. While heparan sulfate (HS) is known to regulate stem cell fate in various contexts, its role in the differentiation of human embryonic stem cells (hESCs) toward the pancreatic lineage remains not well understood. Here, we demonstrate that HS acts as a key effector in the differentiation of hESCs into the pancreatic lineage, particularly during the transition from endocrine progenitor (EP) to endocrine cell (EC). Pancreatic ECs are derived from hESCs through a series of developmental processes, including the definitive endoderm (DE), pancreatic endoderm (PE), and EP stages. To investigate the effect of HS on pancreatic β-cell development, hESCs were treated with HS during the transition from EP to EC, a critical commitment phase. HS treatment enhanced the transcriptional activity of β-cell-associated genes in hESC-derived ECs. Furthermore, pancreatic islet-like organoids (PIOs) generated from HS-treated cells exhibited higher expression of β-cell-associated genes than those derived from untreated cells. Moreover, a glucose-stimulated insulin secretion (GSIS) assay revealed that insulin production was significantly higher in HS-treated PIOs than in untreated controls when exposed to a high glucose concentration (27.5 mM), indicating enhanced functionality. Notably, HS treatment did not alter the morphological characteristics of PIOs derived from hESC-derived ECs. Thus, HS treatment not only improves the differentiation efficiency of hESCs into pancreatic β-cells but also enhances their functionality. Our results suggest that HS is a potent modulator of pancreatic β-cell development.

Funding Source: Korean Fund for Regenerative Medicine (KFRM).

T1139

METABOLIC REGULATION OF STEM CELL FATE THROUGH MODULATION OF THE EXTRACELLULAR MATRIX

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The various functions of stem cells during development, regeneration and in homeostasis are determined by their trajectory towards differentiation, self-renewal, or quiescence, and disrupting these paths can lead to a variety of diseases. While transcription factors and chromatin regulators are known to regulate pluripotency and cell fate, increasing evidence shows that extrinsic factors like the nutrient environment and the extracellular matrix (ECM) can regulate stem cell functions. The ECM is a complex network of proteins that surrounds the cells, provides structural support and transduces mechanical and biochemical signals to influence cell behavior, including proliferation, migration, and differentiation. Despite its importance, the role of ECM in regulating stem cell function remains inadequately explored. Our work aims to address this gap by investigating how nutrient-driven changes in metabolism influence ECM composition and stem cell reprogramming. Specifically, we focus on Vitamin C (VitC), an essential nutrient that enhances pluripotency induction and is commonly used in iPSC culture media. Using metabolomic approaches, we show that VitC rapidly enhances glucose flux towards Pentose phosphate pathway (PPP) and Hexosamine biosynthesis pathway (HBP) early in reprogramming. Further, VitC addition leads to depletion of intracellular Col1 and ColIV and enhances ECM deposition. VitC derived ECM can regulate cell physiology and enhance reprogramming efficiency. Altogether, we propose that VitC modulates ECM deposition through its effect on cellular metabolism, particularly by enhancing glycosylation pathways necessary for glycosaminoglycan and collagen production. This, in turn, alters ECM composition and promotes iPSC generation. These findings provide novel insights into how metabolism and ECM interact to regulate stem cell fate transitions. Our work also advances the understanding of ECM's role in stem cell function, with broad implications for improving stem cell therapies, tissue engineering, and regenerative medicine, as well as addressing diseases linked to ECM dysregulation.

Funding Source: CIRM-BSCRC Postdoctoral Fellowship, BSCRC Innovation Award.

T1141

QUIET ACHIEVERS: A TROPHIC ROLE FOR MACROPHAGES IN THE DEVELOPING HUMAN INNER EAR

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Normal inner ear development requires the sophisticated orchestration of specialised cell differentiation and integration, which ultimately gives rise to the exquisite organs of hearing and balance. We are only just beginning to understand the timeline and dynamic nature of this process in the developing human inner ear. Recent studies using single cell transcriptomics have improved our understanding of the various cellular phenotypes present in the mammalian inner ear. During human inner ear development, macrophages have been identified as early as gestational week 7

by expression of IBA1 and CD45 and clearly populate the adult cochlea. Yet, there is little known about the origin of cochlear macrophages, or their functional contributions to inner ear organogenesis. Using a transcriptional approach, we have identified seven distinct macrophage subtypes present over a broad window of human inner ear development which spans foetal weeks 7.5, 9.2, 18, 18.4 and adult. We describe differential gene expression in each of these unique macrophage subtypes, including how each subtype is closely linked to a specific developmental age. These data support and extend upon existing histological studies in the human inner ear, reporting the presence of resident and non-resident macrophages in both the developing cochlea, and in adult cochleae following cochlear implantation. In addition, we report that human inner ear macrophages are seeded from multiple sources, supporting the conclusions from recent studies in mice indicating yolk sac and foetal liver origins. We discuss the possible functions of the unique macrophage phenotypes identified at different developmental ages, by analysing their ligand-receptor interactions with other key cell types present during inner ear development. Together, these data highlight for the first time, the breadth of macrophage phenotypes present throughout human inner ear development and their possible multi-disciplinary contributions to normal inner ear organogenesis. A more comprehensive understanding of the functional roles of human inner ear macrophages will accelerate novel therapeutic strategies targeting both immune-, congenital and age-related hearing loss.

Funding Source: The University of Melbourne - University of Toronto Joint Collaborative Research Grant.

T1143

XENO/FEEDER-FREE CULTURE USING LAMININ SUPPORTS RESETTING TOWARDS NAIVE PLURIPOTENCY IN HUMAN EMBRYONIC STEM CELLS

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The field of naive human pluripotent stem cells (hPSC) has seen a surge in research activity over the years, particularly their use for generation of blastoids, a powerful 3D model to study human embryogenesis in vitro. A major hurdle today is the lack of a standardized protocol for deriving naive hPSC without the use of mouse embryonic fibroblasts (MEF) or transgene overexpression. In this study, we cultured hPSC on laminin 521 (Biolamina LN521), a key cell adhesion protein in the inner cell mass of human blastocysts, to mimic their natural niche. We established this novel approach of culturing cells on LN521 during epigenetic resetting through histone deacetylation

inhibition following the PXGL protocol (Austin Smith Lab). We used four in-house derived primed hPSC lines to generate feeder-free naive hPSC. After resetting, the cells were cultured in PXGL conditions on LN521 and passaged regularly into a single cell suspension. Two female hPSC lines (VUB14 and VUB26) produced hPSC with naive characteristics. The cells showed expression of naive pluripotency markers KLF17, NANOG and SUSD2 through RT-qPCR while 78.0% of VUB14 cells were SUSD2+/CD75+ and 82.1% VUB26 cells were SUSD2+/CD75+ using flow cytometry at passage 20. Both VUB14 and VUB26 showed presence of KLF17 through immunostaining and differentiated successfully towards primitive endoderm showing GATA4+/NANOG- cells. We also tested genome stability of the reset lines through CNV assays for chromosomal aberrations common in hPSC cultures and found a 20q11.21 duplication for VUB14 and a 12p duplication for VUB26. We hereby report a novel and standardized protocol for the derivation and sustained culture of naive hPSC importantly on a cell-free matrix, without the need for MEFs. Future experiments include resetting more cell lines towards naïve pluripotency on a LN521 matrix as well as investigating their potential to generate blastoids in comparison to MEF derived naïve hPSC.

Funding Source: This research is funded by FWO11H9825N.

T1145

COLLOIDAL SELF-ASSEMBLED PATTERNS (CSAPS) PROMOTE INTESTINAL DIFFERENTIATION VIA TGF-BETA PATHWAY AND SEROTONIN NEURON DIFFERENTIATION VIA SONIC HEDGEHOG PATHWAY

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Colloidal self-assembled patterns (cSAPs) exhibit highly organized surface structures with heterogeneous surface chemistries, generating well-defined physicochemical cues that influence cellular behavior. Binary colloidal crystals (BCCs), a subset of cSAPs, possess intricate surface micro/nanostructures and heterogeneous chemical properties, positioning them as significant regulators of the in vitro microenvironment. Previous research has shown that BCCs can modulate the differentiation of human embryonic stem cells (hESCs). However, limited studies have explored the potential of BCCs in facilitating hESC differentiation into endodermal (intestinal organoids) and ectodermal (serotonin-producing neurons) lineages. Our results demonstrate that one type of BCCs enhances hESC differentiation into endodermal cells and accelerates intestinal organoid formation through the TGF-β signaling and integrin-mediated signaling pathways. Another type of BCCs promotes hESC differentiation into serotonin neurons via the Sonic Hedgehog (SHH) signaling pathway. This study offers valuable insights into the role of physicochemical cues in directing hESC differentiation into specific cell types, enhancing our understanding of mechanotransduction. It also presents a promising strategy for reducing culture time and facilitating the transition from laboratory research to clinical applications.

Funding Source: PYW thanks the support from the Ministry of Science and Technology of China (2022YFA1105101) and the Zhejiang Provincial Natural Science Foundation of China (LZ23C070004).

T1147

ENHANCED EX UTERO ROLLER CULTURE PLATFORM

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Understanding the developmental processes that lead to the formation of tissues and organs is a fundamental issue in developmental biology. In mammals, this process occurs after the embryo is implanted into the uterus, making observation and manipulation relatively difficult. Therefore, the sequence of developmental events from gastrulation formation to organogenesis remains to be fully understood and difficult to manipulate. The conventional mouse embryo culture system from E6.5 to E8.5 has certain applications in embryonic development research, but there are also some obvious drawbacks. Traditional static culture systems usually only provide a two-dimensional culture environment and cannot fully simulate the three-dimensional development process of embryos in the mother's body under natural physiological conditions. The oxygen and nutrients in the culture medium may not be evenly distributed in a static culture environment, resulting in uneven nutrition obtained by the embryo. And the embryos will deposit at the bottom of the culture bottle due to gravity, which may cause morphological irregularities or physical damage to the embryos. The ultimate result is usually a lower embryo development rate. Therefore, with the deepening of research, new cultivation methods, such as rotating culture systems, support embryos to have better in vitro developmental abilities. We further explored and developed the instrument structure and composition of the culture medium in the whole embryo in vitro rotation culture system, and found that our developed system can develop embryos from E7.5 stage to E11.5 stage at a rate of 100%. At the same time, we are also trying to combine the embryo rotation system with other systems modified in our laboratory to culture E11.5 to later stage embryos, and have made some progress.

T1149

GENOMIC SCREEN IDENTIFIES JARID2 AS A CELL CYCLE-ASSOCIATED GENE ESSENTIAL FOR LINEAGE COMMITMENT

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The mechanisms by which the cell-cycle directs embryonic-stem-cells differentiation outcomes are not fully understood. To gain deeper insights, we performed a CRISPR/Cas9 genomic-screen on pre-sorted G1 and G2/M ESCs, identifying hundreds of genes essential for the transition from pluripotency to differentiation. Notably, many of these genes are influenced by the cell-cycle state at the onset of differentiation, even if they are not expressed during pluripotency. By incorporating temporal measurements, we revealed a link between the exit from pluripotency and differentiation decisions, demonstrating that fate selection is already influenced during the pluripotent state. Next, we focused on Jarid2, a subunit of PRC2.2, which emerged as an essential G2/M-dependent differentiation regulator. Interestingly, other PRC2 components, while essential for differentiation, did not exhibit cell cycle sensitivity. The majority of G2/M Jarid2 KO ESCs failed to survive differentiation, while the small fraction that endured exhibited an immature differentiation state, retaining ability to reactivate pluripotency. We showed that Jarid2 selectively recruits RNA polymerase II Ser5 to genes involved in extra-embryonic endoderm-like (XEN-like) differentiation

specifically during G2/M explaining the preference of G2/M cells to activate XEN. Using cell-cycle pre-sorted ESCs and single-cell RNA-seq, we further characterized Jarid2's role in ESCs differentiation; While EpiSC-like cells did not require Jarid2—indicating their status as a premature differentiation state. XEN-like cells originated mostly from persisting G2/M ESCs, exhibited a mixed expression with pluripotent markers. Moreover, the activation of more defined states, such as mesendoderm and pre-epithelium were unable to activate without Jarid2. Additionally, chromatin immunoprecipitation for H3K27ac revealed that the loss of Jarid2 disrupts the activation of enhancers critical for differentiation while promoting H3K27me3 deposition, resulting in the repression of pluripotency. In summary, our screen identified a novel set of cell-cycle-sensitive genes, providing a valuable resource for the stem cells research community. Our focus on Jarid2 further elucidated its pivotal role in regulating early steps of differentiation.

Funding Source: ERC and ISF.

T1151

INFLAMMATORY CYTOKINES TRIGGER THE ACTIVATION OF THE KYNURENINE PATHWAY OF TRYPTOPHAN METABOLISM: EFFECTS ON EMBRYONIC MOUSE NEURAL STEM CELL PROLIFERATION, HEALTH AND NAD STATE

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Neural stem cell (NSC) proliferation regulators underpin future cell therapies, while understanding NSC vulnerabilities during disease states could explain why innate repair in neurodegenerative/inflammatory diseases fails. The kynurenine pathway (KP) regulates essential amino acid tryptophan (TRP)'s bioavailability, notably induced by stimuli including interferons – components of a cell's antiviral defences. In neurodegenerative diseases the KP is dysregulated, producing high levels of metabolites like potent neurotoxin Quinolinic acid (QUIN). QUIN metabolism by enzyme quinolinate phosphoribosyltransferase (QPRT) into cofactor NAD is rate-limited. We characterized KP/NAD genes via PCR, and hypothesized KP modulation by interferons alters embryonic mouse neural stem cell (emNSC) proliferation, neurosphere size and cell health (Muse flow cytometry assays). We found Tryptophan-2,3-dioxygenase (TDO2) is a key regulator of initial TRP catabolism, having substantial basal expression (65.45□27.45 (*1000 versus beta-actin) while modestly upregulated by interferons (type II family member IFN-gamma->2.7-fold)/type I IFN-beta->2.1-fold). IFN-gamma slightly upregulates alloenzyme indoleamine-2,3-dioxygenase (IDO1, 4.1+/-0.38); while IFN-beta has no effect. emNSCs varyingly express all KP genes basally including QPRT; upregulated by both IFNs. While IFN-gamma significantly increased neurosphere size (10IU/mL, 287.3+/-32.73um; p=0.0032; 100IU/mL, 305.7+/-10.64um; p=0.0005) versus controls (180.5+/-42.1um), it increased oxidative stress/caspase-activation, compromising cell health. Conversely, 50nM KYNA significantly grew neurosphere size (307.5+/-11.45um; p=0.0004) and retained cell health. Strikingly, both IDO-1/TDO2 are linked with NAD state, as siRNA with IFN-gamma gave increased NAD+/NADH ratio, typical of an active metabolic state. In conclusion, we did the first KP/NAD gene expression screen. emNSCs are vulnerable to deleterious effects of interferons like IFN-gamma, which preferentially reduces (while IFN-beta enhances) enzymes

linked with producing protective metabolites KYNA/Picolinic acid. Selective KP inhibition, or increasing KYNA could minimize cell death, improve regeneration, and optimize NSC proliferation therapeutically.

Funding Source: We acknowledge funding of the Peter Duncan Neurosciences Unit.

T1153

PROFILING AND PROGRAMMING IN VITRO HUMAN NEURONAL DIVERSITY AT SINGLE-CELL RESOLUTION

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Human neurons programmed through transcription factor (TF) overexpression model neuronal differentiation and diseases. However, the diversity of neuronal subtypes programmable in vitro remains underexplored. Here, we modulate developmental signaling pathways combined with TF overexpression to explore the spectrum of neuron subtypes generated from pluripotent stem cells. We screened 480 morphogen signaling modulations coupled with NGN2 or ASCL1/DLX2 induction using a multiplexed single-cell transcriptomic readout. Analysis of 700,000 cells identified diverse excitatory and inhibitory neurons patterned along the developmental axes of the neural tube. Patterning neural progenitors prior to TF overexpression expands neuronal diversity by enabling access to regulons active in primary tissue counterparts. Our approach provides a strategy for programming diverse human cell subtypes and to investigate how cooperative signaling drives neuronal fate.

T1155

SELF-ORGANIZATION OF POLARIZED NEOCORTICAL ORGANOIDS

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Neocortical organoids derived from human pluripotent stem cells (hPSCs) have emerged as an accessible and invaluable tool to study human neurodevelopment and disease. Neocortical organoids consistently self-organize into the laminar architecture of the human cortex dorsoventrally. However, neocortical organoids currently still lack a controlled induction in the anteroposterior axis. Here, we demonstrate the polarization of neocortical organoids with the activation of anteroposterior signaling activations. We observed organizer formations that in turn self-polarize into distinct areal signatures. Many neurodevelopmental disorders have specific dysregulation in different areas within the neocortex. Thus, the demonstration of the

anteroposterior polarization would serve as a powerful platform to study various neurological disorders that have differential effects on neocortical areas.

Funding Source: This work was supported by the NIH R00HD096105, NSF RECODE 2225624, New Investigator Faculty Award and startup funds to MW, the FRAXA Postdoctoral Fellowship to YCT, the CIRM Postdoctoral Fellowship (EDUC-12822) to HO.

T1157

UNVEILING THE EPIGENETIC ROLES OF PEPTIDYL ARGININE DEIMINASE III (PADI3)

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Citrullination is a post-translational modification catalyzed by the Peptidyl Arginine Deiminase (PADI) enzyme family. By altering the charge of histone tails, citrullination modulates chromatin compaction, orchestrating transcriptional programs epigenetically when occurring at gene regulatory regions. Conversely, global histone citrullination promotes chromatin condensation and extracellular trap formation, contributing to programmed cell death such as NETosis. Over the years, histone citrullination has been implicated in a broad spectrum of biological processes, such as pluripotency maintenance, cell fate determination, cancer progression, and autoimmunity, highlighting its vast biological significance. Despite the structural and functional similarities among Padi enzymes and their nuclear localization, the epigenetic roles of certain members, such as Padi3, remain unexplored. Interestingly, our Proximity Ligation Assay (PLA) result revealed that Padi3 interacts with histone H3 in mouse epidermal progenitor cells primarily in the nucleus, while immunoblotting demonstrated forced Padi3 expression leads to H3 hypercitrullination in human embryonic kidney cells (HEK 293). These results strongly implicate Padi3 in epigenetic regulation, a novel and unknown function. To elucidate the mechanisms underlying Padi3-mediated gene regulation, we will employ Chromatin Immunoprecipitation (ChIP) and SLAM-sequencing to identify direct gene targets, while biochemical assays will investigate its interactions with other Padi enzymes and chromatin regulators. Collectively, this study aims to expand our understanding of the epigenetic functions of Padi3 and pave the way for future research into its role in a wide range of normal biological processes and diseases.

T1159

ADVANCING NEURAL CELL FUNCTIONALITY: LAMININS AS KEY ECM COMPONENTS IN CELLULAR MODELS AND TRANSLATIONAL RESEARCH

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Cells within tissues interact directly with an extracellular matrix (ECM), a protein-rich framework essential for cellular adhesion, proliferation, differentiation, and maturation. The ECM family of laminins is composed of 16 different isoforms all critical for heathy tissue development and homeostasis. The laminin isoforms vary in expression both temporally and spatially, and act as active part in both the stem cell niches and differentiation. The successful recapitulation of in vivo laminin expression has been demonstrated in various neural applications. For example, groundbreaking work by Kirkeby et al. (2016), which demonstrated the use of Biolaminin 521 and -111 to significantly enhance and standardize protocols for PSC-derived dopaminergic neurons. This approach increased cell yield by over 40-fold and facilitated clinical translation, setting a new benchmark for neural differentiation protocols. Biolaminin-111 and -521 have also been shown to enhance neural differentiation and reduce neuroinflammation in human 3D brain organoids, enabling long-term functional midbrain and cerebral organoid. Here we demonstrate that Biolaminin 521 can support PSC-derived neural crest differentiation with high purity and yield. The monolayer culture simplifies the differentiation with equivalent or better result compared to the often-used aggregate differentiation. We further demonstrate that Biolaminin 521 in combination with -221 can support PSC-derived astrocyte differentiation, increase standardisation and accelerate differentiation, shortening the protocol with up to one week. In conclusion, in vitro recapitulation of the natural cell niches, with recombinant laminin proteins, can lead to increased yield of desired target cells, simplified and accelerated protocols, as well as enabling clinical translation.

T1161

ALTERNATIVE PROMOTER DURING MAMMALIAN ORGAN DEVELOPMENT

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Most human protein-coding genes are regulated by multiple promoters, highlighting the importance of promoter selection in gene expression. However, the role of alternative promoters during embryonic development across species remains largely unexplored. To investigate alternative promoter usage during development, we analyzed promoter activity in various organs across seven species using the ProActiv tool. We identified promoters that exhibited significant changes in activity at different developmental time points, despite overall gene expression levels remaining unchanged. We further explored the transcription factors (TFs) driving these changes using UniBind and ChIP-seq data. We detected alternative promoters in multiple organs and species, with the number of genes exhibiting promoter activity changes increasing over developmental time in mouse liver. For example, the gene Lsp1 showed minor changes in overall expression but significant alterations in isoform expression due to alternative promoter usage. We identified key TFs, including NR1D1, ESR1, FOXA2, and RXRA, associated with upregulated promoters in mouse liver at 9 weeks post-birth (9wpb). The canonical motifs for FOXA2 and HNF4A were significantly enriched in upregulated promoters compared to background promoters (p-values ranging from 0.01 to < 0.001). ChIP-seq data showed higher overlap of FOXA2 peaks with upregulated promoters. In contrast, downregulated promoters lacked overlap with these TF peaks.

Additionally, promoter activity assessments in wild-type and HNF4A-knockout liver samples revealed that while overall promoter activity increased in the knockout samples, the activity of specific upregulated promoters decreased. Our study provides insights into the dynamic regulation of alternative promoters during mammalian organ development, highlighting the critical role of specific TFs in driving promoter activity changes. Future work should focus on further elucidating the mechanisms underlying these observations and exploring their functional implications in development and disease.

T1163

BUILDING A HIPSC DERIVED TESTICULAR SOMATIC NICHE: A STEP TOWARDS IN-VITRO SPERMATOGENESIS

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Sertoli cells (SCs) play a crucial role in testicular development and spermatogenesis, providing structural and biochemical support to developing germ cells. However, limited access to human fetal testicular tissue limits our understanding of SC function and their role in germ cell maturation. In vitro gametogenesis (IVG) presents a promising approach to overcoming this challenge. Still current models depend on co-culture of germ cells with fetal or rodent somatic cells, and are therefore practically challenging or impedes proper translation to human, respectively. In addition, achieving full meiotic progression remains challenging. In this study, we aim to generate and characterize Sertoli-like cells (SLCs) from human-induced pluripotent stem cells (hiPSCs) to develop a human-derived testicular soma for IVG. Using directed differentiation, 2 hiPSC lines were induced through an intermediate mesodermal lineage and further differentiated into SLCs using FGF9, PGD2, and Activin A. Molecular analyses of the derived SLCs confirmed the upregulation of key SC markers (SOX9, WT1, VIM, AR), downregulation of pluripotency genes (NANOG, OCT3/4), thereby validating lineage commitment. Additional functional assessment through a fluorescent bead uptake assay demonstrated phagocytic activity, a defining feature of SCs in clearing apoptotic germ cells. These results establish a foundation for generating human SLCs in vitro, providing a relevant model to support germ cell maturation. This work advances the field by contributing to a fully humanized IVG system, with implications for fertility research and potential therapeutic applications in male infertility.

Funding Source: Funding for this work was provided via ZonMW PSIDER program no

10250022120001 (HipGametes).

T1165

EFFECT OF THE PI3K/AKT SIGNALING PATHWAY DURING EARLY PORCINE EMBRYOGENESIS

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The phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathway is known to be a key signaling pathway in various cell types, playing critical roles in cell proliferation, survival, and particularly in zygotic genome activation (ZGA) and anti-apoptosis. Although the role of the PI3K/AKT pathway in mouse embryonic development is well established, its function in porcine embryonic development remains unexplored. This study aimed to investigate the effects of the PI3K/AKT signaling pathway during preimplantation development in porcine embryos. We performed immunofluorescence to detect total and phosphorylated AKT (pAKT), as well as total and phosphorylated RPS6 (pRPS6), commonly used downstream markers of the PI3K/AKT pathway, throughout early development. Our results revealed that both AKT and pAKT were expressed during early embryonic development. Notably, pAKT translocated to the nucleus at the 2-cell stage, coinciding with minor ZGA, and was strongly expressed in the nucleus at the 4-cell stage during major ZGA. RPS6 and its phosphorylated form were localized in the cytoplasm, consistent with their role in translation. These results were consistent in embryos derived from both parthenogenetic activation (PA) and in vitro fertilization. Then, we treated early embryos with varying concentrations (0, 1, 10, and 100 μM) of LY294002, a PI3K inhibitor, during in vitro culture after PA. The cleavage rates were not significantly different, but the blastocyst formation rates were significantly decreased in 1 and 100 μM LY294002-treated groups compared to the control group. TUNEL assay revealed that total cell numbers in blastocysts decreased in a concentration-dependent manner with LY294002 treatment. While the number of apoptotic cells was not significantly different among groups, the apoptotic index was significantly increased in LY294002-treated groups. These findings demonstrate that the PI3K/AKT signaling pathway is essential for porcine preimplantation embryo development by regulating ZGA and apoptosis. Further studies will investigate the molecular mechanisms of PI3K/AKT signaling during ZGA.

Funding Source: “Technology Innovation Program funded by the MOTIE, Korea (20023068)”, and “IPET in Food, Agriculture, Forestry and Fisheries funded by MAFRA (RS-2024-00398561, RS-2024-00399475)", Republic of Korea.

T1167

ENERGY PROFILE OF IPSC-DERIVED HUMAN PGCLCS: A MULTI-OMICS APPROACH

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Primordial germ cells (PGCs) are the embryonic precursors of gametes and play a crucial role in reproductive biology. The in vitro differentiation of human PGC-like cells (hPGCLCs) from induced pluripotent stem cells (hiPSCs) offers a valuable model for studying germ cell development and potential applications in regenerative medicine. However, a detailed understanding of the molecular differences between hiPSCs and their differentiated hPGCLC counterparts remains elusive, particularly in terms of their proteomic and metabolomic profiles. While genomics offers valuable information on genetic potential, ; proteomics and metabolomics offer crucial insights into the functional state of cells, allowing for a more accurate representation of their phenotype and physiological state. With the goal of characterizing the proteomic and metabolomic changes during early hPGCLC induction, we employed a multi-omics approach that combined metabolomics and proteomics mass spectrometry. For this, we performed a previously published hPGCLC in vitro differentiation protocol, and collected for analysis the progenitor hiPSCs and the differentiated hPGCLC and non-hPGCLC fraction, as a differentiation-effect control. The results showed distinct metabolic and proteomic profiles between the progenitor cells and differentiated counterparts, and a distinct proteomic profile between hPGCLCs and the non-hPGCLC fraction. Several alterations were detected in the glycolysis pathway and oxidative phosphorylation (citric acid cycle and the electron transport chain), between the three groups. The data indicate that the hPGCLCs present a more quiescent energy state, with decreased oxidative phosphorylation in comparison to hiPSCs and non-hPGCLCs, and a decreased glycolytic activity versus non-hPGCLC. The integration of a multi-omics approach study not only contributes to the fundamental characterization of iPSC-derived PGCLCs, but also offers potential insights for optimizing in vitro differentiation protocols that are needed to generate human gametes from stem cells in vitro. Moreover, it may provide a more inclusive understanding of the biological processes underlying human PGC differentiation in general.

Funding Source: This study was funded by ZonMW PSIDER 10250022120001.

T1169

GUINEA PIG STEM CELL REVOLUTION: FIRST-EVER CAPTURE OF PRIMED PLURIPOTENCY

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Guinea pigs serve as valuable models for human disease research, yet the lack of established pluripotent stem cell (PSC) lines has limited their utility in biomedical applications. In this study, we report the first-ever capture of primed pluripotency in guinea pigs through the successful derivation of guinea pig epiblast stem cells (gpEpiSCs) from post-implantation embryos. These cells exhibit stable self-renewal, differentiation into the three germ layers, and normal karyotypes, marking a significant breakthrough in stem cell research. Transcriptomic and epigenetic analyses confirm that gpEpiSCs share key features with human and mouse primed stem cells while also revealing species-specific pluripotency characteristics. We demonstrate that gpEpiSCs rely on FGF2 and ACTIVIN A signaling, with WNT inhibition essential for maintaining their pluripotency state. This pioneering achievement establishes a novel pluripotent model, broadening the landscape of stem cell research and expanding the potential of guinea pigs as preclinical models for disease modeling, regenerative medicine, and drug discovery. The successful establishment of gpEpiSCs unlocks new possibilities for comparative pluripotency studies and translational research, positioning guinea pigs as an indispensable tool for biomedical innovation.

Funding Source: This work was supported in part by the National Key Research and Development Program of China (2018YFE0204800), NSFC (U20A2013, 32370791, 32100594, and 32200695), Guangdong Basic and Applied Basic Research Foundation (2020A1515110122).

T1171

IMPACT OF EXTENDED IN VITRO CULTURE ON HUMAN DENTAL STEM CELLS

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This study examines the effects of prolonged in vitro cultivation on SCAP, DPSC, and BMSC stem cells, focusing on their proliferative capacity and cellular senescence markers to evaluate their potential for regenerative medicine. Stem cells from the apical papilla (SCAP), dental pulp (DPSC), and alveolar bone (BMSC) exhibit strong proliferative and differentiation abilities under controlled conditions, making them valuable for regenerative therapies. However, the impact of long-term cultivation on these cells remains unclear. This study aims to assess how extended in vitro culture affects SCAP, DPSC, and BMSC, with an emphasis on cellular senescence markers. Stem cells were isolated from healthy third molars (SCAP, DPSC, and BMSC) and cultured in DMEM with 10% fetal bovine serum for approximately four months. Cultivation was categorized into early (1st–3rd), intermediate (10th–12th), and late passages (18th–20th). Gene expression analysis was performed using the RT2 Profiler PCR Array – PAHS – 178ZE-4 for Human Aging. SCAP, DPSC, and BMSC were successfully isolated and expanded. Gene analysis focused on telomere maintenance, DNA damage response, oxidative stress, mitochondrial function, cellular

senescence, apoptosis, inflammation, and metabolic regulation. A slight increase in apoptotic cells was observed at later passages, while telomerase activity remained stable throughout. SCAP, DPSC, and BMSC exhibit strong potential for regenerative medicine. Despite prolonged cultivation, these cells maintained their proliferative capacity and showed minimal signs of senescence, reinforcing their suitability for regenerative therapies and tissue engineering. This study provides a foundation for future research and clinical applications of dental-derived stem cells.

Funding Source: This study is financed by the European Union-NextGeneratio- nEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project № BG-RRP-2.004-0004-C01.

T1173

IN-GEL CULTURE OF NEURAL ORGANOIDS WITH POROUS HYDROGEL SCAFFOLDS FOR DYNAMIC CONTROL OF NETWORK FORMATION AND CELLULAR COMPLEXITY

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An ideal neural organoid culture system should possess sufficient scalability and flexibility in modulating cellular diversity and developmental timing to better model human brain development. However, existing culture systems are often insufficient to achieve both scalability and flexibility at the same time. In this study, we developed a hydrogel scaffold-based culture system specifically designed to address these challenges. The interconnected pore network within the hydrogel facilitates efficient media perfusion, enhancing long-term viability and sustained culture. Through adjustment of seeding cell density, we observed tunable patterns in organoid quantity and size distribution, enabling scalable production. Furthermore, this system permits controlled temporal integration of biological or chemical components, creating a versatile platform for experimental modulation. We demonstrated the platform's capability to establish temporally controlled neural organoid networks and achieve multi-lineage integration within composite organoids. These findings highlight our hydrogel scaffold system's potential as a scalable and adaptable platform for investigating neural development, network formation, and cellular interactions within a controlled 3D environment.

Funding Source: Brain Korea 21 Plus Program For Biomedical Science.

T1175

MOLECULAR MECHANISM OF PLURIPOTENCY INDUCTION VIA PARK7

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Induced pluripotent stem cells (iPSCs) are critical for disease modeling and drug development, yet the mechanisms driving pluripotency induction are not fully resolved. We have found that PARK7 (DJ-1), a regulator of oxidative stress and transcription, promotes differentiation in mouse

embryonic stem cells (mESCs) and hinders somatic cell reprogramming, though its precise role remains unclear. We investigated PARK7's function during pluripotency induction in low-KLF4 (LowK) paused iPSCs, a stable partially reprogrammed iPSCs. Park7 knockdown (KD) in LowK paused iPSCs upregulated the expression of pluripotency markers (Nanog and Rex1) and increased glycolysis through an increase in reactive oxygen species (ROS) and HIF1α stabilization. In contrast, PARK7 overexpression (OE) at an early stage of somatic cell reprogramming suppressed these markers but enhanced them at a later stage, revealing reprogramming stage-specific effects. These findings suggest PARK7 inhibits early pluripotency induction by limiting ROS and HIF1α-driven glycolysis yet aids later stages. This dual role elucidates PARK7's impact on reprogramming efficiency, offering insights into optimizing iPSC generation for therapeutic applications and understanding pluripotency dynamics.

Funding Source: JSPS KAKENHI No. JP23H02407.

T1177

OPTIMAL MEDIUM COMPOSITION FOR PORCINE BLASTOID FORMATION

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Blastoids are three-dimensional (3D) structures resembling blastocysts, serving as ideal models for ethically challenging embryo studies. Unlike rodents, pigs exhibit greater developmental similarity to humans, making porcine blastoids a valuable model for studying early developmental mechanisms. In this study, we aimed to establish the optimal culture medium for porcine blastoid formation using primed-state porcine embryonic stem cells (pESCs) and to generate blastocyst-like structures through 3D culture. pESCs were cultured in media containing different combinations of factors to identify the optimal medium composition. pESCs cultured in FAB5i/X medium supplemented with FGF2, Activin A, BMP4, and small-molecule inhibitors targeting SRC, GSK3, TGFβ, ROCK, HDAC, and WNT signaling differentiated into the three distinct lineages of the blastocyst, namely epiblast, hypoblast, and trophoblast, as demonstrated by qPCR and immunofluorescence. This finding established FAB5i/X as the optimal medium for porcine blastoid formation. Utilizing the FAB5i/X medium, pESCs were cultured in AggreWell for approximately five days, resulting in the generation of a 3D structure closely resembling natural embryos, as confirmed through immunostaining. Future studies will aim to evaluate the efficiency of blastoid formation across various embryonic stem cell types and develop strategies for long-term culture. This research offers valuable insights into porcine embryo development, expands the possibilities for embryo-related experimental approaches, and contributes to advancing the field of early developmental biology.

Funding Source: Funded by the Korean Government (2020R1A2C2008276, NRF-2021R1C1C2007132), funded by the MOTIE, Korea (20023068), funded by MAFRA (RS-2024-00398561, RS-2024-00399475).

T1179

VCAM1-MEDIATED REGULATION OF DOPAMINERGIC NEURON FUNCTION IN PARKINSON'S DISEASE

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Parkinson's disease (PD) is the second most common neurodegenerative disorder, characterized by the degeneration of dopaminergic neurons, striatal dopamine deficiency, and the accumulation of intracellular α-synuclein aggregates. In this study, we employed induced pluripotent stem cell (iPSC) technology to generate dopaminergic neurons from somatic cells of both PD patients and healthy controls. Our results demonstrate that patient-derived neurons exhibit elevated expression of vascular cell adhesion molecule 1 (VCAM1), which correlates with altered synaptic plasticity, mitochondrial dysfunction, and impaired Rac1 and FAK2 signaling. These findings suggest that VCAM1 plays a pivotal role in PD pathogenesis and may serve as a potential therapeutic target.

T1181

3D TRANSCRIPTOMIC RECONSTRUCTION HIGHLIGHTS HUMAN HEART-FORMING ORGANOID DEVELOPMENT AS A MODEL OF EARLY ORGANOGENESIS

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The human embryo undergoes gastrulation 14-21 day's post-fertilisation, establishing the body plan and initiating organogenesis. Difficulty accessing samples has hindered our understanding of this critical window, particularly the developing tissue crosstalk and the morphological changes that drives. Recent in vitro models have made significant progress in replicating the co-development of the cell types involved. However, one of the first morphological processes in human organogenesis, the caudal invagination of the primitive gut tube dorsal to the cardiac mesoderm, remains unexplored. In 2021, we described the human heart-forming organoid (HFO), which recapitulates the co-development of heart, foregut and vasculature in a robust and reproducible manner. To study the developmental mechanisms from pluripotent to proper heart and foregut patterning at day 10, we performed daily 10x Visium HD spatial RNAseq on multiple HFO sections, these where then spatially contextualised using 3D confocal imaging of whole mount immunofluorescence stained and cleared HFOs. This revealed a primitive streak like Brachyury+ population surrounding an ectodermal core at day 3, which provided a starting point for endodermal migration, with definitive endoderm (FOXA2+/SOX2-) cells migrating inwards and gaining SOX2 expression, committing to the anterior foregut (AFE) fate (FOXA2+/SOX2+). From the same location, day 6 HNF4a+ posterior foregut endoderm (PFE) cells migrated outwards. HFOs therefore mimic the migration and development of the foregut and its anlagen in a human model, which has previously only been possible in model organisms. We next questioned HFOs

ability to mimic in vivo genetic and compound induced teratogenic phenotypes. Firstly, we show that GATA4-/- HFOs replicated the loss of PFE seen in GATA4-/ mouse embryos. Secondly, HFOs reproduced the characteristic anti-angiogenic effects and cardiac abnormalities seen in clinical data from thalidomide exposed pregnancies. Notably we also saw inhibition of AFE migration, highlighting the models ability to study transient teratogenic processes. Together, we provide independent experimental evidence that HFOs model key developmental processes, enabling a deeper mechanistic understanding of previously inaccessible areas of human development.

T1183

A DEEP LEARNING MODEL FOR SINGLE-CELL RNA SEQUENCING ANNOTATION IN MONKEY AND HUMAN PERI-IMPLANTATION TO GASTRULATION EMBRYONIC DEVELOPMENT

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Human embryonic development, from peri-implantation to gastrulation, represents a critical period for understanding cell lineage and tissue generation. With the growing number of stem-cell-based embryo models designed to replicate this stage in vitro, the need for annotating and evaluating these models using a well-curated, integrated post-implantation cell reference has become pressing. In this study, we employed a systematic approach to construct comprehensive reference atlases for human and monkey embryos. To facilitate automated cell type recognition, we further coupled our human reference with label transfer algorithms, which effectively identify cell types in both spatial and single-cell transcriptomic data. Furthermore, we curated annotations for nine human embryo models and benchmarked them using a series of metrics. Our reference-based annotation and benchmarking frameworks demonstrated their exceptional utility in characterizing embryonic models, assessing their fidelity, and revealing the extent of lineage development synchrony in current in vitro embryo models.

T1185

A NOVEL PYROGEN DETECTION METHOD BASED ON TRANSGENIC MONOCYTES DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

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Pyrogens, including endotoxins and nonendotoxins, are among the key factors that impact the safety of parenterally applied drugs serve as contaminants. At present, conventional pyrogen detection methods are predominantly dependent on the Rabbit Pyrogen Test (RPT) and the

Limulus Amebocyte Lysate (LAL) assay, both of which are animal-based. However, both methods have limitations: the RPT exhibits poor reproducibility, a high false-positive rate, and is cost-intensive; whereas the LAL test is restricted to detecting endotoxins and is constrained by the precipitous decline in the horseshoe crab population. The Monocyte Activation Test (MAT), based on monocytes, has become the most promising alternative to traditional pyrogen detection methods. However, the MAT is complex to operate, time-consuming, has significant individual differences, and usually requires a large amount of human blood, which greatly limits its application. Therefore, we have developed a rapid pyrogen detection method based on monocytes derived from human pluripotent stem cells (hPSCs) using a luciferase reporter system. Owing to the stability and pluripotency of hPSCs, we can obtain a large number of hPSC-derived monocytes (eMo) that are stable between batches and similar to peripheral blood monocytes through directed differentiation in vitro. We have discovered that eMo exhibits sensitive reporting capabilities for at least two types of pyrogens: lipopolysaccharide (LPS), a major component of the cell wall of Gram-negative bacteria, and lipoteichoic acid (LTA), a major component of the cell wall of Gram-positive bacteria. Further, we confirmed that eMo exhibits stable expression of TLRs, which are one of the main factors determining whether pyrogens can be stably detected. Thus, we have developed and validated a new pyrogen detection method based on the reporting system and directed differentiation of hPSCs in vitro, providing a new direction and approach for the replacement of traditional pyrogen detection methods.

Funding Source: Supported by the Strategic Priority Research Program of the Chinese Academy of Sciences, Grant No. XDC0200000.

T1187

ADVANCEMENTS IN THE GENERATION OF FUNCTIONAL RED BLOOD CELLS

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Red blood cell (RBC) generation from human pluripotent stem cells (hPSCs) offers potential for innovative cell therapy in regenerative medicine as well as developmental studies. Ex vivo erythropoiesis from PSCs is currently limited by the low efficiency of functional RBCs with β-globin expression in culture systems. During induction of β-globin expression, the absence of a physiological microenvironment, such as a bone marrow niche, may impair cell maturation and lineage specification. Here, we suggest two important systems: one for RBC culture protocol and another for gene editing for Gene A knock-out. First, we describe a simple and reproducible culture system that can be used to generate erythroblasts with β-globin expression preparing a two-dimensional defined culture with ferric citrate treatment based on definitive hemogenic endothelium. Upon maturation, the erythroblasts cultured in the presence of ferric citrate showed high transcriptional levels of β-globin and enrichment of genes associated with heme synthesis and cell cycle regulation, indicating functionality. Second, Terminal erythropoiesis involves a complex differentiation process, encompassing erythropoietin (EPO) signal transduction and intracellular gene regulation. We identify gene A as a novel EPO-responsive Ca2+ channel that negatively KLF1 transcription. Using hPSCs-derived CD71+ erythroblasts, we demonstrate the down-regulation of Gene A and subsequent Ca2+ homeostasis during erythropoiesis in stage-specific manner. This gene A inactivation positively manipulated erythroid-specific genes by promoting KLF1 gene expression and erythroid maturation including increased globin expression and enucleation. Overall, when treated with Fe and modified gene A, erythroblasts exhibited advanced

maturity and high β-globin transcription. These findings can aid in developing a stable protocol for generating clinically applicable RBCs.

Funding Source: Korean Cell-Based Artificial Blood Project funded (RS-2023-KH141086) the KRIBB Research Initiative Program Grants (KGM4562532) the National Research Council of Science and Technology grant by the Korea government (GTL24021-100).

T1189

AGING IN REPRODUCTIVE SYSTEM: PLACENTA

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Our research endeavors to reveal novel genetic factors and mechanisms involved in placental aging, with a particular emphasis on the function of specific genes identified during trophoblast stem cell differentiation. Initial findings suggest the involvement of several genes that play a critical role in the aging of reproductive tissues. Our previous research identified unique gene expression patterns shared between trophoblast differentiation and the aging process, such as CDK7, TFCP2L1, CDO1, NR6A1, and TACSTD2. To further explore the significance of these genes in cell senescence, we plan to conduct gene knockout experiments in trophoblast stem cells, followed by differentiation of these knockout cell lines into EVT and ST. Our research aims to uncover new mechanisms related to placental aging through gene editing and high-throughput sequencing technologies. Situated at the intersection of reproductive biology and genomics, our work provides fresh perspectives on the genetic foundations of aging within the reproductive system. The outcomes of this research could potentially lead to the development of innovative strategies to support healthy aging in women and enhance our understanding of the intricate genetic processes governing reproductive aging.

T1191

AN ESSENTIAL ROLE OF DDX21 IN FETAL HEMATOPOIESIS BY AN EPIGENETIC MECHANISM

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Fetal hematopoietic stem cells (HSCs) and their committed progenitors (HSPCs) are highly proliferative cells that rely on active ribosome biogenesis to support their rapid expansion during fetal hematopoiesis. Insufficient ribosome biogenesis, a pathological condition known as ribosomopathy, activates the p53 pathway and induces cell cycle arrest in immature progenitor cells, leading to disrupted hematopoiesis and erythroid aplasia (anemia). DDX21 is an RNA helicase that regulates ribosome biogenesis by modulating rRNA splicing, processing, and ribosomal protein transcription. To test whether ribosome insufficiency is a cause of defective hematopoiesis, we generated Ddx21 conditional knockout (Ddx21cKO) in hematopoietic cells

using Vav1-Cre;Ddx21f/f mice. The Ddx21cKO embryos showed fetal anemia and regression of the fetal liver. We also observed decreased HSPC numbers and their committed progenitors (CMP, GMP, and MEP) at E13.5 onwards. Terminal differentiation of erythroid cells was unbalanced, with more immature erythroblasts accumulated at basophilic and polychromatic stages. Consistently, E13.5 Ddx21cKO fetal liver cells failed to constitute the blood cells when transplanted into lethally irradiated mice, suggesting that Ddx21 is essential for fetal hematopoiesis. Analysis of the DDX21 protein interactome revealed several histone modification enzymes directly interacting with DDX21. Among them, the histone deacetylases (HDAC1/2) are components of the repressive complex for epigenetic regulation. Interestingly, the loss of Ddx21 in HSPC resulted in the downregulation of ATAC peaks at transcription start site, suggesting a more closed chromatin including the promoters of rDNA tandem repeats. These results suggest that DDX21 may prevent the HDAC complex from improper silencing of genes essential for HSPC proliferation. Our findings reveal a new function of DDX21 in regulating hematopoiesis via an epigenetic mechanism.

Funding Source: This study was supported by a grant from Hong Kong Research Grants Council (RGC) General Research Fund (Project No. 14104321), and a grant from Health@InnoHK Program launched by Innovation Technology Commission, Hong Kong S.A.R. China.

T1193

ANALYSIS OF HUMAN UNIPARENTAL EMBRYONIC STEM CELLS REVEALS NEW IMPRINTED LOCI

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Genomic imprinting, an epigenetic process resulting in parent-specific gene expression, is essential for normal development and growth. Disruption of imprinting leads to various developmental disorders and cancers, yet our understanding of the full repertoire of imprinted genes in humans remains incomplete. Here, we utilized androgenetic, parthenogenetic, and biparental human embryonic stem cells and their neural derivatives to identify novel imprinted genes by analyzing their methylome and transcriptome profiles. Our analysis revealed 12 novel imprinted genes distributed across four distinct loci, with six of them clustered in an uncharacterized imprinted region on chromosome 19. We identified potential imprinting control regions regulating this novel cluster, suggesting a coordinated regulatory mechanism. Notably, these imprinted genes are enriched in neurodevelopmental functions and cancer-related pathways, with several showing isoform-specific imprinting patterns. Our analysis also revealed consistent DNA methylation aberrations in pluripotent stem cells at specific imprinted loci, highlighting potential epigenetic instability during culturing. These findings significantly expand our understanding of genomic imprinting regulation and its implications in human development and disease.

T1195

ASSESSING DEVELOPMENTAL COMPETENCE USING MIRNA MARKERS IN TRISOMY 21 HIPSC-GENERATED HUMAN BLASTOIDS

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In 2020, the Centers for Disease Control and Prevention reported that only 45% of embryo transfer procedures result in live birth deliveries. Aneuploidy is a common cause of embryo developmental failure, although low-range mosaicism of aneuploidy has displayed some developmental capabilities. The current methods of preimplantation genetic testing for aneuploidy (PGT-A) often overlook the possibility of genetic mosaicism within an embryo. Furthermore, studies have not demonstrated favorable effects of PGT-A on improving live birth rates. Recent research has identified microRNA (miRNA) markers in trisomy cell lines that may serve as predictors for developmental potential. Similarly, these miRNAs released by mammalian embryos into the culture media are essential, unbiased, and non-invasive markers for evaluating embryo quality. However, using human embryos as a research model raises ethical challenges. To address the ethical considerations of our research, we have successfully generated blastoids from naïve-like human induced pluripotent stem cells (hiPSC) of Trisomy 21 (Tri21) origin. An isogenic subclone with corrected ploidy status (Disomy 21; Di21) was included as an euploid control. Blastoids generated from Tri21 hiPSC exhibited a fluid-filled cavity and the self-organization of three primary cell lineages, comparable to those created from Di21 hiPSC. miRNA markers associated with developmental competence (e.g., miR-191, miR-320a, miR-372, and miR-16) were evaluated in blastoids and their spent culture media fractions using RT-qPCR. Elevated levels of miR-320a, -372, and -16 were observed in the spent culture media fraction of Tri21-origin blastoids compared to those of Di21 origin, suggesting a differential miRNA profile correlating with ploidy status. Our study demonstrates that blastoids generated from hiPSCs of known aneuploid status present a promising and ethically sound model of aneuploid human embryos. We highlight the potential of these models in providing valuable insights into identifying miRNA markers of developmental competency.

Funding Source: Canadian Institutes of Health Research (CIHR), Children's Health Research Institute (CHRI).

T1197

CELL ADHESION REMODELING DRIVES CARDIAC CELL FATE

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Human pluripotent stem cells (hPSCs) can generate specific cell types for therapeutic applications. Cell therapy often requires billions of cells for transplantation, so it is critical to maximize the potential of differentiation to optimize both quantity and quality. Cardiomyocytes are commonly induced in static culture with limited expandability. In this study, we explored the impact of cell adhesion remodeling on hPSC cell fate determination. We reveal that cell dissociation at critical time points drives cardiac cell fate even without traditional cardiac inducers. Cardiac fate is specified while cells proliferate continuously. Cell adhesion remodeling leads to a 10-fold increase in the yield of high-purity cardiomyocytes compared to traditional static culture. Transcriptomic analysis suggests that cell adhesion remodeling enhances the expression of critical cardiac genes associated with maturation. This study highlights that cell adhesion remodeling significantly impacts cell fate during in vitro differentiation. Our study provides an ideal method for high-yield, high-purity cardiomyocyte production, and offers a useful strategy for generating other cell types through directed differentiation.

Funding Source: This work was supported by the University of Macau (File No. MYRG-GRG2023-00137-FHS-UMDF) and by the Science and Technology Development Fund, Macau SAR (File No. 0010/2023/AKP and 0073/2023/ITP2).

T1199

CELLULAR MECHANISMS OF TROPHOBLAST INVASION AT THE EMBRYO-MATERNAL INTERFACE

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In the early days of development of a mammalian embryo, a competent blastocyst adheres to a receptive uterine endometrium and invades it to establish a crucial connection. This process, termed as implantation is achieved by the adhesion of the mural trophectoderm (TE) of the late blastocyst stage embryo to the uterine wall, and its subsequent differentiation to form invasive trophoblast giant cells (TGCs) capable of secreting matrix metalloproteinases (MMPs). A closer look at the TE to TGC differentiation from embryonic day 3.5 to 6.5 revealed an increased presence of actin in the cytoplasm of the mural TE and at the leading, invasive edges of the TGCs. The presence of these actin-rich degrading devices prompted us to look for the presence of invadosomes. Our experiments have shown that differentiation of TGC from TE is accompanied by the assembly and maturation of invadosomes. Further, we set out to understand if invadosomeassembly and maturation relies on TE to TGC differentiation. To study this, we looked at embryos arrested at blastocyst stage, without mural TE differentiation. We observed invadosome assembly in the TE cells, but they failed to mature and release MMPs, and invade the endometrium. In order to learn how TE differentiation and invasion will be affected in the absence of invadosome formation, we looked at embryos lacking a master regulator of invadosome formation. This embryo model failed to adhere and invade into the uterine endometrium, revealing the crucial role played by invadosomes in mediating implantation. Thus, invadosome formation and maturation is a key cellular mechanism that guides the attachment and invasion of a developing blastocyst into the uterine endometrium during implantation.

T1201

CHARACTERIZATION OF THE ROLES OF IRX3/5 GENES USING MEIBOMIAN GLANDS ORGANOID CULTURE

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The meibomian gland is a holocrine gland that secrete lipid meibum and protect ocular surface from desiccation. Meibocytes are lipid synthesizing cells, their maturation process involves lipid accumulation and cell disintegration to release cellular content as meibum. To maintain lifelong meibum production, continuous meibocyte regeneration is required but the understanding of the

meibocyte replenishment from meibocyte stem/progenitor cell is limited. We studied two Iroquois mouse mutants (Irx3 and Irx5) that exhibit meibomian gland atrophy and dry eye disease features. We successfully established a mouse meibomian gland organoid culture that displayed meibocyte differentiation, the organoids could be expanded for over 20 passages. Using this meibomian gland organoid culture platform, we investigated the roles of Irx3/5 genes in meibocyte differentiation and maturation. Mouse meibomian glands were isolated and cultured in three-dimensional environment with a defined medium. Organoids were examined using meibocyte marker PPARγ and ductal cell marker Krt6A. Lipid content was assessed by Nile red oil stain. The differentiated mouse meibomian organoids were spherical in shape (diameter= 88±27μm) and comprised of meibocytes and ductal cells. The central region of the organoids were filled with oil, indicating complete meibocyte maturation. Intriguingly, the Irx3 mutant organoids displayed heterogeneous characteristics. The mutant organoid displayed an enlarged spherical shape (diameter= 162±96μm). Some mutant organoids continued to proliferate in differentiation medium as indicated by positive Ki67 staining. Moreover, some mutant organoids have reduced PPARγ staining, with lipid containing cells identified in the central region. Our results suggest that Irx3 was involved in controlling organoid proliferation and could affect meibocyte maturation. Irx3/5 genes may play a role in maintaining meibocyte homeostasis, and their inactivation may disrupt meibocyte replenishment, leading to atrophic glands and dry eye disease in the mutant animals. Our meibomian gland organoid offers a novel in vitro model for studying the mechanism for meibocyte replenishment and development of therapeutic approaches for managing dry eye disease.

Funding Source: This project is supported by the Health and Medical Research Fund, Hong Kong (HMRF 09203156).

T1203

COMPREHENSIVE ANALYSIS OF DIFFERENTIATION CAPACITY OF EARLY NEUROEPITHELIAL STEM/PROGENITOR CELL LINES DERIVED FROM HUMAN EMBRYONIC BRAIN TISSUE

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Cell lines derived from early embryonic stages have significantly advanced our understanding of mammalian development. However, existing human neural stem/progenitor cell models primarily represent later stages of neurodevelopment, such as rosette-like or radial glial cells, and often fail to maintain long-term clonal self-renewal. Here, we report the successful isolation and establishment of a tissue-derived human neural stem/progenitor cell population that closely mirrors the human neuroepithelium at five weeks of in vivo development. These embryonic neural stem/progenitor cells (eNSPCs) exhibit a distinct naïve, non-polarized, pre-rosette phenotype and demonstrate virtually unlimited self-renewal. Single-cell RNA sequencing revealed that eNSPCs represent a homogeneous population of multipotent stem cells, as opposed to a mixture of

committed neural progenitors, with distinctive expression of early neurodevelopmental markers. Notably, this cell population exhibits broad regional plasticity, differentiating into diverse lineages of both the central and peripheral nervous system, including forebrain and midbrain dopaminergic neurons, V2a progenitors, interneurons, and neural crest cells. In conclusion, our findings establish the eNSPC cell population as the earliest stabilized human neural stem/progenitor cell model to date, characterized by broad plasticity, making them an invaluable tool for studying human neurodevelopment and exploring regenerative medicine applications.

T1205

COMPREHENSIVE SINGLE-CELL PROFILING AND TRAJECTORY MAPPING IN A HIGH-FIDELITY IN VITRO MODEL OF HUMAN POST-IMPLANTATION DEVELOPMENT

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In vitro embryo-like models have emerged as transformative tools for investigating human early development, providing a controlled platform to elucidate processes such as lineage specification, morphogenesis, and body axis formation. Despite their potential, existing models are often hindered by suboptimal efficiency or limited fidelity in recapitulating these complex developmental events. We developed a novel system wherein human pluripotent stem cells (hPSCs), under precise modulation of signaling pathways, autonomously self-organize into embryo-like structures that closely resemble Carnegie Stage (CS) 6–7 human embryos. Single-cell RNA sequencing (scRNA-seq) combined with trajectory inference was employed to dissect the molecular mechanisms underlying the differentiation processes guiding hPSCs toward their terminal cell fates. These analyses revealed distinct differentiation trajectories and dynamic molecular events orchestrating cell fate transitions driven by the trajectory-dependent stepwise activation of lineage-specific genes. Collectively, these findings illuminate the molecular programming underlying transitions to distinct cellular fates. Comprehensive molecular profiling of the embryo-like structures demonstrated their remarkable fidelity to natural CS6–7 embryos, as evidenced by highly similar lineage composition and marker gene expression patterns, as well as accurate recapitulation of key intercellular signaling interactions observed in their in vivo counterparts. These findings highlight the utility of our model as a robust and faithful system for studying the molecular underpinnings of human post-implantation development.

T1207

CONVERSION OF PRIMED PORCINE EMBRYONIC STEM CELLS TO A NAIVE STATE THROUGH CHEMICAL REPROGRAMMING

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Pluripotent stem cells (PSCs) are divided into ‘naïve state’ and ‘primed state’ depending on their differentiation potential. Naïve PSCs represent an earlier developmental state with minimal epigenetic features, greater developmental capacity, and enhanced genomic stability than primed PSCs. These features make naïve PSCs more advantageous in studying developmental biology and for applications requiring high-efficiency genome editing. Notably, porcine naïve embryonic stem cells (ESCs) hold significant value due to their physiological similarity to humans and potential use in transgenic animal production. We have recently established porcine primed embryonic stem cells (ESCs); however, there have been no reports on the successful derivation of naïve ESCs in pigs. This is due to the species-specific characteristics of the pluripotency gene network and the variation in the pluripotent state. Therefore, we aimed to identify a species-specific naïve pluripotency gene network in pigs through chemical reprogramming of porcine primed ESCs. The results showed that the reprogrammed cells had dome-shaped colonies, characteristic of naïve PSCs. Interestingly, leukemia inhibitory factor (LIF) and activin a (Act A) appeared to play a key role in porcine naïve pluripotency similar to human naïve PSCs. These cells exhibited alkaline phosphatase activity and the expression of Oct4, Sox2, Nanog, SSEA-1, and SSEA-4. Significant upregulation of naïve PSC markers such as STELLA($P < 0.05$) and NANOG($P < 0.001$) were also observed compared to porcine primed ESCs. Taken together, we found that porcine primed ESCs can be reprogrammed to the naïve-like state using chemical agents without genetic manipulation. This study will not only be used as preliminary research for establishing embryo-derived porcine naïve ESCs but can also be applied for comparative developmental studies between various species.

Funding Source: This work was supported by the BK21 Four program, the Korea Evaluation Institute of Industrial Technology (KEIT) [20012411]; and the National Research Foundation of Korea (NRF) grant [2023R1A2C1005026].

T1209

DECIPHERING THE EPIGENETIC ROLE OF KDM4A IN PANCREATIC BETA-LIKE CELL DIFFERENTIATION FROM IPSCS

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Pancreatic β cells derived from human induced pluripotent stem cells (hiPSCs) represent a promising therapeutic avenue in regenerative medicine for diabetes treatment. However, current differentiation protocols lack the specificity and efficiency required to reliably produce fully functional β cells, limiting their clinical applicability. Epigenetic barriers, such as histone

modifications, may hinder proper differentiation and the acquisition of essential maturation markers in these cells. In this study, we investigated the role of the histone demethylase KDM4A in the differentiation of insulin-producing pancreatic β cells from hiPSCs. KDM4A specifically removes trimethyl groups from lysine residues H3K9 and H3K36, critical epigenetic marks associated with gene regulation. Modulation of KDM4A expression revealed its pivotal role in the epigenetic landscape of differentiation. The results showed that KD of KDM4A significantly reduced the expression of pancreatic differentiation genes, such as PDX1, NX6.1, and INS, compared to WT iPSCs differentiated under the same conditions. Similarly, glucose-stimulated insulin secretion was reduced by approximately 80%. Contrary to the KD results of KDM4A, its overexpression showed a high expression of pancreatic genes and an increase in glucose-stimulated insulin release. These findings underscore the importance of histone demethylation in optimizing differentiation protocols for hiPSC-derived β cells. Our results provide preliminary insights into the epigenetic mechanisms governing β-cell maturation, laying the groundwork for further exploration of histone demethylases as potential targets to enhance regenerative medicine strategies for diabetes treatment.

Funding Source: Universidad de La Sabana.

T1211

DECIPHERING THE POTENTIAL ROLES OF PPAR-GAMMA IN EARLY HUMAN TROPHOBLAST DIFFERENTIATION

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Successful trophoblast differentiation is essential for proper embryo implantation and placental development. However, as restricted by the ethical concerns of acquiring early human embryo samples, the exact molecular pathways of early human trophoblast development remain poorly understood. Peroxisome proliferator-activated receptor-γ (PPARγ) is a nuclear receptor widely recognized for its transcriptional roles to regulate various metabolism and stem cell differentiation. Although previous human and transgenic mouse studies have reported that PPARγ-mediated signaling is crucial for normal placental and embryo development, the dynamic expression and precise functional effects of PPARγ during early trophoblast differentiation in human have yet to be elucidated. In this study, we employed human expanded potential stem cell (hEPSC)-derived trophoblastic cells and the hEPSC-derived early trophoblastic spheroid to explore the detailed molecular roles of PPARγ during early trophoblast differentiation. The study has initially confirmed a progressive increase in PPARγ mRNA levels during induced trophoblast differentiation. Treatment of PPARγ agonist L-165041 enhanced extravillous trophoblast differentiation with enhanced matrix metalloproteinase-2 (MMP2) secretion while the antagonist T0070907 significantly inhibited syncytiotrophoblast differentiation with reduction in Human chorionic gonadotropin secretion. To further validate the functional effects of PPARγ, the study has conducted functional assays on the modulated trophoblastic cells. The results showed that L-165041 induced invasive ability of trophoblast cells and enhanced attachment ability of BAP-EB onto endometrial cell lines, suggesting the critical roles of PPARγ-mediated signaling on

trophoblast differentiation and its involvement in normal implantation processes. Collectively, these findings have advanced our understanding of the roles of PPARγ in early trophoblast differentiation and potentially broadened our knowledge of the molecular mechanisms regulating early implantation in human trophoblasts in general.

T1213

DEFINING STEMNESS OF PORCINE TROPHOBLAST STEM CELLS THROUGH OPTIMIZATION OF CULTURE CONDITIONS

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The derivation of trophoblast stem cell (TS) lines from porcine embryos produced in vitro provides a powerful platform for studying placental development and embryo-maternal interactions in ungulates. Porcine TS offer a valuable model for studying placental development and implantation in ungulates due to their dynamic nature and ability to form non-invasive epitheliochorial placentas. In this study, we aimed to optimize culture conditions for inducing and maintaining porcine TS from in vitro-produced embryos. Initially, we replicated culture conditions based on previous studies, which led to an increase in the expression of epithelial-mesenchymal transition (EMT)-related genes. As the cells were sub-cultured, noticeable changes in cell morphology were observed, prompting us to identify specific chemical combinations that could either maintain the original cell shape or revert the cells to their initial morphology. Taken together, we confirmed the expression of TS-specific markers in both the pig embryos and the outgrown cells, validating their identity and lineage. To establish stable TS lines, we systematically evaluated various media compositions, growth factors, and signaling modulators to determine their effects on cell adhesion, colony formation, and stemness maintenance. During the derivation process, we identified distinct subpopulations of TE cells and classified the subtype that exhibited the highest capacity for maintaining stemness as trophoblast stem cells (TS). Combinations of specific signaling molecules were identified as critical for preserving TS-specific marker expression, while promoting robust attachment and colony formation. This comprehensive analysis provides new insights into the molecular mechanisms governing TS behavior, offering a foundation for further studies on trophoblast differentiation and placental development in pigs. Our results contribute to a broader understanding of TS biology, with potential applications in improving reproductive efficiency and addressing placental dysfunctions in livestock.

Funding Source: This work was supported by the BK21 Four program, the Korea Evaluation Institute of Industrial Technology (KEIT) [20012411]; and the National Research Foundation of Korea (NRF) grant [2023R1A2C1005026].

T1215

DEPLETION OF TP53 IN HUMAN PLURIPOTENT STEM CELLS TRIGGERS MALIGNANT-LIKE BEHAVIOR

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Human pluripotent stem cells (hPSCs) tend to acquire genetic aberrations upon culture in vitro. Common aberrations are mutations in the tumor suppressor TP53, suspected to confer a growth-advantage to the mutant cells. However, their full impact in the development of malignant features and safety of hPSCs for downstream applications is yet to be elucidated. Here, TP53 is knocked out in representatives of both an embryonic (H9) and an induced pluripotent stem cell line (LU07) using CRISPR-Cas9 technology. These were compared with their isogenic wild-type counterparts, as well as with malignant human germ cell tumor lines (2102EP, NCCIT) used as models of malignancy. While no major changes in proliferation, pluripotency, and transcriptomic profiles are found, mutant lines display aberrations in some of the main chromosomal hotspots for genetic abnormalities in hPSCs. Additionally, enhanced clonogenic and anchorage-free growth, alongside resistance to chemotherapeutic compounds is observed. The results indicate that TP53-depleting mutations in hPSCs, although commonly occurring and potentially overlooked by standard analyses, can impact their behavior by inducing a greater resilience to stressors, causing malignancy-associated traits hampering their safety in a clinical setting.

T1219

DEVELOPMENT OF PLURIPOTENT STEM CELL-DERIVED B CELL THERAPY APPLICATIONS

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B cells play a crucial role in mounting rapid immune responses against pathogens and have the potential to be powerful tools for cell-based therapies. However, their use is limited by a lack of robust methods for in vitro generation. To address this, we developed a serum- and feeder-free culture system for generating B cells from human pluripotent stem cells (hPSCs). Utilizing hPSCs provides both an unlimited source of starting material and an opportunity for cell engineering. Human induced pluripotent stem cells derived from B cells (B-hiPSCs) provide a foundation for the generation of antigen-specific B cells as they possess a rearranged B cell receptor (BCR) and have undergone selection checkpoints in vivo. hPSCs were differentiated to CD34+ hematopoietic stem and/or progenitor cells (HSPCs) following a 12-day STEMdiff™ Hematopoietic - EB workflow, then were differentiated to B cells following a 5-week serum- and feeder-free protocol using StemSpan™ SFEM II medium and multi-stage B cell differentiation supplements. B-hiPSCs produced CD19+ B cells with a frequency of 72 ± 4% and a yield of 13 ± 6 cells per input CD34+ cell and CD19+IgG+ B cells with a frequency of 65 ± 5% and a yield of 11 ± 5 cells (mean ± SEM; n = 9). hPSC lines derived from other cell sources generated CD19+ B cells with a frequency of 19 ± 5% (n = 15) and CD19+IgM+ B cells with a frequency of 1.6 ± 0.5% (n = 15). B cells showed functional secretion of IgG or IgM antibodies by ELISpot assay, where B-hiPSC cultures generated 1,461 ± 247 (n = 3) antibody secreting cells (ASC) per 10,000 day 35 input cells. Non-B cell derived hPSCs generated 27 ± 20 IgM, and 19 ± 13 IgG ASCs per 10,000 day 35 input cells (n = 5). This novel serum- and feeder-free culture system generates hPSC-derived B cells that express and secrete antibodies. This work enables more efficient B lineage-specific research and provides a path to in vitro generation of B cells as potential tools in clinical applications.

T1221

DISCOVERY BIOLOGY OF NEUROPSYCHIATRIC SYNDROMES

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Severe mental illness (SMI) is a significant cause of disability worldwide affecting 1 in 8 people worldwide. The clinical management of these patients poses significant challenges both in terms of diagnosis and the availability of therapeutic agents and better tools are needed in both these domains. The development of such tools in turn would be facilitated by a better understanding of the aetiology of these illnesses as well as the cellular and developmental alterations in the brain of these patients. Both genetic and environmental factors are known to contribute to the development of SMI. Although known to be polygenic, the genetic loci involved are not well understood. Likewise due the non-accessibility of human brain tissue to biopsy, studying cellular changes associated with SMI has not been possible. In order to address these challenges, the Centre for Brain and Mind has assembled a prospective cohort of clinically dense families affected by some of the key SMI (schizophrenia, bipolar disorder, obsessive compulsive disorder, substance dependence and dementia). The program includes three verticals (i) clinical characterization of affected patients along with unaffected family controls at multiple levels of brain structure and function at 3 years intervals over a 20 year period (ii) genetic analysis of cohort members using next generation sequencing and informatics (iii) generation of a biorepository of patient and control derived induced pluripotent stem cells (iPSC). This last vertical is expected tp facilitate the cellular and developmental analysis of the brain using 2D and 3D brain organoid models. To date the program has generated a collection of ca. 120 iPSC lines which along with the relevant metadata are available for “disease-in-a-dish” analysis of SMI. Genome engineered reporter lines derived from these iPSC have also been generated to facilitate the analysis of key sub-cellular and molecular processes in various human brain cell types in SMI. The availability of this comprehensive resource is expected to facilitate an enhanced understanding of altered brain structure and function, in turn leading to the development of better diagnostics and therapeutics for SMI.

Funding Source: The Centre for Brain and Mind is supported by Rohini Nilekani Philanthropies. Full details of the program can be found at https://www.ncbs.res.in/cbm/home.

T1223

DNA REPLICATION TIMING SHAPES GENOME STABILITY AND EPIGENETIC REGULATION IN EARLY MAMMALIAN EMBRYOS

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The mammalian oocyte confers dynamic changes to erase cellular memory and re-establishes the patterns of gene expression, epigenetic modifications of DNA and histones, and of nuclear architecture de novo. Thereby, the oocytes prepares the genome for development of all tissues and cell types in an adult organism. As a crucial element of development is the duplication of the genome. In somatic cells, this duplication occurs through a temporal order termed DNA replication timing. However, the principles of establishment and the regulation of DNA replication timing is not

well understood, in particular in human cells. Here we show using single cell whole genome sequencing of human embryos throughout preimplantation development, that a DNA replication timing program is established from the zygote stage, during the period of methylation erasure of DNA and histones. Late replicating regions are positioned in less accessible regions of the genome, characterized by low GC content, sparse replication origins, and an inverse relationship with the active histone marker H3K4me3. Remarkably, these same features determine the pattern of chromosomal fragility under replication stress. This result highlights the basic role of DNA replication timing in shaping genome stability during the earliest stages of mammalian embryo development. Our findings reveal that DNA replication timing is among the earliest layers of epigenetic regulation established after fertilization, acting prior to and potentially upstream of the regulation of gene expression and of most epigenetic modifications.

T1225

DOSE-DEPENDENT GSK3-INHIBITION INDUCES A QUIESCENT-LIKE STATE IN MOUSE EMBRYONIC STEM CELLS VIA A POSSIBLE B-CATENIN-INDEPENDENT MECHANISM

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Maintenance of a robust, naïve pluripotent state in mouse embryonic stem cells (mESCs) is typically established in 2i conditions (LIF, MEKi and GSK3i). Although GSK3i results in WNT-activation, the pathway seems to be dispensable in pluripotency. Since GSK3 can target over 40 substrates, and research primarily focused on its role in β-catenin stabilization, other GSK3-mediated functions in pluripotent mESCs are currently overlooked, as well as the individual roles of the different GSK3 isozymes (GSK3α and β). In this study, we unveil that inhibition of GSK3 induces a quiescent cellular state in mESCs in a dose-dependent manner, characterized by the absence of Ki67 (G0), increase in P21 and P27kip1, while maintaining pluripotency. Surprisingly, the expression of WNT/β-catenin target genes, c-Myc and n-Myc, are transcriptionally downregulated upon GSK3i in both WT and β-catenin-/- cells, indicating a β-catenin-independent mechanism controlling the downregulation of these genes in pluripotent mESCs. Functionally, priming mESCs with GSK3 inhibitors provides a protective state (increased healthy cell fraction) after cell death induction. The CRISPR/Cas9-mediated generation of GSK3α-/- and GSK3β-/- mESC cell lines revealed a differential role for the isozymes in terms of proliferation, while maintaining their pluripotent state. SP1 was predicted to be the crucial regulator of the majority of differentially expressen genes derived from our RNA-sequencing dataset of GSK3 inhibited mESCs. SP1-/- cells display a reduction in cell number, decreased c-myc at protein level, but no increase in cell death (Annexin V-positive cells). SP1-/- cells were not able to further reduce cell number in response to GSK3i. Future experiments will further elucidate the role of GSK3i in WT mESCs (proteomics), in mouse embryos (CRISPR/Cas9 KO SP1) and in human blastoids. Our results propose a new molecular mechanism for the induction of pluripotent quiescence through the inhibition of GSK3 in a β-catenin-independent manner, possibly via SP1.

Funding Source: 11L8824N.

T1227

DRAFTING A SINGLE CELL AMNIOTIC FLUID ATLAS AS IT CHANGES ACROSS HUMAN DEVELOPMENT AND IN PRESENCE OF CONGENITAL DISEASE

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The Amniotic Fluid (AF) surrounds and protects the human fetus during gestation. Highly heterogeneous, the AF is rich in primary fetal cells shed from multiple developing fetal tissues as well as fetal membranes and immune system. Despite this, the precise AF cellular composition remains largely unexplored. Here we applied single-cell technologies (i.e. scRNAseq) to over 150,000 uncultured AF cells from 30 AF samples (15-39GA) to generate a comprehensive single-cell atlas of the human AF. The epithelial cluster compose 60-70% of all AF cell identities. Through our work we provided initial annotation of every epithelial tissue contributing to the AF, as well as for each immune cell cluster. The analysis presented here, confirms that AF contains fetal epithelial populations from numerous fetal tissues. This includes specialised cells and progenitors from the fetal lung, kidney, gastrointestinal tract, some of which were capable of forming AF-derived Organoids (AFO). Moreover, progressing to a more comprehensive AF mapping, we annotated the multiple immune cell clusters present in the AF, in order to highlight what identities are consistently present in the AF at each single developmental time. Overall, this work allowed us to track the changes occurring in the AF cell composition along with the progression of fetal and organ development. Finally, to investigate the translational potential of AF single-cell mapping, we generated atlases from the fluids of pregnancies affected by Congenital Diaphragmatic Hernia (CDH, n=8 27-34GA) and myelomeningocele (Spina Bifida; SB n=5 23-25GA). When comparing these samples with our GA-matched reference AF atlas we observed variations in both cell cluster ratio and overall cellular composition. Interestingly we observed the appearance of disease-related AF cell clusters not detected in our reference data. We are currently reviewing the clinical files of the patients who donated the CDH/SB AFs, to investigate possible correlations between clinical outcomes and AF-cell composition. Overall, this work advances our knowledge on the biology of the AF, and its use to study congenital disease. Once completed, this will open the door to the translational use of AF mapping and, in combination with AFO, pave the way for advanced diagnostics and personalised disease modelling.

Funding Source: This work was supported by the Academy of Medical Science, UCL Therapeutic Acceleration Support, GOSH-CC, Kidney Research UK, CDH-UK, NIHR GOSH BRC and Rosetrees Trust. GD is supported by an EMBO Scientific Exchange Grant.

T1229

DUAL ROLE OF OCT4 AND SOX2 IN CONTROLLING THE DEVELOPMENTAL CAPACITY AND DEVELOPMENTAL TIMING OF TISSUE MORPHOGENESIS OF THE EMBRYONIC LINEAGE

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Time is a dimension where events occur in a linear sequence, governed by cause-and-effect, avoiding causality paradoxes. Living systems obey the basic laws of nature that define the cause-and-effect sequence of events. Fundamental processes such as cell fate specification, differentiation and morphogenesis occur in a precise order to avoid conflicts. This indicates the existence of molecular factors that temporally coordinate these events. If their function is disrupted, developmental events could be initiated prematurely. The identification of such factors, their roles and effects of their disruption are of fundamental biological importance. Using the mouse embryo model system, we studied the temporal control of tissue morphogenesis during blastocyst formation. Defects in cell lineage segregation and tissue scale organisation during this process can severely impair further embryonic development. Yet, the molecular control that coordinates tissue morphogenesis and cell fate dynamics in the developing blastocyst is poorly understood. A major driver of embryonic morphogenesis is the establishment of epithelial polarity which forms tissue barriers and luminal spaces at defined developmental time points. While the extraembryonic tissues establish epithelial polarity during the preimplantation stages, the epiblast cells undergo epithelialisation only after implantation, upon exiting naive pluripotency. The developmental significance and factors that control this temporal sequence remain elusive. To decipher this process we focussed on the transcriptional regulation and tissue morphogenesis of the pluripotent lineage. Analysis of genome occupancy dynamics and loss of function effects at sequential stages of the early embryogenesis revealed that the developmental timing of epiblast polarisation is controlled by the cooperative activity of the core pluripotency transcription factors Oct4 and Sox2. Our results indicate that the pluripotent cells are actively sustained in an apolar state, allowing timely segregation of the epiblast and the primitive endoderm, hence laying the foundation to build on the further development of the embryo. Thus, our study has revealed a novel, physiological role of Oct4 and Sox2 in tissue morphogenesis and cell fate dynamics in the early mouse embryo.

Funding Source: European Research Council.

T1231

DYNAMIC TRACKING AND ENRICHMENT OF EARLY FUNCTIONAL HEMATOPOIETIC STEM CELLS INDUCED FROM HUMAN PLURIPOTENT STEM CELLS

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The transplantable therapeutic properties of HSCs and the differentiation potential of mature blood cells have attracted many hematological researchers for years. iPSC cells have made it possible to generate HSCs in vitro. The visualization of hematopoietic stem cells can certainly provide a solution to accurately grasp the time window of their emergence, track their dynamics and development, optimize the hematopoietic stem cell niche, and establish an effective expansion model in vitro. By combining traditional knowledge with bioinformatics and deep machine learning, we have selected highly probable long-term and short-term HSCs fate-determining transcription factors, HOXA9, HLF, TCF16, CD34, and CD82 etc, to be integrated with fluorescent proteins through CRISPR/Cas9 gene editing. Subsequently, we established a new ex vivo differentiation and developmental system by optimizing the induction process and growth factors as well as small molecules to mimic the three-dimensional embryonic development in vivo, that effectively enhances the endothelial-to-hematopoietic transition. hematopoietic transition using an embryoid body (EB) model. In developmental tracing ex vivo of HSC, DsRed+ cells began to appear in the EB organoids from day 10 onwards, followed by outgrowth and shedding, which was showed at day 13, DsRed-dim positive hematopoietic progenitor subpopulation, CD34+CD43+CD45+ HSC cells, with strong multicellular type colony forming capability. The floating cells on day 16, on the other hand, formed more homogeneous erythroid colonies. The HSC visualization iPSC line established in this study provides a powerful tool for ex vivo tracking and expansion of HSC and also enables further in vitro simulation of the optimal spatial location of the HSC niche with EBs.

T1233

E-CADHERIN HOMOPHILIC BINDING IS INVOLVED IN BLASTOCYST-ENDOMETRIUM INTERACTION IN HUMANS

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Understanding human blastocyst-endometrium interaction helps fertility treatment. E-cadherin (CDH1) on the lateral membranes of adjacent epithelial cells is a well-known adhesion molecule for maintenance of epithelial integrity through adherens junction formation between adjacent epithelial cells. The endometrial luminal epithelial cells (EEC) are the first maternal cells that interact directly with blastocyst. Here, we demonstrated that CDH1 expressed in apical region of EEC contributed significantly to the interaction. We revealed that homophilic binding of CDH1 between the

trophectoderm of blastocyst and the EEC mediated attachment of blastocyst surrogate (BAP-EB) onto EEC. Knockdown of CDH1 and its stabilizer CTNND1 in the EEC reduced the expression of adhesion-related molecules, and BAP-EB attachment. Embryo-derived human chorionic gonadotrophin enhanced apical expression of CDH1 in EEC. The data suggested that embryonic signals induced apical expression of endometrial CDH1 to facilitate implantation via hetero-cellular CDH1 homophilic binding.

Funding Source: Innovation Technology commission (Health@innoHK); Health and Medical Research Fund (#04151546, #10212996, #11222296); Grant for Fertility Innovation 2016 (Merck).

T1235

EFFECTS OF TGFB INHIBITORS ON TROPHOBLAST DIFFERENTIATION FROM HUMAN EXPANDED POTENTIAL STEM CELLS

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Abnormal placentation is one of the underlying causes of pregnancy failure such as miscarriages and pre-eclampsia. Substantial progress has been made in deriving trophoblasts to study the early placental development. Human expanded potential stem cells (hEPSC) represent an efficient cellular platform for studying the early differentiation events during human embryo development. By using a human Expanded Potential Stem Cell (hEPSC) line with expanded potentials to both embryonic and extraembryonic lineages, this study aimed at identifying compounds that modulated early human trophectoderm/trophoblast development. A high throughput drug screening was carried out using hEPSC with GATA2 reporter (hEPSC-GATA2). Among the drugs that induced trophoblast development, the effects of three TGFβ inhibitors including RepSox, IN-1130 and SB-525334 on trophoblast differentiation were validated and the minimal effective dose was selected for further study. Real time qPCR and western blotting demonstrated that RepSox-, IN-1130- and SB-525334 treatments significantly induced the mRNA and proteins expressions of trophoblast markers. Besides, ELISA method indicated that RepSox and IN-1130 alone -induced the secretion of MMP2, but not hCG level. Furthermore, the addition of RepSox, IN-1130 and SB-525334 increased the attachment rate of the treated trophoblastic spheroid onto endometrial epithelial cells. In conclusion, RepSox, IN-1130 and SB-525334 can alone induce trophoblast differentiation from hEPSC. The TGFβ inhibition is indispensable for acquiring the attachment potential of trophoblastic spheroids. The identified novel pharmacological drugs and the related cell signalling pathways can provide new information of the early trophoblast differentiation and for future drug development for placental diseases.

T1237

ELUCIDATING THE ROLE OF DELETED IN LIVER CANCER 1 (DLC1) IN HUMAN NEURAL CREST DEVELOPMENT

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Neural crest cells (NCCs), a unique population of multipotent embryonic stem-like cells originating from the neural plate border (NPB), are fundamental to vertebrate development and give rise to diverse cell types including neurons, glia, melanocytes, and craniofacial structures. While the transcriptional network governing NC development is well-characterized, we previously identified an unexpected role for RhoGAP Deleted in liver cancer 1 (DLC1) in chick cranial NC formation. Here, using human induced pluripotent stem cells as a model system, we demonstrate that DLC1 isoforms play distinct, sequential roles in human NC development. By conducting detailed temporal expression analysis, we show that DLC1 isoform 2 is specifically expressed in NPB-like cells and early NCCs, while isoform 1 expression precisely correlates with NC specification markers. Functional studies reveal that knockdown of both isoforms significantly reduces expression of NPB and NC markers, while DLC1 isoform 2 overexpression maintains cells in an NPB state and isoform 1 overexpression promotes NC specification. These results establish that DLC1 isoform switching is essential for the NPB-to-NCC transition. Mechanistically, using mass spectrometry, we identified IGF2BP2 as a potential DLC1 interaction partner. RNA immunoprecipitation studies suggest that both DLC1 and IGF2BP2 could associate with FOXD3 mRNA, a key regulator of NC stem cell self-renewal. This association may be crucial for FOXD3 mRNA stabilization, as functional studies show that DLC1 knockdown reduces both FOXD3 expression and crestosphere size, while FOXD3 overexpression enhances NC self-renewal capacity. Together, these findings reveal a conserved role for DLC1 in NC development between human and chick and suggest a potential post-transcriptional regulatory mechanism controlling NC stem cell maintenance. This work provides new mechanistic insights into human NC development and may inform strategies for developing cellular therapies for treating NC-related diseases.

T1239

ELUCIDATING THE ROLES OF INTESTINAL NICHE FACTORS AND THEIR INTERACTIONS IN HIRSCHSPRUNG DISEASE THROUGH INTEGRATED ANALYSIS

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Hirschsprung disease (HSCR) is a congenital disorder characterized by the absence of ganglion cells in the distal colon. Autologous or allogenic transplantation of enteric progenitor cells/neurons are considered as promising regenerative therapies for HSCR. However, the migration, differentiation, and functional integration of the grafted progenitor cells into the host gut are highly dependent on the interaction with the intestinal neural stem cell niche constituting the postnatal gut microenvironment. To date, the essential intestinal niche factors required for enteric neural crest cell migration and differentiation remain largely uncharacterized. In this study, we conducted a pilot

integrated analysis of whole-genome sequencing (WGS) and bulk RNA sequencing (RNA-seq) on aganglionic and ganglionic colonic segments of a small cohort of HSCR patients to identify the molecular signatures characterizing the niche environment of the colon of HSCR patients. The comparison of expressions in gene and transcript level between aganglionic and ganglionic segments revealed a related set of differentially expressed genes (DEGs). Enrichment analysis of the DEGs further emphasized signaling pathways pertinent to enteric nervous system development and function. In addition, scRNA-seq of colonic tissues from HSCR patients, including neuronal cells and other cell types, demonstrated complementary insights, showing the granularity of gene expression changes in individual cell populations. Moreover, to better understand the factors guiding early ENS development, we also employed a scRNA-seq dataset of mouse embryonic ENS models and through this model we analyzed cell-cell interactions and characterized the expression of key markers in the early stages of ENS generation—insights that can inform how transplanted cells might integrate into the gut in human HSCR. By integrating WGS, RNA-seq, and scRNA-seq datasets, this study provides a glimpse into the potential intestinal niche factors dysregulated in HSCR patients.

T1241

ENDOGENOUS PROTEASE ACTIVITY REGULATES METABOLIC AND CELL FATE PATTERNS

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Pattern formation is one of the most important processes during embryogenesis. It is commonly accepted that pattern formation is mainly driven by morphogen gradient. However, heterogeneous pattern still exists in 2D monolayer culture system that does not have obvious growth factor gradient. In this project, we use 2D monolayer human pluripotent stem cell (hESC) culture to study pattern formation in hPSC colonies. We observe that cell fate pattern is determined by the location of individual cells in a colony, and the spatial distribution can be associated with the pattern of metabolic activities in a colony. We show that endogenous protease influences metabolic pattern through the activation of protease-activated receptor (PAR). PAR pathway can further interact with Calcium and metabolism related signaling pathway that is important for fate pattern. Although under 2D monolayer culture condition, metabolic changes caused by PAR itself are not enough to affect or change cell fate, it can still activate a series of signaling and regulate gene expression. This study highlights novel autocrine feedback important for pattern formation and cell fate determination.

T1243

ENGINEERING A CONTROLLED CARDIAC TRILINEAGE CO-DIFFERENTIATION PROCESS FROM PLURIPOTENT STEM CELLS USING STATISTICAL DESIGN OF EXPERIMENTS

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Pluripotent stem cell-derived cardiomyocytes hold great potential for regenerative medicine and drug screening, but their immaturity remains a major barrier to practical applications. During cardiac development, cardiomyocytes are induced together with non-cardiomyocyte cells from cardiac mesoderm cells (CMCs), and their interactions play a crucial role in functional maturation. Inspired by this principle, we aimed to engineer a co-differentiation process for the simultaneous generation of three major cardiac cell types–cardiomyocytes, mural cells, and endothelial cells–from induced pluripotent stem cells (iPSCs). The key challenge was whether these cell types could be co-differentiated in a controlled manner to achieve a desired cell composition favorable for cardiomyocytes. To address this, we employed design of experiments (DoE), a powerful statistical process engineering approach. Our study consisted of developing two subprocesses: (1) differentiation of iPSCs into CMCs and (2) differentiation of CMCs into the three cardiac cell types. For (1), we used response surface methodology (RSM) with CHIR99021 and Activin A, achieving approximately 95% efficiency of CMCs. For (2), we applied RSM with Wnt inhibitors and VEGF to construct predictive models for co-differentiation into the three cell types, enabling us to select ideal co-differentiation conditions. Validation experiments confirmed that the predicted and actual outcomes were comparable, underscoring process controllability. Furthermore, gene expression analysis on day 40 of differentiation revealed enhanced maturation for cardiomyocytes from the controlled co-differentiation compared to those without non-cardiomyocyte cells. The results highlight the effectiveness of our approach in engineering controlled cardiac co-differentiation processes and suggest broader applicability for developing other differentiation processes where heterotypic cell interactions are important.

T1245

ENHANCED REGENERATIVE AND DEVELOPMENTAL POTENTIAL OF EMBRYONAL AND STEM CELL-DERIVED PLATELETS COMPARED TO ADULT PLATELETS

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The molecular features and multifaceted roles of platelets have been extensively studied in adult mammals, but little is known about platelets at other developmental stages, such as in embryos. In this study, we performed transcriptomic and proteomic profiling of platelets isolated from mouse embryos, revealing that while classic immune-regulatory and procoagulant features were reduced, development-supporting characteristics were particularly prominent in embryonic platelets compared to adult ones. Notably, embryonic platelets showed significantly stronger interactions with multiple cell types, including fibroblasts, and were markedly more effective in accelerating refractory wound repair. We identified a subpopulation of CD59a+ platelets that mirrored the effects of embryonic platelets. Furthermore, human induced pluripotent stem cell (hiPSC)-derived platelets exhibited molecular profiles similar to those of embryonic platelets. Our research underscores the unique multi-omics characteristics of embryonic and hiPSC-derived platelets, highlighting their superior capacity for tissue repair compared to adult platelets. These insights pave the way for advancing therapeutic applications by prioritizing the use of platelets from different developmental stages and sources tailored to specific medical needs.

T1247

ESTABLISHMENT OF A NOVEL NON-INTEGRATED HUMAN PLURIPOTENT STEM CELL-BASED GASTRULOID MODEL

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Embryo loss and pregnancy disorders are prevalent worldwide, with both conditions critically associated with dysfunctioning gastrulation processes. Gastrulation and post-gastrulation organogenesis are crucial stages of embryonic development that establish the blueprint for body part formation. These processes involve the sequential generation of three germ layer cells and primordial germ cells, as well as the assembly of the precursor tissues for body parts. However, due to ethical limitations associated with studying human embryogenesis, a more detailed understanding of gastrulation and post-gastrulation organogenesis remains elusive. To ensure that the knowledge obtained from gastruloids is biologically meaningful and clinically relevant, it is critical to create high-fidelity human embryo models that closely mimic embryogenesis in vivo. Here, we developed a two-stage derivation gastruloids in vitro based on human pluripotent stem cells. Morphological tracking mimicks the developmental processes of models from Carnegie Stage 4 (CS4) to early CS7. Our gastruloids exhibit key structures characteristic of human embryos, including amniotic cavity, embryonic disc, primitive streak, primary yolk sac, secondary yolk sac, and blood islets. Comparison of our cell lineage development maps showed that gastruloids closely resembled human natural CS7 gastrula. Our gastruloids exhibited transcriptional

characteristics that mimicked the molecular pathways observed in natural embryos development. Importantly, we found that in our model, extraembryonic mesoderm originates from the yolk sac and primordial germ cells originate from the posterior epiblast of the embryonic disc. Moreover, we found that thalidomide affects the differentiation of three germ layer cells, resulting in the arrest of human gastruloid development. In conclusion, by establishing a human gastruloid, we were able to gain valuable insights into the mechanisms responsible for human gastrulation and shed light on the causes of early embryo loss and pregnancy disorders.

Funding Source: National Natural Science Foundation of China grant 82221005 (J.S.), 82122025 (Y.Y.). National Key Research and Development Program grant 2022YFC2702800 (Y.Y.), 2021YFC2700302 (Y.Y.), 2021YFC2700200 (Y.C.).

T1249

EXPRESSION OF PIWIL PROTEINS IN HUMAN EMBRYONIC STEM CELLS-DERIVED FOLLICLE-LIKE CELLS

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The ability to generate ovarian follicle/oocyte-like cells from human embryonic stem cells (hESCs) opens up exciting possibilities for reproductive medicine. Understanding the intricacies of female gamete development is crucial for addressing issues such as infertility and ovarian aging, which affect many women worldwide. The utilization of Piwil family proteins in regulating germ cell development adds another layer of complexity and interest to the study. These proteins have been implicated in various aspects of germ cell development, particularly through their interaction with piRNAs. Their stage-specific expression and variation during differentiation suggest their crucial roles in guiding the differentiation process. An undifferentiated human ESC line, SNUhES34, was maintained under the feeder-free condition. The cells were formed into embryoid bodies (EBs) and cultured in suspension for 8 to 10 days. After 10 days, the EBs were attached and further differentiated. During differentiation, the expressions of female germ cell development specific- and the piwil family genes were examined by RT-qPCR. Additionally, the differentiation of hESCs into follicle/oocyte-like cells was confirmed by morphological and immuno-specific analyses. Human ESCs differentiated into follicles/oocytes successfully. The differentiation was monitored by expressions of stage-specific genes. Furthermore, the secretion of E2 was confirmed. The Piwil gene family, Piwil-1,-2,-4, expression was stage-specific and varied during differentiation. Further investigation into the functional aspects of these hESC-derived cells and the specific roles of Piwil proteins could uncover valuable insights into female gamete development and possibly lead to novel therapeutic strategies for infertility or reproductive disorders

Funding Source: 2022R1A2B5B01002541.

T1251

FIBROBLAST GROWTH FACTOR 10 ACCELERATED THE MATURATION OF HAIR-BEARING SKIN ORGANOID BY ACTIVATING A KERATINOCYTE SUBPOPULATION

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Skin serves as a protective barrier against the harshness of the external environment. Advances in stem cell and organoid technologies have enabled the generation of hair-bearing skin organoids and an unprecedented way to probe human skin biology in vitro. However, the long lead time required to make them hampers their ease of application to a wide range of medical research. Here, we aim to reduce the time frame to generate skin organoids. We tested growth factors that are known to influence skin development and identified FGF10 that could reduce the time required for hair placodes to emerge from skin organoids by up to 14 days. Despite FGF10 treatment taking place only transiently in early organoid formation, its effect persisted till later timepoints and resulted in skin organoids developing mature hair follicles earlier than untreated counterparts. Importantly, FGF10 treatment did not cause any defects in mature skin organoids and their cellular composition. Mechanistically, single-cell RNA sequencing analysis revealed a specific intermediate keratinocyte cluster that appeared just before hair placode formation in untreated organoids and which occurred only one week after FGF10 treatment, suggesting that its formation may be a key driver of accelerated skin organoid maturation. Collectively, the present study improved the protocol of skin organoid without any notable abnormality, boosting the potential of skin organoids for future investigation of human skin development and their use as therapeutic grafts.

T1253

FTO AS A KEY REGULATOR OF MESODERM AND CARDIAC MESODERM SPECIFICATION IN HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTE DIFFERENTIATION

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Cardiomyocyte differentiation from human induced pluripotent stem cells (hiPSCs) provides an unlimited resource for studying developmental processes and disease progression. N6-methyladenosine (m6A), the most abundant modification in mammalian mRNA, is implicated in various biological processes, yet its specific function in cardiac commitment remains elusive. Here, we found a significant increase in the expression levels of the demethylation enzyme FTO during the early stages of hiPSC-derived cardiomyocyte (hiPSC-CM) differentiation, followed by a decrease in terminally differentiated cells, suggesting a dynamic regulation of m6A modification during cardiac fate determination. By using the FB23-2, a compound that selectively inhibits the demethylation function of FTO, we confirmed the stage-specific requirement of FTO during hiPSC-CM differentiation. Our findings indicated that FTO is crucial for the determination of the mesoderm

and cardiac mesoderm, essential stages in early cardiac lineage specification, while it appears to have no significant influence on the induction of cardiac progenitor cells. Enrichment analyses of gene ontology (GO) showed that downregulated genes in FTO inhibition group are enriched in heart development, cardiac chamber formation, and ventricular cardiac muscle cell differentiation. Mechanistically, we demonstrated that FTO is vital for the activation of key cardiac transcription factors, such as NKX2-5 and TBX5. These results highlight the intricate regulatory network governed by m6A modification in cardiac differentiation. In conclusion, our study identifies FTO as an essential regulator of hiPSC-CM differentiation and underscores the critical role of precise tuning of m6A modification in facilitating successful cardiac differentiation.

T1255

GENERATION OF A COMPREHENSIVE HSPC ATLAS ACROSS SOURCES AND AGES

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Hematopoietic Stem and Progenitor Cells (HSPCs) are heterogeneous populations composed by more primitive subsets and committed progenitors responsible for supporting hematopoiesis. Thanks to their repopulating potential these cells are extensively exploited in clinical applications such as HSPC gene therapy (GT). Thus, understanding HSPC biological properties is of paramount importance for enhancing both their collection and genetic engineering, with the ultimate goal of improving the outcome of HSPC GT. Recent technological advances considerably increased the throughput of single-cell RNA-sequencing (scRNA-seq) experiments hence, rather than analysing each HSPC dataset independently, data integration enhances the characterization of each subpopulation and enables the analysis of low abundant cell types. Although several scRNA-seq atlases have been released none of them focuses on the most primitive HSPC subset that are crucial for the long-term outcome of HSPC GT. Since CD34 marker is commonly exploited for HSPC enrichment in clinical protocols, we collected and integrated in-house (SR-TIGET) unpublished and publicly available CD34+ FACS-sorted scRNA-seq datasets. Such atlas transcriptionally profiles over 277 thousand HSPC derived from healthy donors throughout ages (pediatric to elderly) and clinically relevant sources (bone marrow, cord blood, peripheral blood, mobilized peripheral blood). Such gene expression matrix was normalized, scaled by the number of transcripts and cell cycle stage, and integrated removing batch effects such as dataset, donor and source. The resulting object was then projected onto lower dimensional space, and transcriptionally homogeneous cells were identified by Louvain algorithm. We found a cluster of low-cycling long-term HSCs, composed by cells from all sources and ages, expressing stem-related genes such as AVP, MLLT3 and CRHBP suggesting the existence of a “universal” HSC population with unique gene signature. Sub-clustering of this compartment will allow to identify the most primitive HSC gene signature, that will be tested across HSPC sources. Overall, this work will allow to identify novel pathways associated with stemness and self-renewal that could be also

exploited to improve the ex-vivo manipulation of HSPCs for their clinical application.

T1257

GENERATION OF HUMAN BLASTOIDS FROM PRIMED HUMAN PLURIPOTENT STEM CELLS USING A ZONA PELLUCIDA MIMICKING HYDROGEL

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Human blastoids provide a promising model for investigating early embryonic development and pregnancy failures—challenges that were previously unattainable to address without the use of human embryos. However, recent protocols for blastoid generation have predominantly relied on human naïve pluripotent stem cells (PSCs). Here, we present a new approach to produce blastoids from the more stable primed hPSC state. The method employs a thermoresponsive hydrogel composed of poly(N-isopropylacrylamide) and polyethylene glycol (PNIPAAm-PEG) to mimic the physical characteristics of the zona pellucida. The hydrogel provides gentle mechanical cues to hPSC aggregates without requiring cell adhesion, thereby preventing cell migration into the gel. The sol-gel transition, mediated by temperature control, facilitates blastoid placement and retrieval at low temperatures (20°C), followed by culture at 37°C. Fine-tuning the hydrogel's viscoelastic properties and reducing cell adhesiveness proved essential for efficient blastoid formation. Single-cell RNA sequencing and RNA velocity analysis revealed the emergence of the three principal cell lineages of the blastocyst, underscoring the physiological relevance of the model. This advance improves the capacity to recapitulate early human embryonic development in vitro and offers a promising platform for future studies on human embryogenesis and reproductive biology.

Funding Source: The Japan Society for the Promotion of Science (17H02083, 21H01728 and 23K17345) New York University Abu Dhabi (NYUAD) Faculty Research Fund (AD366 to KK).

T1259

GENERATION OF STABLE GFP-EXPRESSING IPSCS VIA CRISPR-CAS9 TARGETING OF THE AAVS1 LOCUS

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The integration of stable transgenes into induced pluripotent stem cells (iPSCs) is essential for their application in research and therapy. However, conventional methods often result in random transgene insertion, leading to variable expression and instability over prolonged culture. To address this, we generated iPSCs expressing green fluorescent protein (GFP) by using the CRISPR-Cas9 system to target the AAVS1 safe harbor locus. This approach ensures precise integration and long-term transgene stability, providing a robust platform for studying gene expression consistency during extended cell culture. We successfully targeted the AAVS1 locus and inserted a donor plasmid containing a GFP transgene and a puromycin resistance gene using CRISPR-Cas9. Following puromycin selection, the selected clones were expanded and characterized to confirm their pluripotency. The genome-modified iPSCs (GM-iPSCs) displayed compact colonies with a high nucleus-to-cytoplasm ratio, distinct borders, and well-defined edges, consistent with the morphology of pluripotent stem cells. In addition to these morphological characteristics, the selected clones also expressed GFP. Immunocytochemistry analysis confirmed that the GM-iPSCs were positive for pluripotency markers TRA-1-60, OCT3/4, and SSEA4. Additionally, RT-qPCR demonstrated the expression of core pluripotency genes, including OCT4, SOX2, NANOG, LIN28A, REX1, and E-CAD. The potential of GM-iPSCs to differentiate into all three germ layers was confirmed through positive staining and gene expression of FOXA2 (endoderm), TBXT (mesoderm), and PAX6 (ectoderm). Notably, at passage 10, GM-iPSCs continued to express the GFP protein, confirming the stability of the transgene. These results collectively validate the successful establishment of GM-iPSCs with preserved pluripotency and differentiation potential. This work demonstrates a precise and reliable strategy for stable transgene integration, providing a robust platform for long-term gene expression studies and advancing applications in regenerative medicine and genetic research.

Funding Source: This research is fully funded and supported by PT. Kalbe Farma Tbk.

T1261

HAPLOID EMBRYONIC STEM CELL MUTANT SCREEN IDENTIFIES THE INHIBITORS OF PLURIPOTENT-TO-TOTIPOTENT STATE TRANSITION

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Totipotency is defined as the ability of a single cell to give rise to all cell types within an organism, including both embryonic and extraembryonic lineages. However, the rarity of totipotent embryos and their inability to self-renew in vitro pose significant challenges for elucidating the regulatory

mechanisms underlying totipotency. Two-cell-like cells (2CLCs), which exhibit totipotent-like features, have emerged as a valuable model for studying the transition from pluripotency to totipotency. In this study, we utilized a genome-wide loss-of-function haploid mutant library to identify key regulators of totipotency and identified Periphilin 1 (Pphln1) as a novel factor modulating the conversion of embryonic stem cells (ESCs) to 2CLCs. Pphln1-deficient ESCs demonstrated enhanced potential for differentiation into extraembryonic lineages, as evidenced by embryoid body formation and trophoblast stem cell induction assays. Single-cell RNA sequencing analysis revealed that Pphln1 knockout leads to the emergence of a distinct cell population in an intermediate state between pluripotency and totipotency, which is closely associated with energy metabolism pathways. Mechanistically, we demonstrated that Pphln1 suppresses the pluripotent-to-totipotent transition by reinforcing the epigenetic barrier tri-methylation of lysine 9 on histone H3 (H3K9me3) at LINE-1 retrotransposon loci, thereby inhibiting their reactivation. Collectively, our findings identify Pphln1 as a critical regulator that constrains the dynamic transition from pluripotency to totipotency, providing new insights into the molecular mechanisms governing cellular plasticity during early development.

Funding Source: The CAMS Innovation Fund for Medical Sciences (2021-I2M-1-019 and 2021-I2M-1-024); National Natural Science Foundation of China (32370898); Beijing Natural Science Foundation (5232024).

T1263

HEPSCS-DERIVED EARLY EMBRYO MODEL RECAPITULATES HUMAN EMBRYOGENESIS FROM MORULA TO POST-IMPLANTATION STAGES

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Novel stem cell technologies to generate preimplantation embryo-like structures in large numbers and for high-content screens would help alleviate the need for human embryos to gain fundamental knowledge on human early embryo development and implantation. Using human expanded potential stem cells (hEPSCs), we demonstrate that aggregates of hEPSCs in 3D culture recapitulate key signatures of the human morula, particularly in terms of polarity establishment and activated YAP signalling in the polarized outer cells. These hEPSC-derived morula-like structures (moruloids) develop into blastocyst-like structures (blastoids) composed of three pre-implantation lineages including the epiblast, the trophectoderm and the hypoblast via the triple inhibition of the MEK/ERK, TGFβ and Hippo pathways. Mechanistically, we revealed that TFAP2C regulates the aPKC-dependent polarity establishment in moruloids, which is necessary for blastoid generation. Moreover, the hEPSC-derived blastoids further develop into human pre-gastrulation embryoids with the respective amniotic and yolk sac cavities upon in vitro implantation, recapitulating human peri- and post-implantation developmental milestones. Overall, our newly established human early embryo models from hEPSCs provide an in vitro platform for elucidating the molecular mechanisms orchestrating early human embryogenesis.

Funding Source: This project is supported by Health@InnoHK, Innovation Technology Commission, HKSAR.

T1265

HPSC WITH A GAIN OF 1Q OR 20Q11.21 RETAIN THEIR GROWTH ADVANTAGE DURING NEUROECTODERM DIFFERENTIATION AND MIS-SPECIFY TOWARDS NON-NEURAL LINEAGES

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hPSC frequently acquire chromosomal abnormalities during culture. While these abnormalities provide cells with a selective growth advantage, their impact on differentiation and lineage specification remains poorly understood. This knowledge is critical, as it has significant implications for our understanding of embryonic development, for the use of cells as research tools and for their clinical application. Here we studied 6 aneuploid hPSC lines with gains of 1q or 20q11.21 and 4 isogenic genetically balanced control lines. Competition assays were done by co-culturing fluorescently labelled hPSC with different karyotypes during directed neuroectoderm differentiation in monolayer cultures and embryoid bodies. We found that both aneuploidies retain their selective advantage, with mutant cell loads tripling in 8 days of culture. Bulk and single-cell RNA sequencing showed that both aneuploidies result in cell mis-specification. While 1q cells specified to cranial placode, 20q11.21 cells progressed to surface ectoderm and, remarkably, to amnion, showing that both mutants bypassed the neural lineage and mis-specified to other ectodermal fates. To explore the mechanisms underlying their growth advantage, we performed competition assays with siRNA-treated cells and studied the apoptosis rates of 1q cells over time after bleomycin-induced DNA damage. We found that the 1q gene MDM4 mediates a reduced sensitivity to p53-driven apoptosis in 1q gain cells. Additionally, quantification of γH2AX foci and tracking the ratio of aneuploid cells in culture over time, revealed that high cell density in culture lead to increased levels of DNA damage, promoting the takeover by the mutant cells. The higher BCL2L1 expression in 20q cells not only confers resistance to apoptosis, but also is sufficient to drive the alternative cell fates via TGFβ and BMP signaling. Our work shows that aneuploid cells not only retain their growth advantage during differentiation, but also consistently mis-specify during directed neuroectoderm differentiation, suggesting that this lineage is particularly vulnerable to genetic aberrations. Further, we identify MDM4 as the key driver gene of the selective advantage of gains of 1q, and uncover a novel role for BCL2L1 in modulating TGFβ superfamily pathways to promote alternative cell fates.

Funding Source: Fonds Wetenschappelijk Onderzoek (FWO) and Methusalem Grant.

T1267

HYPOIMMUNOGENIC UNIVERSAL HUMAN IPSC-DERIVED ENDOTHELIAL CELLS FOR IMMUNE EVASION AND BLOOD FLOW RESTORATION IN HUMANIZED PERIPHERAL ARTERY DISEASE MOUSE MODEL

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Despite the increase in the number of peripheral artery disease (PAD) patients, cultivating primary endothelial cells (ECs) from patients for therapeutic angiogenesis is difficult due to time and cost constraints. Human-induced pluripotent stem cells (hiPSCs) can be an attractive substitute because they can be easily induced in vitro while maintaining in vivo efficiency. However allogeneic transplantation of hiPSCs derivatives has the potential to elicit the patient's immune response leading to graft rejection. To address these challenges, we diminished the immunogenicity of hiPSCs through the inactivation of major histocompatibility complex (MHC) class I and II genes to evade T cells. As MHC class knockout renders cells susceptible to NK cell attack, we over-expressed the 'don't eat me signal' CD24. We conducted T cell proliferation assay and activation assay using CD3+, CD4+, and CD8+ T cells. To verify the abilities to evade NK cell attack, we co-cultured NK cells with hiPSC-derived ECs. Based on these in vitro data, we transplanted hiPSC-derived ECs into humanized mouse models generated by injecting CD34+ hematopoietic stem cells from human umbilical cord blood into immune-deficient mice. Using luminescence of luciferase-expressing In Vivo Imaging Systems, we observed that U-ECs survived for longer periods after transplantation compared to WT-ECs, while reducing immune response. We induced humanized PAD model by ligating and excising the femoral artery of the left hindlimb. Then we injected U-ECs, demonstrating the therapeutic efficacy of U-ECs. The U-ECs demonstrated improved survival and contributed to improvement in blood perfusion. We also observed that the alleviation of muscle degeneration was most significant in the U-EC group. These findings suggest that human-engineered U-ECs can be utilized as an "off-the-shelf" cell therapy in PAD patients.

Funding Source: This study was partially supported by the Research Institute for Veterinary Science, Seoul National University, and Korean Fund for Regenerative Medicine (KFRM) grant (22A0101L1-11).

T1269

IDENTIFICATION OF POTENTIAL TRANSCRIPTION FACTORS ENHANCING HUMAN IPSC GENERATION AND CELLULAR INTEGRITY

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Reprogramming human somatic cells into induced pluripotent stem cells (iPSCs) using the well-known OSKM transcription factors holds promise for regenerative medicine. However, the efficiency of this process remains low, with only a small proportion of cells undergoing successful reprogramming. Disease-related mutations are one the considerable factors that affect reprogramming efficacy. Here, we employed RNA-seq to identify five transcription factors (GBX2, NANOGP8, SP8, PEG3, and ZIC1) that significantly increased iPSC colony formation from Parkinson's disease samples. Additionally, we identified three candidate genes (CCN3, POSTN, and PTHLH) associated with enhanced colony stability and genomic integrity in iPSCs. Bioinformatics analyses indicated that GBX2, NANOGP8, SP8, PEG3, and ZIC1 play crucial roles in pluripotency and self-renewal, interacting with key regulators like OCT4, SOX2, NANOG, and KLF4. CCN3, POSTN, and PTHLH were found to contribute to iPSC stability through interactions

with components of the extracellular matrix, Wnt signaling, and factors related to apoptosis and cell cycle regulation. RT-qPCR validation confirmed these findings. Our results underscore the potential of these transcription factors and candidate genes to improve reprogramming efficiency and preserve genomic integrity, prompting further exploration of their underlying mechanisms.

T1271

ILLUMINATING PLURIPOTENT AND DIFFERENTIATION CELL STATES USING THE ALLEN CELL COLLECTION

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The Allen Institute for Cell Science aims to understand the principles by which human induced pluripotent stem cells (hiPSCs) establish and maintain robust dynamic localization of cellular structures, and how they transition between states during differentiation and disease. To accomplish these goals, we used CRISPR/Cas9 to create a collection of endogenously tagged hiPSC lines to illuminate organization in various cell states from pluripotency through differentiation. The tagged proteins include notable membrane-bound and membrane-less cellular organelles, signaling complexes, phase transition markers, transcription factors and differentiation-specific structural and cell identity markers. To date, the Allen Cell Collection consists of 56 tagged cell lines that have undergone extensive quality control testing to ensure genomic, cell biological, and stem cell integrity. Here, we describe the development and application of these cell lines with live dynamic 3D imaging to study key biological processes including cell division, migration, and differentiation into distinct cell types. For example, we have generated TBR2-mEGFP (EOMES) and TBXT-Halo (Brachyury) to follow the early differentiation process during the epithelial-mesenchymal transition, CDH5-mEGFP (Vascular E-cadherin) to characterize endothelial cells, and MYH7-mEGFP (Myosin heavy chain 7) to visualize myofibrils in cardiomyocytes. These endogenously tagged hiPSC lines have allowed us to study the intracellular organization, heterogeneity, and dynamics of various cell states and cell state transitions over time. In addition, we have generated 8 disease cell line collections to study cardiac, skeletal and nuclear disease phenotypes. We will describe the workflows used to generate these collections and highlight some of the applications leading to novel insights on disease progression. Our cell lines, plasmids, 3D images, image analysis and visualization tools, integrated cell models and biological findings are openly available for the research community (www.allencell.org).

T1273

IMPROVING NEURAL TRANSPLANTATION SAFETY AND FUNCTIONAL PREDICTABILITY THROUGH SELECTIVE PROTECTION OF DEFINED POPULATIONS

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Parkinson’s disease continues to be at the centre of hPSC replacement therapy with several clinical trials using human pluripotent suicide cell (PSC)-derived dopamine progenitors currently

underway. While promising, there remains an ongoing need for technology/therapeutic refinement. There persists a need to address the purity of these transplants and the potential for tissue overgrowth risk, regardless of how small. One strategy to perturb concerns of cellular overgrowth is through genomic integration of a suicide-gene systems into the PSC donor material to enable ablation of proliferative cells or the graft entirely post-transplantation. In an advancement, here we developed a PSC line containing a novel suicide cell-based system that can be regulated by tissue-specific promoters to activate the suicide gene switch within both cell and non-cell autonomous identities at specific developmental trajectories of the grafted neural progenitors through administration of a chemical pro-drug. We validate selective ablation of immature cell lineage and non-neuronal populations in vitro when the new line was differentiated toward dopamine neurons, resulting in a significant 3-fold enrichment of MAP2+ neurons and total DA neurons (2.6-fold). We similarly demonstrate the ability to selectively ablate non-neuronal and proliferative populations at 10 weeks following transplantation of midbrain-derived progenitors into immunecompromised PD rodents, with functional recovery restored by 12 weeks. Histological assessment of grafts at 18 weeks revealed a significant reduction in the presence of proliferative cells, glia and non-neural cells complimented by a 35% increase in neurons and DA neurons. This new stem cell tool holds significant implications for improving the safety, purity and predictability of hPSC-derived neural transplants to treat diseases such as PD.

T1275

IN-VITRO LPS ACTIVATION OF MSCS INHIBIT BACTERIAL GROWTH THROUGH CYTOKINE MEDIATED OXIDANT PRODUCTION

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Immunomodulatory protective effects of Mesenchymal stem cells (MSCs) are well established. Physiological activation of resident MSCs during a bacterial infection tends to protect the tissues by neutralizing the bacterial growth or spread. The exact mechanism is not clear however, it is speculated that the transcriptional activation of the pro-inflammatory cytokines and antimicrobial secretomes like reactive oxygen species (ROS) are the possible reason for its antibacterial activity. We currently investigated role of indigenous inflammatory cytokines augmenting ROS both secreted by activated MSCs which were in-turn activated though LPS signifying initial infection effecting subsequent inhibition of bacterial spread. Umbilical cord blood MSCs were activated with low dose LPS (10ng/ml) for 12 hrs. The supernatant was collected post 24 hours for assessment of pro-inflammatory cytokine levels and ROS/RNS and other oxidants. The mRNA expression of the cytokines and tissue bound ROS were determined. The antibacterial studies were done by disc diffusion method using standard reference stains and clinical isolates of selected gram positive and negative bacteria. The results of the LPS activation showed 4 to 7 folds rise in pro-inflammatory cytokines TNF-□, IL-□, IL-6. We did not see significant rise of or IL-8 levels. The anti- bacterial evaluation by disc diffusion showed profound zone of clearance for certain stains of gram positive and negative bacteria in a concentration of cell homogenate dependent manner. The bacterial cells recovered post 6 hrs of disc diffusion showed raise in NADPH oxidase and ROS along with increased lipid peroxidation, DNA damage and carbonylation of proteins. Pro-inflammatory cytokines are known to directly act on the bacterial cells. Further, like any other immune cells activated resident MSCs can eliminate bacteria like innate immune cells at the site of infection or in systemic circulation. In the current in-vitro observation, the increased secretion of the pro-inflammatory cytokines by MSCs have led to increased production of oxidants which in-turn directly

killed bacterial cells.

Funding Source: King Khalid University, Saudi Arabia.

T1277

INHIBITION OF NOTCH SIGNALING DISRUPTS CARDIAC PROGENITOR CELL PROLIFERATION AND CARDIOMYOCYTE FUNCTION IN IPSC-DERIVED CARDIAC ORGANOIDS

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The heart is the first organ to form in the human body and is regulated by genetic, epigenetic, and molecular signaling dynamics. When these processes are disrupted, it can result in the formation of Congenital Heart Defects (CHD), which are the most common and severe birth defect affecting 1% of newborns each year. Notch signaling is a conserved signaling pathway crucial for cell fate decisions during mammalian development, although the exact mechanism it plays in cardiogenesis is not fully understood. Dysfunction in Notch signaling has been linked to congenital defects such as Hypoplastic Left Heart Syndrome. By understanding the molecular mechanisms driving cardiogenesis, we can apply these finding to advancing regenerative medicine applications and developing therapies for congenital heart defects. Previous induced pluripotent stem cell (iPSC) modeling approaches for studying cardiac development have employed 2D cell culture, but full understanding of the molecular mechanisms involves spatial and molecular profiling of multiple cell types which are involved in coordinating the development of the heart. Recent advances using organoid modeling has further advanced our ability to understand the cellular and morphological events which occur during heart development. Here, we utilize pharmacological inhibition of Notch signaling in a human iPSC derived cardiac organoid developmental model to further evaluate the role notch signaling plays in cardiogenesis. Cardiac organoids were treated with Notch inhibitor DAPT throughout the course of a 12-day cardiac differentiation. Organoids were imaged and collected throughout various developmental stages. DAPT treated cardiac organoids exhibited decreased size, impaired chamber formation and reduced contractility. Whole organoid immunofluorescence showed changes in cardiac progenitor and cardiomyocyte populations compared to DMSO control. Overall, these findings further highlight the advantage of cardiac organoid modeling for studying cardiogenesis and will allow us to gain a greater understanding of the complex interactions of Notch signaling and better understand its role in proper cardiac development.

Funding Source: This work was supported by California Institute for Regenerative Medicine (CIRM EDUC4-12751).

T1279

INVESTIGATING THE CONSEQUENCES OF MITOTIC ERRORS IN HUMAN PLURIPOTENT STEM CELLS

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For human pluripotent stem cell (hPSC)-based therapies to enter routine clinical use, ensuring their efficacy and safety is critical. A significant challenge lies in the recurrent chromosomal abnormalities frequently acquired during hPSC in vitro expansion. These abnormalities lead to altered cell behaviours such as enhanced proliferation or reduced differentiation potential, raising concerns for hPSC usability in the clinic. Understanding the mechanisms driving these abnormal variants is essential for eliminating them from cultures. One proposed mechanism for variant emergence is mitotic error. hPSCs are known to exhibit poor mitotic fidelity compared to somatic cells or differentiated counterparts, likely due to an impaired mitotic checkpoint. However, the mechanisms of aneuploidy propagation and consequences of these in hPSCs, are still poorly understood. In this project, we explore chromosome mis-segregation in pluripotent versus isogenic differentiated cells. Using chemical and genetic manipulation techniques combined with timelapse microscopy, we study the acute and long-term cellular responses to aneuploidy induction. Additionally, using aforementioned techniques, we successfully generate a panel of novel aneuploid hPSC lines, and utilise these to study chromosome-specific effects of aneuploidy on cell fate and differentiation. This work provides critical insights into the origins and implications of chromosomal abnormalities in hPSCs, advancing our understanding of how to mitigate these risks for safe and effective clinical applications.

T1281

KDM3A CONTROLS POSTNATAL HIPPOCAMPAL NEUROGENESIS VIA DUAL REGULATION OF THE WNT/B-CATENIN SIGNALING PATHWAY

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Hippocampal neurogenesis, the generation of new neurons in the dentate gyrus (DG) of mammalian hippocampus, plays a crucial role in cognitive and emotional processes. While significant progress has been made in identifying transcription factors and signaling pathways that regulate DG neurogenesis, the epigenetic machinery enabling molecular changes for functional neuron generation from NSPCs remains elusive. The histone demethylase Kdm3a regulates genes that are involved in spermatogenesis, mammalian sex determination, lipid metabolism and cancer development. It was also found to orchestrate the expression levels of pluripotency genes, such as Tcl1 and Nanog, thereby establishing its vital role in maintaining the self-renewal capacity of embryonic stem cells. Despite these findings, the function of Kdm3a in the brain and particularly neurogenesis remains poorly understood. Employing reporter mice and knockout mice models will provide further insights into the specific contributions of Kdm3a to neurogenesis and potentially uncover therapeutic strategies for neurological disorders and injuries. We demonstrate that KDM3A is predominantly expressed in neural stem/progenitor cells (NSPCs) during postnatal DG development using Kdm3a-tdTomato reporter mice. Global or conditional knockout (cKO) of

Kdm3a in NSPCs hinders postnatal neurogenesis, leading to compromised learning and memory abilities, as well as impaired brain injury repair in mice. Intriguingly, Kdm3a regulates the Wnt/β-Catenin signaling pathway through both transcriptional and posttranslational mechanisms. Moreover, we identify that quercetin, a geroprotective small molecule, upregulates Kdm3a and promotes adult hippocampal neurogenesis following brain injury. In conclusion, our study highlights Kdm3a as a crucial regulator of postnatal hippocampal neurogenesis, influencing NSPC proliferation and differentiation via the Wnt/β-catenin signaling pathway. These findings provide important insights into the epigenetic mechanisms governing hippocampal neurogenesis, with potential implications for the development of new therapeutic approaches for neurological disorders and injuries.

Funding Source: This study is supported by GRF/UGC (14112223) and GRF/UGC (14116622).

T1283

LONG-TERM CELL-INTRINSIC DISRUPTION OF OLIGODENDROCYTE PRECURSORS AFTER FETAL GROWTH RESTRICTION

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Fetal growth restriction (FGR) associates with long-term white matter disruption and neurological disabilities but the underlying mechanisms are unclear. Here, we establish mouse FGR models of maternal malnutrition and placental insufficiency that have largely normal neurogenesis but long-term adult undermyelination. We find that the adulthood forebrain myelin deficit is not associated with reduced number of myelin-making cells, oligodendrocyte precursor cells (OPCs) and their progeny, the oligodendrocytes themselves. Instead, FGR triggers a post-mitotic defect in oligodendrocyte growth and myelination. Surprisingly, this defect can be recapitulated with in vitro cultures of newly-formed OPCs from FGR mouse neonates, indicating that it is cell-intrinsic. Our findings together demonstrate that the life-long myelination potential of OPCs is strongly influenced by the exposure to environmental stressors during early development. They also open up a new avenue of mechanistic research into the neurological aspects of the non-genetic developmental origins of health and disease (DOHaD).

Funding Source: The project is funded by a Wellcome Early Career Award (Wellcome Trust) awarded to KHT Mau.

T1285

MAGIK: A RAPID AND EFFICIENT METHOD TO CREATE LINEAGE-SPECIFIC REPORTERS IN HUMAN PLURIPOTENT STEM CELLS

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Precise insertion of fluorescent proteins into lineage-specific genes in human pluripotent stem cells (hPSCs) presents challenges due to low knockin efficiency and difficulties in isolating targeted cells. To overcome these hurdles, we present the modified mRNA (ModRNA)-based Activation for Gene Insertion and Knockin (MAGIK) method. MAGIK operates in two steps: first, it uses a Cas9-2A-p53DD modRNA with a mini-donor plasmid (without a drug selection cassette) to significantly enhance efficiency. Second, a deactivated Cas9 activator modRNA and a 'dead' guide RNA are used to temporarily activate the targeted gene, allowing for live cell sorting of targeted cells. Consequently, MAGIK eliminates the need for drug selection cassettes or labor-intensive single-cell colony screening, expediting precise gene editing. We showed MAGIK can be utilized to insert fluorescent proteins into various genes, including SOX17, NKX6.1, NKX2.5, and PDX1, across multiple hPSC lines. This underscores its robust performance and offers a promising solution for achieving knockin in hPSCs within a significantly shortened time frame.

Funding Source: This work was supported by NSF CBET-1943696 (to X.L.L.), NSF CBET-2319913 (to X.L.L.), NIH R21EB026035 (to X.L.L.), NIH R56DK133147 (to X.L.L.), Huck Innovative and Transformational Seed (HITS) Fund (to X.L.L.).

T1287

METTL3 DEFICIENCY IMPAIRS NEURONAL DIFFERENTIATION IN HESC-DERIVED CEREBRAL ORGANOIDS

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N6-methyladenosine (m6A) modification represents a crucial epitranscriptomic regulatory mechanism in stem cell fate determination. However, its role in human neural development remains largely unexplored. Here, we first systematically profiled m6A modifications during human embryonic stem cell (hESC) differentiation into three germ layers, revealing dynamic m6A patterns associated with lineage specification. Through CRISPR-mediated knockout of METTL3, the catalytic subunit of m6A methyltransferase complex, we identified extensive transcriptional changes during germ layer differentiation, with particularly profound effects on ectodermal lineage commitment. Further investigation using hESC-derived brain organoids demonstrated that METTL3 depletion resulted in severe developmental defects, particularly characterized by reduced organoid size and decreased expression of cortical neuron markers TBR1 and CTIP2. Single-cell transcriptome profiling of day 60 organoids revealed distinct alterations in cellular composition and neuronal differentiation trajectories upon METTL3 deletion. Our findings demonstrate that METTL3-mediated m6A modification is indispensable for human neural development and provide new insights into the epitranscriptomic regulation of brain development.

T1289

MITOCHONDRIAL TRANSCRIPTION FACTOR A CONTRIBUTES TO THE MATURATION OF EMBRYONIC STEM CELL-DERIVED CARDIOMYOCYTES

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Mitochondrial transcription factor A (TFAM) is pivotal for mitochondrial DNA transcription and plays a critical role in regulating mitochondrial biogenesis. Embryonic stem cell-derived cardiomyocytes (ESC-CMs) are recognized as a promising cell source for drug screening and cell replacement therapy; however, their maturation presents significant scientific challenges. The processes of mitochondrial biogenesis and maturation are essential for cardiomyocyte function, yet the underlying mechanisms remain largely unexplored. This study aims to elucidate the influence of TFAM on the maturation of ESC-CMs and to investigate the mechanisms through which mitochondrial biogenesis regulates this maturation. Our preliminary findings showed that knockdown of TFAM decreased mitochondrial biogenesis in ESC-CMs, while overexpression of TFAM significantly enhanced it. Furthermore, knockdown of TFAM negatively regulates the maturation of ESC-CMs. TFAM knockdown was found to reduce upstroke and decay kinetics of calcium transients, and decrease the maximum depolarization rate of action potentials. In addition, TFAM knockdown also decreased the cell size and sarcomere length of ESC-CMs. Collectively, these results indicate that TFAM positively regulates mitochondrial biogenesis and contributes to the functional and morphological maturation of ESC-CMs. In summary, our research highlights the positive role of TFAM in the maturation of ESC-CMs, provides new insights into the development of ESC-CMs and facilitates the future application of ESC-CMs in disease modeling and drug screening.

T1291

GENOMIC SCREEN IDENTIFIES JARID2 AS A CELL CYCLE-ASSOCIATED GENE ESSENTIAL FOR LINEAGE COMMITMENT

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The mechanisms by which the cell-cycle directs embryonic stem cells (ESCs) differentiation outcomes are not fully understood. To gain deeper insights, we performed a CRISPR/Cas9 genomic screen on pre-sorted G1 and G2/M ESCs, identifying hundreds of genes essential for the transition from pluripotency to differentiation. Notably, many of these genes are influenced by the cell cycle state at the onset of differentiation, even if they are not expressed during pluripotency. By incorporating temporal measurements, we revealed a link between the exit from pluripotency and differentiation decisions, demonstrating that fate selection is already influenced during the pluripotent state. Next, we focused on Jarid2, a chromatin factor and key subunit of PRC2.2, which emerged as an essential G2/M-dependent differentiation regulator. The majority of G2/M Jarid2 KO ESCs failed to survive differentiation, while the small fraction that endured exhibited an immature differentiation state, retaining the ability to reactivate pluripotency expression. We showed that Jarid2 selectively recruits RNA polymerase II Ser5 to genes involved in extra-embryonic endoderm-like (XEN-like) differentiation specifically during G2/M explaining the preference of G2/M cells to activate XEN. Using cell-cycle pre-sorted ESCs and single-cell RNA-seq, we further characterized Jarid2's role in ESCs differentiation; While EpiSC-like cells did not

require Jarid2—indicating their status as a premature differentiation state. XEN-like cells originated mostly from persisting G2/M ESCs, exhibited a mixed expression with pluripotent markers. Moreover, the activation of more defined states, such as mesendoderm and pre-epithelium were unable to activate without Jarid2. Additionally, chromatin immunoprecipitation for H3K27ac revealed that the loss of Jarid2 disrupts the activation of enhancers critical for differentiation while promoting H3K27me3 deposition, resulting in the repression of pluripotency.In summary, our screen identified a novel set of cell-cycle-sensitive genes, providing a valuable resource for the stem cells research community. Our focus on Jarid2 further elucidated its pivotal role in regulating early steps of differentiation

T1293

MONITORING, PREDICTING AND IMPROVING CELL FATE AND DIFFERENTIATION NON-DESTRUCTIVELY USING THE AI PLATFORM FATEVIEW

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Identifying the regulatory inputs that control and improve cell differentiation to a target cell fate is a fundamental requirement to unravel the mechanisms of organismal development and design strategies to produce cell therapy products efficiently, reproducibly and cost-effectively. Endpoint analytical methods - like immunohistochemistry and omics – are unable to yield continuous insights into cell fate dynamics and transitions, which can only be inferred. By contrast real-time analysis using e.g. genetically-encoded fluorescent reporters can highlight cell fates ‘live’ in a population and yield continuous fate dynamics but is lengthy to implement, cell-type specific and can report only on few cell states simultaneously. We developed FateView, a technology platform that leverages live-cell imaging, computer vision and AI to enable real-time, non-invasive scalable and predictive monitoring of cell fate dynamics at single-cell level in heterogeneous cell populations. FateView works flexibly across cell types and applications and uses label free imaging for cell predictions, thus requiring no lengthy cell line generation and enabling multiple cell fates to be co-monitored. FateView provides current cell state readouts, predicts future cell states, measures Critical Quality Attributes (CQAs) and differentiation potential and acts as visual digital twin that can evaluate millions of processes in silico and suggest actions to improve outcomes. Using human Pluripotent Stem Cells (hPSCs), cardiac progenitor cell (CPC) differentiation and retinal pigment epithelial (RPE) cell maturation as exemplars we show that FateView can detect differentiation bias differences across multiple stem cell lines within hours of applying differentiation triggers, significantly faster than conventional endpoint assays. We also show that optimal cell differentiation conditions vary between cell lines, enabling precise line-specific protocol optimisation. Remarkably, all this can be conducted without sacrificing or disaggregating cells or interrupting cell differentiation processes. By providing a robust flexible and non-invasive platform for monitoring cell fate live, FateView paves the way for novel discoveries and offers a powerful technology to improving cell-based therapies and disease modelling.

T1295

MULTIPOTENT NEURAL STEM CELLS ORIGINATION FROM NEUROEPITHELIUM EXIST OUTSIDE OF THE MOUSE CENTRAL NERVOUS SYSTEM

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It has been widely accepted that mammalian neural stem cells (NSCs) exist only in the central nervous system. Here, we report that peripheral NSCs (pNSCs) exist outside of the CNS and can be isolated—from mouse embryonic limb, postnatal lung, tail, dorsal root ganglia, and adult lung tissues. Derived pNSCs are distinct from neural crest stem cells, express multiple NSC-specific markers and exhibit cell morphology, self-renewing capacity, genome-wide transcriptional profile, epigenetic features, and differentiation ability similar to those of control brain NSCs. pNSCs are composed of Sox1+ cells and originate from neuroepithelial cells. Single-cell RNA sequencing identifies that pNSCs in situ have similar molecular features to NSCs in the brain. Furthermore, many pNSCs that migrate out of the neural tube can differentiate into mature neurons and limited glial cells during embryonic and postnatal development. Our finding of the existence of pNSCs provides previously unidentified insight into the mammalian nervous system development and presents an alternative potential strategy for neural regenerative therapy.

Funding Source: This project is supported by Health@InnoHK, Innovation Technology Commission, HKSAR.

T1297

N6-METHYLADENOSINE SAFEGUARDS FORMATIVE COMPETENCE FOR LINEAGE ENTRY

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The role of N6-Methyladenosine (m6A) abundances in formative pluripotency remains undefined. We examined mouse and human in vitro models enabling primordial germcell-like-cell (PGCLC) specification and somatic differentiation. Depletion of m6A by Mettl3 or Mettl14 knock-down or pharmacological METTL3 inhibition impairs germline entry. In mouse epiblast-like cells, m6A depletion increases embryonic Ras (Eras) dependent PI3K-AKT signaling. ERAS up-regulation lowers EZH2 coordinated H3K27me3 histone mark deposition, disrupting Oct4 distal enhancer silencing required for germline competence. In m6A depleted mouse formative stem cells, Eras facilitates the precocious up-regulation of somatic and germline transcription factors (TFs), including the germline repressor Otx2. Depletion of m6A in human formative cells elevates FGF dependent ERK activation. ERK up-regulates OTX2, repressing germline TFs upon PGCLC induction. Both mouse and human germline entry deficiency is accompanied by skewed somatic lineage differentiation. With the aid of genome-wide sequencing approaches, we identify how m6A

orchestrates signaling dependent enhancer silencing and TF expression, critical for robust lineage competence.

Funding Source: Helse Sør-Øst Norwegian Research Fund (Project No. 39907) and the Norwegian Research Council 661 (Project No. 247656).

T1299

NUCLEOLAR RNA HELICASE DDX21 IS REQUIRED FOR EMBRYONIC STEM CELL RENEWAL

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DDX21 is a nucleolar RNA helicase known to regulate RNA metabolism, including ribosomal RNA (rRNA) transcription and processing, ribosomal protein gene transcription, mRNA splicing, RNA modifications, and resolving R-loop structure. Knockout (KO) of Ddx21 in mice is embryonic lethal, suggesting that the helicase is essential for embryonic survival. To elucidate the function of DDX21 in embryonic stem cells (ESCs), we performed gene knockout and knockdown in ESCs. No homozygous ESC KO clones could survive during expansion. DDX21 knockdown (DDX21-KD) ESCs showed poor clonogenicity, which can be rescued by overexpressing wild-type DDX21 but not helicase-dead DDX21, suggesting that the helicase activity is required for the ESC self-renewal and proliferation. Transcriptomic analysis indicated the downregulation of ribosome biogenesis, nuclear division, and activation of the p53 pathway. Inhibition of p53 could also rescue ESC clonogenicity. Thus, loss of DDX21 may trigger a p53-dependent nucleolar stress that attenuates ESC proliferation. We also found a change in the nuclear heterochromatin in DDX21-KD cells. Analysis of chromatin accessibility by ATAC-seq revealed a decreased open chromatin near the transcription start sites. We thus hypothesized that DDX21 regulates ESC self-renewal through an epigenetic mechanism. By co-immunoprecipitation, we demonstrated that DDX21 could bind the Polycomb repressive complex 2 (PRC2). As PRC2 is known to maintain stem cell pluripotency by repressing lineage-specific gene expressions, our results suggested that DDX21 plays a role in maintaining an epigenetic state for ESC self-renewal by interacting with PRC2 complex, besides its established function in ribosome biogenesis.

T1301

OPTIMIZING NON-VIRAL GENOME EDITING WORKFLOW FOR INDUCED PLURIPOTENT STEM CELLS (IPSCS) WITH ADVANCED ELECTROPORATION TECHNOLOGY

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Induced pluripotent stem cells (iPSCs) have become an essential research platform to study various human diseases and hold significant potential for clinical development. The CRISPR/Cas9 system represents another breakthrough in biomedical research, enabling precise genome editing in mammalian cells. In our current workflow, we utilized the Neon™ NxT Electroporation System with the 8-Channel Pipette for rapid optimization of electroporation parameters for editing iPSCs. This system is a powerful tool for streamlining the optimization of electroporation programs and conditions for primary cells and cell lines. We demonstrated the system's performance in efficiently optimizing CRISPR/Cas9-based non-viral genome editing of iPSCs. Using the Neon™ NxT 8-Channel Pipette, we screened different gene delivery and editing conditions in high throughput format and generated chimeric antigen receptor (CAR)-iPSCs by delivering CD38-targeted anti MESO3 donor DNA and CTS™ HiFi™ Cas9 to iPSCs. Editing efficiency and cell viability were measured using flow cytometry and Next Generation Sequencing. Our results highlight the capacity to simultaneously test multiple electroporation conditions, significantly reducing the time required for optimization experiments. Through systematic screening of voltage, pulse duration, and pulse number, we optimized the delivery of CRISPR/Cas9 ribonucleoprotein (RNP) complexes while maintaining high cell viability. Furthermore, we evaluated different electroporation buffers and achieved up to 70% CD38 knockout and up to 30% CAR-positive iPSCs. These findings emphasize the critical role of buffer components in transfection efficiency and cell viability. In summary, the Neon™ NxT Electroporation System has proven to be an effective tool for the rapid and efficient optimization of non-viral genome editing in iPSCs. This capability significantly advances iPSC-based research and therapeutic applications, addressing scalability issues and improving patient outcomes.

T1303

PCBP1 ORCHESTRATES AMINO ACID METABOLISM INTENSIFICATION DURING THE NAÏVE-TO-PRIMED PLURIPOTENCY TRANSITION

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Mammalian embryogenesis from fertilization to implantation represents a series of cleavages without significant embryo growth. The connection established between the embryo and the uterus after implantation in turn provides the necessary nutrients for further development and extensive growth of the body. This process also implies drastic metabolic changes in the cells of the epiblast, which undergo transition from naïve to primed pluripotency. Previously, embryos lacking the poly(rC)-DNA/RNA-binding protein Pcbp1 have been shown to exhibit a peri-implantation lethal phenotype, but the mechanisms underlying this phenotype are still unknown. In our study, using

the multi-omics assays RNA-seq, ChIP-seq, shotgun proteomics, and metabolomics, we show that Pcbp1 is dispensable for the characteristics of naïve and primed pluripotency, but is crucial for the intensification of amino acid metabolism during the transition between the two states. Naïve embryonic stem cells deficient for Pcbp1 are viable, but their priming results in failed upregulation of genes related to amino acid uptake, serine/proline biosynthesis, and tRNA charging. As a result, the level of several amino acids decreases and protein synthesis declines, causing proliferation slowdown and apoptosis. In vivo, this phenotype manifests itself in a severe reduction in size and subsequent death of the embryos within a few days after implantation. In conclusion, our study describes a key role of Pcbp1 in early embryogenesis and helps to understand the anabolic mechanisms that enable substantial embryo growth after implantation.

Funding Source: The study was supported by the Russian Science Foundation (RSF) grant № 23-75-10096, https://rscf.ru/en/project/23-75-10096/.

T1305

PHENOTYPIC AND TRANSCRIPTOMIC CHARACTERIZATION OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED SCHWANN CELLS/SCHWANN CELL PRECURSORS FOR PERIPHERAL NERVE REGENERATION

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Peripheral nerve injury (PNI), a common form of peripheral neuropathy, is a widespread and debilitating health problem that affects millions of patients globally. Despite the limited nerve regenerative capacity of peripheral nerves and current advances made in nerve reconstruction, a majority of patients with PNI does not regain satisfactory functions after surgical intervention. Limitations have also been found in current surgical approaches for PNI, and therefore, new therapeutic strategies are urgently required to improve not only the treatment outcomes but also the quality of patients' lives. Schwann cells (SCs) are the most abundant glial cells of the peripheral nervous system and are crucial for axon growth, neuronal support, and nerve repair. They and their precursors (i.e. Schwann cell precursors SCPs) are considered potential cell types for embedding into nerve guidance conduits for transplantation to promote nerve regeneration after PNI. However, sources of human autologous SCs/SCPs are limited. Here, we presented an induction strategy for generating a stable source of functional SCs/SCPs from human induced pluripotent stem cells (hiPSCs) for incorporation to nerve guidance conduits. A combination of phenotypic assays and single-cell RNA sequencing (scRNA-seq) analyses of induced SCs/SCPs enabled comprehensive classification and understanding of the cellular profiles and transcriptomes of hiPSC-derived SC/SCP subtypes. Our study provided evidence for the robust induction of functional SCs/SCPs and demonstrated the important roles of different biological and biophysical cues provided by SCs/SCPs for peripheral nerve regeneration. These cell types may serve as therapeutic tools to improve iPSC-based therapies for peripheral neuropathy.

Funding Source: The work was supported by the General Research Fund (Ref.: 14120522 and 14118818) from the Research Grants Council, and the research fund from Health@InnoHK program by the Government of Hong Kong Special Administrative Region, China.

T1307

PLAKOGLOBIN AS A REGULATOR OF PLURIPOTENCY - WHAT HAVE WE OVERLOOKED?

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Biomechanical cues are instrumental in guiding embryonic development and cell differentiation. Understanding how physical stimuli regulate transcriptional programs provides key insights into mechanisms underlying mammalian pre-implantation development and pluripotency transitions. We explore this regulation using a microfluidic platform to encapsulate mouse embryonic stem cells in agarose microgels, exerting precise microenvironmental control. This approach stabilizes the naive pluripotency network and specifically induces the expression of Plakoglobin (Jup), a vertebrate homolog of β-catenin and potential modulator of WNT/β-catenin signaling. Overexpression of Plakoglobin re-establishes the naive pluripotency gene regulatory network under metastable conditions, as confirmed by single-cell transcriptome profiling. Finally, we find that, in the epiblast, Plakoglobin was exclusively expressed at the blastocyst stage in human and mouse embryos – further strengthening the link between Plakoglobin and naive pluripotency in vivo. Our findings reveal Plakoglobin as a pivotal mediator of the interplay between biomechanical signals and transcriptional regulation, offering new perspectives on WNT pathway dynamics and cell-fate decisions during early embryogenesis.

T1309

PLURIPOTENT STEM CELL DERIVED CULTURED MEAT WITH MULTIPLE TISSUES

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Cellular cultivated meat (CM) as a sustainable meat production method holds significant potential to reduce challenges associated with environmental sustainability, global public health, and animal welfare. The acquisition of seed cells is a key aspect in the research and application of CM, and embryonic pluripotent stem cells (EPSCs), with their potential for continuous self-renewal and multi-directional differentiation, are considered ideal seed cells for CM development. However, long-term stable passage and directed differentiation of these cells in vitro have always been technical bottlenecks. Guided by insights from a large-scale single-cell transcriptome analysis of preimplantation embryos, we successfully established technical systems for isolation and culture of porcine, bovine, and sheep stable EPSCs, which enables long-term stable passage of EPSCs (over hundred generations) while maintaining pluripotency and genomic stability. Impotently, using these pig EPSCs, we developed a serum-free directed induction differentiation system, and achieve serum-free myogenic differentiation of the EPSCs. The EPSC-derived muscle progenitor cells and muscle fibers show typical muscle cell characteristics and display skeletal muscle transcriptional features. We further establish a three-dimensional differentiation system for shaping cultured tissue by screening plant-based edible scaffolds of non-animal origin, followed by the generation of EPSC-derived cultured meat. Additionally, we further realized the directed induction differentiation of PSCs into multiple tissues such as fat and endothelium, providing possibilities for the creation of nutritionally customized CM. We hope that our study could enrich our understanding for the self-renewal mechanisms of pluripotent stem cells between different species, as well as for the application of stem cells in the food field.

Funding Source: This work was supported by the Biological Breeding-National Science and Technology Major Project (2023ZD04075).

T1311

PREDICTING BLASTOCYST FORMATION USING 3D LIVE EMBRYO IMAGING

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We present quantitative whole-embryo imaging of live mouse embryos for systematic 3D feature analysis of preimplantation development. To this end, we introduce low-coherence holotomography, a method capable of 3D refractive index imaging of unlabeled live embryos. Leveraging the measured 3D embryo tomograms, we identified cellular and subcellular 3D features associated with the morphology, spatial arrangement, and protein concentration of internal structures. Using these features, we then set out to establish a predictive model for blastocyst formation, achieving an accuracy of 95.71 %. This work highlights the potential of these features as

noninvasive biomarkers of embryo quality and provides 3D subcellular insights into preimplantation development.

Funding Source: National Research Foundation of Korea grant funded by the Korea government (MSIT) (RS-2024-00442348); a grant of the Korea Health Technology Research and Development Project through the KHIDI funded by the Ministry of Health and Welfare (HR22C1605).

T1313

RANDOM MONOALLELIC GENE EXPRESSION IN EARLY EMBRYONIC LINEAGES

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Recent studies based on allele-specific RNA-sequencing analysis revealed clonal random monoallelic expression (clonal RME) of autosomal genes in different cell types. Clonal RME represents stable maintenance of paternal-monoallelic or maternal-monoallelic expression of genes in specific clonal cell populations, along with stable biallelic expression in other clones. Thus, clonal RME creates stable yet heterogeneous allelic expression patterns within a particular cell type. Considering the potential of clonal RME in shaping heterogenous gene expression during early embryonic development, we wondered whether clonal RME contributes to the dynamic cell fate transitions. To address this, we have profiled the clonal RME landscape in early embryonic lineages, namely extraembryonic endoderm (XEN) stem cells, trophoblast stem cells (TSC) and epiblast stem cells (EpiSC) through allele-specific RNA-sequencing analysis. Importantly, our genome-wide analysis revealed that the majority of the RME genes in XEN, TSC and EpiSC were lineage-specific. Interestingly, we found only a few common RME genes across XEN, TSC and EpiSC, all of which are highly crucial for early embryonic development. Furthermore, our preliminary analysis depicts that the clonal RME landscape gets altered with cell state transition in XEN cells, suggesting the plausible relevance of clonal RME in cell fate specification. Separately, we also found that clonal RME is stable across multiple passages in the XEN cells. Altogether, we delineate that stable clonal RME might quantitatively fine-tune gene expression dosage to drive cell fate specification events during early embryonic development. These findings could foster the broader understanding of how clonal RME may contribute to gene expression variability potentially influencing the incomplete penetrance and variable expressivity of autosomal disorders.

T1315

RE-ENGINEERING THE METHYLATION READER FUNCTION OF KLF4 ENABLES PLURIPOTENCY INDUCTION WITHOUT THE SOX2/OCT4 DUO

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KLF4 is a pioneer transcription factor directing reprogramming towards pluripotent and multipotent stem cells. This activity is tied to its capacity to bind silenced chromatin, such as methylated CpG and nucleosome core particles. To dissect the basis for KLF4's reprogramming activity and to enhance its function, we designed saturation mutagenesis libraries by randomizing positions that are critical to binding epigenetically modified DNA. Using pooled screens in mouse pluripotency reprogramming, we identified several evolved KLF4 (eKLF4) variants that change the reprogramming activity of wild-type KLF4. Notably, two double mutants in KLF4 support iPSC generation without the otherwise essential SOX2/OCT4 duo. The iPSCs derived from eKLF4 and c-MYC (eKM) exhibit all the molecular, cellular, and functional characteristics of embryonic stem cells. The eKM iPSCs display robust developmental potential and capacity for germline transmission. Mechanistically, eKLF4 differentially activates mesenchymal-to-epithelial transition, early pluripotency marker genes at the onset of eKM reprogramming and opens up chromatin regions that are less accessible to wild-type KLF4, potentially facilitating the induction of pluripotency. In vitro, eKLF4 shows a high affinity for nucleosome core particles and methylated DNA binding elements compared to the wild-type protein. Optimized pioneer factors are poised to transform personalized regenerative medicine, amplify the potential of stem cells, and facilitate rejuvenation by partial reprogramming.

T1317

RESETTING STEM AND SOMATIC CELLS TOWARD A STATE WITH ENHANCED POTENTIAL

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Human pluripotency with expanded potential can be generated through chemically defined cultures, enabling cells to form both embryonic and extraembryonic tissues, thereby advancing developmental potential and genome engineering. However, current human pre-implantation stage stem cell models face challenges, such as genomic instability, reliance on harsh chemicals and inter-batch variations and lack of culture consensus across laboratories. Blastoid and embryo models based on these chemically induced cells sometimes show incomplete germ layer specification and developmental arrest at early stages. Therefore, we sought to develop an alternative genetic strategy for reset somatic cells or primed pluripotent stem cells into a pre-implantation pluripotent stage. Through systematic optimization, we enhanced the performance of key reprogramming factors while minimizing their genetic payloads. We then developed an efficient system to fast convert human fibroblasts into induced expanded potential stem cells using these optimized factors. We further extended this system to wildlife species where pluripotency reprogramming was unavailable. Lastly, we screened multiple transcription factor combinations to achieve direct conversion of primed human pluripotent cells to a state with enhanced developmental potential. This rapid and robust approach for generating developmentally potent stem cells has broad implications for embryology research, genetic therapies, endangered species preservation, and regenerative medicine applications.

Funding Source: This research is supported by Health@InnoHK, the Innovation and Technology Commission of the Government of the HKSAR.

T1319

RFX3 IS ESSENTIAL FOR THE DEVELOPMENT AND MATURATION OF HUMAN PANCREATIC ISLETS DERIVED FROM PLURIPOTENT STEM CELLS

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We recently identified the role of Regulatory Factor X 6 (RFX6) in human islet development, revealing how biallelic mutations in RFX6 contribute to monogenic diabetes and hypoplastic pancreas formation. However, the role of RFX3 in pancreatic islet development remains unexplored. In this study, we investigated RFX3 function using iPSC-derived islet organoids. RFX3 knockout (KO) iPSCs were generated via CRISPR/Cas9 and differentiated into pancreatic islet organoids. We evaluated gene expression, cell markers, apoptosis, proliferation, and function. Single-cell RNA sequencing (scRNA-seq) and bulk RNA sequencing were performed to examine RFX3 expression and transcriptomic changes, with RFX3 overexpression used to reverse dysregulated gene expression. RFX3 was highly expressed in pancreatic endocrine cell populations at various stages, including pancreatic progenitors (PPs), endocrine progenitors (EPs), and mature islets derived from hESCs and iPSCs. scRNA-seq further confirmed RFX3 expression across different endocrine cell clusters during differentiation. Loss of RFX3 disrupted pancreatic endocrine gene regulation, reduced islet cells, and impaired beta-cell function and insulin secretion. Despite a significant reduction in all pancreatic islet hormones, the pan-endocrine marker CHGA remained unchanged, due to an increase in enterochromaffin cells (ECs), which are also known to express CHGA. This was further supported by elevated expression of EC markers, including SLC18A1, FEV, CDX2, and LMX1A, in RFX3 KO EPs and islets, suggesting a shift in cell lineage commitment. Furthermore, RFX3 loss resulted in smaller islet organoids, and increased apoptosis, associated with elevated levels of the pro-apoptotic gene TXNIP in EPs and islets. Restoration of RFX3 expression in KO pancreatic cells rescued the dysregulated expression of endocrine genes and corrected the defective phenotypes, underscoring its critical role in pancreatic islet development. Our study highlights RFX3 as a key regulator of human pancreatic islet cell differentiation and a suppressor of EC lineage specification, which is linked to beta cell immaturity. These findings highlight RFX3's role in islet biology and diabetes, with potential to improve islet cell differentiation for diabetes therapies.

Funding Source: This work was funded by grants from QBRI (QBRI-HSCI Project 1) and from Sidra Medicine (SDR400217).

T1321

SCA-1-BASED PROCEDURE FOR PURIFYING ADIPOSE-DERIVED MESENCHYMAL STEM CELLS WITH ENHANCED PROLIFERATIVE AND DIFFERENTIATIION POTENTIAL

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Adipose-derived mesenchymal stem cells (ADSCs) hold great promise for regenerative medicine due to their accessibility and multipotent differentiation capabilities. However, the purification of high-purity ADSCs remains a major challenge, hindered by inconsistent surface marker profiles and contamination with non-target cells. The inconsistency in results from preclinical and clinical studies largely arises from experimental models, especially mice, with developing ADSC surface markers. In this study, we developed a novel Sca-1-based purification strategy—adherence to the third generation followed by magnetic cell sorting (ADSC-AM)—to enhance the yield, purity, and functionality of ADSCs. Our comparative analysis of three purification methods—direct adherence (ADSC-A), magnetic cell sorting followed by adherence (ADSC-M), and ADSC-AM—demonstrated that the ADSC-AM method consistently outperformed the others. ADSC-AM cells exhibited a uniform spindle morphology, enhanced proliferative capacity, and superior adipogenic differentiation potential. Additionally, ADSC-AM achieved over 95% expression of Sca-1 and CD29, a critical improvement in purity. RNA sequencing and pathway enrichment analysis further revealed that ADSC-AM cells uniquely exhibited increased potential for osteogenic differentiation, angiogenesis, and immune regulation, highlighting their enhanced functional properties. This optimized Sca-1-based procedure represents an innovative and efficient approach for isolating ADSCs with superior purity and functional properties, providing a solid foundation for advancing research in stem cell-based therapies.

Funding Source: Support by the Fundamental Research Funds for the Central Universities [2022CDJYGRH-014], and Chongqing Medical Scientific Research Project [2023ZDXM012].

T1323

SEQUENCE DETERMINANTS FOR NUCLEOSOME BINDING BY POU FACTORS LINKED TO CELL FATE CHANGES

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Pioneer transcription factors (PTFs), are a special class of TFs that can bind directly to their target DNA sites even when located on closed chromatin. PTFs bind silent chromatin and initiate regulatory events. However, a disconnect exists between a PTF's cellular function and its biochemical ability to bind nucleosomes. This highlights a key gap in our understanding of what constitutes a PTF and whether the nucleosomal context of its binding site influences its pioneering activity.Oct4 and Brn2 present an interesting pair of homologous TFs with seemingly disparate pioneering activity. Oct4, a well-known PTF essential for stem cell maintenance and pluripotency, contrasts with Brn2, a collaborating TF in neural reprogramming. This provides an opportunity to more clearly dissect the characteristics that define a pioneer factor by contrasting Oct4 with Brn2. Here, we performed binding assays with Widom 601 nucleosomes and free DNA with various POU motifs (Octamer, CpGpal, MORE). We demonstrate that Oct4 exhibits significantly higher affinity and specificity for nucleosomes containing the Octamer motif compared to Brn2. Structural modelling reveals that Oct4's binding mode varies depending on the motif: a monomeric configuration for the Octamer, a potential dimer for CpGpal, and a monomer for MORE. In contrast,

Brn2 demonstrates non-specific binding to nucleosomes regardless of the motif. These findings emphasize the critical role of specific DNA sequences in mediating selective nucleosome targeting by PTFs, potentially differentiating them from collaborating factors. Further research into POU factor-nucleosome interactions will clarify their implications for gene regulation and potentially enable the engineering of improved cell fate-driving proteins.

T1325

SERINE SYNTHESIS PATHWAY CONTROLS CARDIAC INDUCTION OF HUMAN PLURIPOTENT STEM CELLS

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Metabolism is well known as an active driver of physiological changes rather than merely a housekeeping process during development. With the expanding understanding of metabolic control over cell fate specification, metabolic regulation is now recognized as a potential signaling axis in the regulation of development. Human pluripotent stem cells (hPSCs) hold significant potential for advancing our understanding of heart development. Differentiating hPSCs into cardiac cells allows for modeling early cardiac developmental processes and exploring key signaling pathways. However, variations in differentiation efficiency and poor reproducibility of hPSC-derived cardiomyocytes (hPSC-CMs) production have remained a challenge. Here, we report a unique metabolic method to promote hPSC-CM differentiation that involves marked suppression of the mitochondrial oxidative phosphorylation from the mesendoderm to the cardiac mesoderm, which is regulated by PHGDH, a rate-limiting enzyme in the serine synthesis pathway (SSP). Mechanistically, the analysis of metabolomics and single-cell RNA-sequencing revealed that SSP inhibition impaired mitochondrial respiration by blocking the electron transport chain, resulting in elevated reactive oxygen species (ROS) levels and promoting the cardiomyocyte lineage specification by disrupting the cardiopharyngeal mesoderm lineage differentiation. Additionally, antioxidant supplementation can scavenge ROS and eliminate the effects of SSP inhibition. Collectively, our findings show that SSP can regulate cardiac lineage specification and have implications in providing a cellular source for transplantation and elucidating the potential mechanisms of heart development and pathogenesis of heart disease.

T1327

SEX CHROMOSOME OVERDOSAGE ALTERS EPIGENOMIC AND TRANSCRIPTOMIC LANDSCAPES OF NEURAL STEM CELLS AND IMPAIRS THEIR NEUROGENIC POTENTIAL

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Sex chromosome overdosage in Klinefelter Syndrome (KS; 47,XXY), Jacobs Syndrome (JS; 47,XYY), and high-grade sex chromosome aneuploidies (SCAs; 48,XXXY and 49,XXXXY) results in a spectrum of clinical manifestations, including intellectual disabilities and delays in motor, speech, and language development. The severity of neurological symptoms correlates with the number of supernumerary sex chromosomes; however, the in vitro modeling of these diseases remains largely unexplored. In this study, we employed an iPSC-based approach to elucidate the effects of sex chromosome aneuploidies on early neurodevelopment. Differentiation of iPSCs from individuals with KS, JS, high-grade SCAs, and 46,XY controls into neural stem cells (NSCs) and neurons revealed X chromosome dosage-sensitive impairments in NSC differentiation and survival. Neurons derived from high-grade SCAs exhibited reduced neurite branching, diminished number of MAP2-positive dendrites, and failed to establish functional networks. Integrated methylation and transcriptomic profiling of NSCs demonstrated widespread epigenomic and transcriptomic alterations in X aneuploidies, characterized by X-linked hypermethylation proportional to X chromosome dosage. In contrast, Y chromosome aneuploidy was associated with subtle epigenomic changes. We quantified gene expression changes across X and Y chromosome complements using linear regression modeling. Our analysis uncovered modular transcriptomic alterations specific to X or Y chromosomes, with ~30% of these changes shared between KS and JS. Notably, a subset of X-linked genes escaping X-inactivation, located in the pseudoautosomal (PAR) regions of X and Y chromosomes, defined both X and Y transcriptomic signatures and may contribute to the overlapping neurodevelopmental deficits observed in SCAs. To further dissect the molecular mechanisms, we developed 46,XY NSCs overexpressing individual candidate PAR genes, identifying their roles in transcriptomic dysregulation associated with aneuploidies. Additionally, we created an open-access, web-based platform to facilitate exploration of the epigenetic and transcriptomic landscapes of SCAs, offering a valuable resource for investigating the genetic basis of X and Y overdosage during early neurodevelopment.

T1329

SINGLE-CELL AND SPATIAL MULTIOMIC INFERENCE OF GENE REGULATORY NETWORKS USING SCRIPRO

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The burgeoning generation of single-cell or spatial multiomic data allows for the characterization of gene regulation networks (GRNs) at an unprecedented resolution. However, the accurate reconstruction of GRNs from sparse and noisy single-cell or spatial multiomic data remains challenging.Here, we present SCRIPro, a comprehensive computational framework that robustly infers GRNs for both single-cell and spatial multiomics data. SCRIPro first improves sample coverage through a density clustering approach based on multiomic and spatial similarities. Additionally, SCRIPro scans transcriptional regulator (TR) importance by performing chromatin reconstruction and in silico deletion analyses using a comprehensive reference covering 1292 human and 994 mouse TRs. Finally, SCRIPro combines TR-target importance scores derived from multiomic data with TR-target expression levels to ensure precise GRN reconstruction. We benchmarked SCRIPro on various datasets, including single-cell multiomic data from human B-cell

lymphoma, mouse hair follicle development, Stereo-seq of mouse embryos, and Spatial-ATAC-RNA from mouse brain. SCRIPro outperforms existing motif-based methods and accurately reconstructs cell type-specific, stage-specific, and region specific GRNs. Overall, SCRIPro emerges as a streamlined and fast method capable of reconstructing TR activities and GRNs for both single-cell and spatial multiomic data. SCRIPro is available at https://github.com/wanglabtongji/SCRIPro.

T1331

SOX AND POU TRANSCRIPTION FACTORS ARE OLDER THAN STEM CELLS AND ANIMALS

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Stem cells are regarded as a key feature of animal multicellularity. The transcription factors SOX and POU, crucial regulators of pluripotent stem cells, were traditionally thought to be unique innovations of animals. Our research challenges this notion by identifying Sox and POU homologs in the closest unicellular relatives of animals. We found that unicellular SOX proteins bind DNA similarly to mammalian Sox2 and can induce pluripotent stem cells, a capability also exhibited by ancestrally reconstructed Ur-Sox proteins dating back 700 million years. In contrast, unicellular POU proteins have distinct DNA-binding profiles and cannot induce pluripotency. Our findings suggest that the evolution of stem cells utilized pre-existing set of transcription factors, involving significant changes in POU DNA-binding specificity and the exaptation of ancestral interactions with SOX factors. This study expands our understanding of the origins of stem cell-associated transcription factors and illuminates the evolutionary processes that facilitated the transition from unicellular to multicellular life, providing new insights into the molecular foundations of cellular differentiation and regeneration.

T1333

SPATIOTEMPORAL MOLECULAR ARCHITECTURE OF WHOLE MOUSE EMBRYOS AT GASTRULATION AND EARLY ORGANOGENESIS

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During mammalian embryogenesis, temporal and spatial regulation of gene expression and cell signaling influences lineage specification, the patterning of tissue progenitors and the morphogenesis of embryo. Gastrulation and organogenesis are the processes that the cells of the three germ layers transform into an embryo that includes most of the major internal and external organs within a short timeframe. In previous research, we have reconstructed the molecular trajectory of the gastrulating embryos. Here, we performed Geo-seq+ technology to reconstruct a spatial molecular atlas encompassing embryonic and extraembryonic tissues in mice, ranging from the egg cylinder (E5.5) to the early stage of organogenesis (E8.75), at 6-hour intervals for a total of 14 stages. This spatiotemporal transcriptome provides genome-wide digitized gene expression profiles at the resolution of a few cells and defines the molecular characteristics of the genealogy of lineages and continuum of organic primordium in time and space. Remarkably, we systematically revealed the molecular regulatory network of patterning along the anterior-posterior and dorsal-ventral axes during endodermal organogenesis and uncovered interactions between the splanchnic mesoderm and gut endoderm during their differentiation into various organ primordia. Our findings shed light on the spatial patterning of axes, the systematic temporal regulation of developmental progression, and the spatial interactions among inter-germ layer lineages that orchestrate gut organogenesis, providing insights into the intricate mechanisms underlying organogenesis.

T1335

STEM CELL-NICHE INTERACTIONS IN TISSUE MAINTENANCE AND ENGINEERING

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Stem cells are a group of cells with unique capacities to self-renew and differentiate into specialized cells, which are vital for the maintenance of tissue homeostasis throughout the lifespan under the regulation of their niche. Despite the well-known importance of stem cell-niche interactions in tissue maintenance, the molecular mechanisms underlying their roles in various biological processes and diseases are not yet fully understood. Here, we made use of fruit fly (Drosophila melanogaster), zebrafish (Danio rerio), nematode (Caenorhabditis elegans), mouse (Mus musculus), and human induced pluripotent stem cells (iPSCs) as a model to comprehensively investigate the stem cell-niche interactions in tissues maintenance and ultimately engineer niche and stem cell-based tissues. In the last year of the project, 1) we have identified new secreted niche factors (NetA and NetB) for germline stem cells (GSC) self-renewal, new niche factors for GSC progeny differentiation (condensin II and Mirror), and 29 new niche factors (e.g., Tet, BAP55, BAP111, and Pus1) involved in controlling germline stem cells (GSC) aging using Drosophila ovary. 2) Besides, we also investigated intrinsic factors, ATF3 and H2B in muscle stem cell (MuSC) activation and proliferation and uncovered two intrinsic factors, JUNB and CCR5, for driving MuSC aging using Zmpste24 mutant mice and human samples. 3) On the other hand, biotechnologies, including somatic niche cell co-culturing, B12-dependent photo-regulatable proteins, and SpyTagged spider silk protein have been developed in our project to build up the artificial niche for stem cell maintenance and expansion in vitro. Taken together, these findings enable a deeper understanding of stem cell-niche interactions in biological processes and diseases, providing solid

evidence for further development of therapeutic strategies targeting stem cells and their niche.

Funding Source: RGC Theme-based Research Scheme (T13-602/21N).

T1337

STRAIGHTFORWARD IN VITRO EMBRYO MODEL BY CONFLICTING TRANSCRIPTIONAL DETERMINANTS OF EARLY MOUSE EXTRAEMBRYONIC AND EMBRYONIC DEVELOPMENT THROUGH INCREASED STAT3 SIGNALING

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The segregation of inner cell mass (ICM) and trophectoderm (TE) marks the first cell fate defining milestone in mouse early embryogenesis. The ICM gives rise to embryo proper, while TE conduct implantation via direct interaction with the uterus and forms the fetal portion of the placenta. During early embryo development, ICM and TE derivatives exhibit dynamic regulative interactions and inter-conversion, where TE derived lineages play a pivotal role in tuning embryonic development. However, the suboptimal research approaches and ethical concerns hinder the feasibility to dynamically monitor these interactions from pre-implantation to post-implantation stages. Although several studies have shed light to the field by generation of in vitro embryo model mimicking key developmental events, these embryo models mostly rely on the mixture of cell types cultured in distinct culture conditions and often fail to sustain TE-derived lineages or progress beyond the pre-implantation stage. Here we report a straightforward mouse embryo model generated exclusively via increased JAK/STAT3 signaling in single sourced embryonic stem cells (ESCs). Through modulating JAK/STAT3 activation level, ESCs efficiently reprogram into intermediates co-expressing conflicting cell fate determinants OCT4, CDX2 and GATA6. The intermediates exhibit molecular features of both ICM and TE lineages, which further undergo spontaneous cell fate determination in petri-dish. The resulting ICM and TE -like cells share synchronized developmental status where transcriptomic analysis confirms the activation of pre-implantation TE-specific signaling pathways. Upon aggregation, the intermediates can self-assemble into a pre-implantation embryo-like structure containing both embryonic and extraembryonic tissues in vitro with high efficiency. This model holds great developmental potential, exhibiting strong morphological and molecular interaction resemblance to a gastrulating embryo upon prolonged culture, with coordinating embryonic and extraembryonic compartments. Our embryo model provides a robust, single-cell-derived system to recapitulate synchronized early embryo development, offering a simplified yet effective platform to study critical embryogenesis events in vitro.

T1339

THE CONNECTION BETWEEN FGF SIGNALING PATHWAY AND ANTIOXIDANTS IN HUMAN EMBRYONIC STEM CELL MAINTENANCE

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The FGF signaling pathway maintains pluripotency and promotes self-renewal in human embryonic stem cells (hESCs). Previous research suggests that hESC differentiation upon FGF deprivation is followed by ROS production and expression of antioxidant enzymes. Therefore, there may be a link between the FGF signaling pathway and antioxidants. In this report, we have established a new culture condition that maintains the pluripotency of hESCs without the support of FGF2, instead incorporating a combination of antioxidant compounds. We found that these antioxidant compounds endowed hESCs with the ability to withstand FGF inhibition-induced differentiation by MEK inhibitor. Besides, They are also essential for cell survival. Further analysis showed that AKT2 overexpression helped hESCs survive without the help of antioxidants in the new culture system. Therefore, both AKT phosphorylation levels and downstream signaling of the AKT pathway remain to be tested. This study suggests that antioxidants may regulate cell death-related pathways to replace the function of the FGF signaling pathway.

T1341

THE LAMININ-511-FUNCTIONALIZED FIBRIN GEL SUPPORTS IN-GEL PROLIFERATION OF HUMAN PLURIPOTENT STEM CELLS

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Three-dimensional (3D) gel culture systems bridge the gap between in vivo microenvironments and conventional two-dimensional in vitro culture, enabling more physiologically relevant cell behaviors. Fibrin, a hydrogel widely used in surgical applications, is also used in regenerative medicine as a scaffold for 3D culture of stem cells because of its biocompatibility and biodegradability. However, fibrin gels exhibit limited cell adhesion, except for platelets, restricting their utility in cell-based therapies. To overcome this limitation, we engineered a bi-functional fusion protein, Chimera-511, by linking the N-terminal self-polymerization domain of fibrinogen to the C-terminal integrin-binding domain of laminin-511 via their heterotrimeric coiled-coil domains. Chimera-511 binds fibrinogen in a thrombin-dependent manner and retains its integrin-binding activity in a fibrinogen/fibrin-bound form. Fluorescence imaging showed that GFP-labeled Chimera-511 colocalized with fibrin, confirming its integration into the gel. When cultured in the Chimera-511-functionalized fibrin gel, human iPS cells stably proliferated into a sphere-like structure and maintained their undifferentiated state (Oct3/4, Nanog, SSEA-4 expression) and pluripotency (trilineage differentiation potential) for at least five passages. These findings demonstrate that Chimera-511-functionalized fibrin gel provides a 3D microenvironment suitable for stem cell cultivation, offering a versatile platform for both basic research and clinical applications involving stem cell manipulation.

T1343

THE USE OF EMIRATI INDUCED PLURIPOTENT STEM CELLS (IPSCS) AS AN IN-VITRO MODEL TO STUDY AND CONTROL OBESITY

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Obesity remains a pressing public health issue in the Gulf region including UAE, with studies identifying a strong association between the FTO gene variant rs9939609 and increased obesity risk. Individuals with the A/A genotype are more prone to obesity, while those with the T/T genotype are more likely to maintain a healthy weight. The FTO gene encodes an m6A RNA demethylase, which regulates key RNA metabolic processes involved in adipogenesis. While human studies on FTO function yield mixed results, animal models consistently show that FTO deficiency leads to reduced body weight, whereas overexpression results in increased adiposity. This study aims to examine the role of FTO genetic variants in adipogenesis, using induced pluripotent stem cells (iPSCs) derived from individuals with the A/A and T/T genotypes. These iPSCs will be differentiated into adipocytes, including brown and beige subtypes, to explore the impact of FTO-related m6A modifications on adipocyte function. To broaden the scope of our analysis, we will also investigate additional obesity-linked FTO variants, such as rs1421085 and rs8050136, known to influence thermogenesis and adipose tissue gene expression. Finally, we will evaluate the effects of small molecules that modulate FTO activity to better understand their potential to influence adipogenesis and restore metabolic balance. By integrating genetic, molecular, and pharmacological approaches, this research study aims to uncover the mechanisms linking FTO genetic variants to obesity and identify potential therapeutic targets for metabolic disorders.

T1345

TRACING HYPOBLAST FOR BLOOD ORIGIN IN HUMAN EMBRYONIC MODELS
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Previous reports suggest that hypoblasts acquire plasticity in their cell fate through epigenetic remodeling. To date, however, whether human hypoblasts contributed to other cell types than yolk sac endoderm, whether hypoblasts are transient cell populations or contribute to embryo part is unclear. Even more important, how hypoblast cell fate is epigenetically regulated through the interaction with other embryonic tissues is also unknown. Based on our preliminary data and previous observations in stem cell-derived embryo models and human embryos, we hypothesize that hypoblast might directly contribute to hematopoietic lineage by opening the loci of hematopoietic programs. To investigate the hypothesis, we use novel molecular barcoding system and computational methods to determine the lineage of hypoblast in stem cell-derived embryo models with expressive barcodes and cross-comparison with human and non-human primate embryos. We also investigate the epigenetic regulation of hypoblast cell fate which provide insights into how to instruct their lineage by forced expression of transcription factors. Besides deepening our knowledge of human embryonic development, these insights will help us to understand how to derive functional cells from human stem cells for cell therapy, disease modeling, and drug discovery.

Funding Source: GRF.

T1347

TRANSDIFFERENTIATING OF GABA RECEPTOR-POSITIVE CEREBELLAR GRANULE CELL PRECURSORS FROM JDP2-NULL MICE INTO PURKINJE CELLS
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Jun dimerization protein 2 (Jdp2) gene is expressed in mouse cerebellar granule cell progenitors (GCPs), where its encoded protein plays a pivotal role in cerebellum lobule formation through programmed cell death. However, the precise role of Jdp2 in cellular differentiation, pluripotency, and its interaction with antioxidative mechanisms in the cerebellum remains poorly understood. In the present study, we investigate the impact of N-acetyl-L-cysteine (NAC) on the early commitment of granule cell precursors (GCPs) to neuronal fates, particularly Purkinje cells, through the γ-aminobutyric acid type A receptor α6 subunit (Gabra6) axis. We find that Jdp2-depletion enhances the differentiation of GCPs towards Purkinje cells in vitro. The antioxidative action of NAC drives this differentiation process, particularly in the presence of Gabra6, suggesting that NAC can potentiate the transdifferentiating of GCPs into Purkinje cells. These findings highlight the potential of antioxidative treatments as therapeutic strategies for rescuing oxidative stress-induced damage in GCPs and guiding their differentiation into Purkinje cells.

T1351

UNRAVELLING THE ROLE OF CDON IN MESODERMAL SPECIFICATION AND HEART DEVELOPMENT USING A HUMAN ORGANOID MODEL

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CDON (Cell adhesion molecule-related/downregulated by oncogenes) is a key regulator in the Sonic Hedgehog (Shh) and N-cadherin signaling pathways, essential for heart development. Whole-body Cdon knockout mouse models display significant embryonic lethality, highlighting Cdon's critical role in early embryogenesis. Additionally, Cdon-deficient mouse embryonic stem cells show impaired cardiomyocyte differentiation, underscoring its importance in cardiac development. However, insights into CDON's role in human cardiac development remain limited due to the inherent differences between human and animal models, as well as the lack of robust in vitro systems to recapitulate early heart morphogenesis. To address this gap, we generated human heart organoids from CDON-knockout induced pluripotent stem cells (iPSCs) to study its role and underlying mechanisms in human heart development. Our findings reveal that CDON deficiency leads to abnormal lateral plate mesoderm specification, leading to aberrant germ layer patterning and disrupted cardiogenesis. These defects were associated with dysregulated Wnt signaling gradients, emphasizing CDON's crucial role in maintaining Wnt signaling balance during mesodermal and cardiac development. Furthermore, the organoids revealed structural and functional abnormalities, including reduced cardiac marker expression and disrupted organoid architecture. By leveraging human heart organoids, this study provides novel insights into the essential role of CDON in human cardiac development and offers potential therapeutic strategies to address cardiac dysfunctions resulting from CDON deficiency.

T1353

DECIPHERING THE MECHANISMS OF DNMT3A REGULATION ON HUMAN EPSC CELLULAR POTENCY

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Human enhanced pluripotent stem cells (ePSCs) represent a significant advancement in stem cell research, offering a unique platform for exploring cellular potency and differentiation potential. The epigenetic landscape of human ePSCs is pivotal for understanding their unique cellular potency and differentiation capabilities. However, the specific epigenetic mechanisms that govern the unique properties of ePSCs compared to traditional human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) remains elusive. DNA methylation, a key epigenetic modification, plays a crucial role in regulating gene expression and cellular identity during development and cellular differentiation. DNMT3A is a key enzyme involved in establishing DNA methylation patterns, crucial for maintaining pluripotency and guiding differentiation. This study investigates the role of DNMT3A-mediated de novo methylation in regulating the epigenetic landscape and cellular potency of human ePSCs. By employing genome-wide methylation profiling and Cleavage Under Targets and Tagmentation (CUT&TAG) assay, we elucidate the specific contributions of DNMT3A to the epigenetic regulation of ePSCs. Using tandem affinity purification coupled with mass spectrometry, we identified a few ePSCs-specific transcription factors that interact with DNMT3A for its specific localization in ePSCs. Furthermore, we explore the interplay between DNMT3A and other epigenetic modifiers, highlighting its role in shaping the dynamic epigenome during lineage specification. This study provides critical insights into the mechanisms by which DNMT3A-mediated de novo methylation influences the cellular potency of ePSCs, underscoring its potential as a therapeutic target for enhancing stem cell functionality in regenerative medicine and disease modeling.

T1355

SPATIOTEMPORAL CONTROL OF SOX2 DOSAGE DEFINES PROSENSORY VERSUS NONSENSORY CELL FATE DURING OTOGENESIS

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Cell fate determination is orchestrated by precise key regulators expression. Sox2, one of the stemness factors, is both necessary and sufficient to drive prosensory fate during inner ear development. Through the study of the genomes of two Sox2 hypomorphic mutants, Sox2Ysb and

Sox2Lcc, novel early acting otic regulatory elements were identified in the disrupted region downstream of Sox2 in these mutants. The data indicate the dynamic otic expression of Sox2 is controlled by multiple temporal- and spatial- specific enhancers. We have demonstrated the dosage-dependent requirement of Sox2 on otic prosensory lineage decision. Reduced SOX2 level does not only change prosensory cell fate to a nonsensory ES/ED progenitor-like cell fate, as evidenced by the molecular signatures of the mutant cells starting from early OV stage, but also promotes apoptosis in mutant cells at later stage. Additionally, we showed BMP/TGFbeta signalling is required for both activating and restricting Sox2 expression during early and late otic development, respectively. The results are consistent with a temporal-specific role of Sox2 expression, under many delicate layers of both cis and trans regulations.

Funding Source: Health and Medical Research Fund #05163176, Research Grants Council and University Grants Council of Hong Kong (HKU7385/02M; AoE/M-04/04), National Institute of Health USA (RO1 DC 005590) and the Medical Research Council.

T1357

DECIPHERING ENDOTHELIAL AND MESENCHYMAL ORGAN SPECIFICATION IN VASCULARIZED LUNG AND INTESTINAL ORGANOIDS

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To explore the co-development of vasculature, mesenchyme, and epithelium crucial for authentic organogenesis and organotypic characteristics acquisition, we developed a human pluripotent

stem cell-derived organoid system encompassing lung or intestinal epithelium surrounded by organotypic mesenchyme and vasculature. Mesoderm-endoderm co-differentiation, which is essential for subsequent gut tube organoid vascularization, was exclusively modulated by BMP. Simultaneously, the BMP signal also dictated anterior-posterior gut specification prior to the conventional primitive gut tube formation period. Single-cell RNA-seq analysis uncovered organ-specific endothelium and mesenchyme from vascularized organoids, identifying key ligands driving endothelial specification in lung and intestine. Organotypic endothelium exerted tissue-specific barrier function, enhanced epithelium maturation, and supported alveolar structure formation on bioengineered scaffolds. Upon transplantation in mice, these organoids matured further, forming perfusable organ-specific human capillaries. The spatial transcriptomic analysis confirmed the similar spatial organization of ligand-receptor pairs in the transplanted vascularized lung organoid compared to those found in human fetal lung tissues, which facilitated the characterization of lung endothelium and epithelium. Additionally, our model recapitulated abnormal endothelial-epithelial crosstalk in patients with FOXF1 deletion or mutations. Multilineage organoids provide a unique platform to study developmental cues guiding endothelial and mesenchymal cell fate determination, and investigate intricate cell-cell communications in human organogenesis and disease.

T1359

CHARACTERIZATION OF GMP-COMPLIANT INDUCED PLURIPOTENT STEM CELL (IPSC) MASTER CELL BANK FOR CLINICAL APPLICATIONS

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The generation of induced pluripotent stem cells (iPSCs) by reprogramming adult somatic cells, such as skin or blood cells, has recently unlocked numerous applications in disease modeling, personalized medicine, regenerative medicine, and cell therapies. These include treatments for skin regeneration, neurodegenerative diseases, cardiac conditions, and retinal disorders. Characterization of iPSC Master Cell Banks (MCBs) is critical to ensuring the quality, safety, and efficacy of iPSC-derived products, particularly for clinical use. Comprehensive characterization ensures compliance with stringent global regulatory standards. uBriGene has successfully established a GMP-compliant iPSC Master Cell Bank (MCB) by reprogramming fibroblast cells collected from a clinically qualified donor using a DMF-registered mRNA-LNP cocktail. The process involved clone selection, seed bank generation, and MCB manufacturing. The iPSC bank underwent a comprehensive testing panel to evaluate morphology, cell viability, growth, stability, stemness, and pluripotency. Stem cell markers, including OCT4, SOX2, NANOG, TRA-1-60, SSEA-4, and TRA-1-81, were analyzed using flow cytometry. An embryoid body formation assay was conducted to confirm the pluripotency of the iPSC bank, demonstrating its ability to differentiate into all three germ layers. Additionally, karyotyping analysis was performed to ensure genetic stability by detecting chromosomal abnormalities. The iPSC bank's safety testing panel included assessments for sterility, mycoplasma, and endotoxins, along with in vitro and in vivo screenings for adventitious agents. The GMP-compliant iPSC banks, generated using mRNA-LNP and thoroughly characterized, will serve as a valuable tool for advancing safe and effective iPSC-based clinical applications.

T1361

TARGETING USP11 COUNTERACTS SFTPCI73T-ASSOCIATED PULMONARY FIBROSIS IN HIPSCS-DERIVED ALVEOLAR ORGANOIDS AND IN VIVO MODELS

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Interstitial lung disease (ILD) is a progressive pulmonary disease characterized by inflammation and fibrosis in the lung parenchyma, often driven by dysfunction of alveolar type 2 epithelial cells (AT2s). Surfactant protein C (SFTPC), produced exclusively by AT2s, plays a critical role in maintaining lung homeostasis by regulating the surface tension of pulmonary fluids. However, mutations in SFTPC, particularly I73T, lead to its misfolding and toxic accumulation, triggering subsequent pulmonary fibrosis (PF) associated with toxic gain of function. Although the association between mutant SFTPC and fibrosis is well established, the mechanism regulating its stability remains poorly understood. In this study, we aimed to provide a detailed understanding of the mechanism that regulates the stability of the SFTPCI73T mutant protein during the progression of PF and to suggest a novel therapeutic approach for fibrosis. To address this, we identified USP11 as a novel deubiquitinase (DUB), which is an important protein stabilizer of SFTPC, and confirmed the strong interaction including the deubiquitinating and stabilizing effect on SFTPCI73T mutant protein. To mimic SFTPCI73T-induced fibrosis, we generated human induced pluripotent stem cell (hiPSC)-derived alveolar organoids (AOs) carrying the I73T mutation and successfully recapitulated key fibrotic features. Interestingly, we confirmed that the spontaneous fibrotic changes caused by the mutation were significantly attenuated by the reduction of USP11. Furthermore, we demonstrated that pharmacological inhibition of USP11 alleviates the fibrotic lesions of PF in hiPSCs-SFTPCI73T-AOs and BLM-induced mouse model, underscoring its therapeutic potential. Our study establishes a novel ILD model using gene-edited hiPSC-derived AOs and paves the way for targeted treatment of PF by regulating SFTPC stability through USP11 inhibition, suggesting a novel therapeutic strategy for patients with PF.

Funding Source: This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MSIT) (RS-2022-NR067319).

Poster Session 2 (EVEN)
5:00 PM – 6:00 PM

TRACK: DISEASE MODELING AND DRUG DISCOVERY (DMDD)

T1002

INVESTIGATING THE NEUROTOXICITY MECHANISM OF SODIUM CHLORATE USING IPSC-DERIVED DOPAMINERGIC NEURONS AND ZEBRAFISH

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Sodium chlorate ($NaClO_3$), a strong oxidizing agent widely used in industrial and agricultural applications, poses potential risks to both human health and the environment. This study aimed to investigate the dose-dependent neurotoxic effects of $NaClO_3$ using human induced pluripotent stem cell (iPSC)-derived dopaminergic neurons (DAn) and zebrafish as models, with a focus on understanding implications for environmental pollution. We treated iPSC-derived DAn and zebrafish with 10, 30, and 50 mM concentrations of $NaClO_3$, observing significant effects only at the highest concentration (50 mM). In DAn, 50 mM $NaClO_3$ treatment led to a marked increase in apoptosis, accompanied by upregulation of cell cycle regulators P16 and P21, indicating stress-induced senescence and apoptosis specifically in iPSC-derived neurons. Additionally, zebrafish treated with 50 mM $NaClO_3$ exhibited notable reductions in movement distance and speed, suggesting impaired motor function. qPCR analysis revealed that $NaClO_3$ at this concentration significantly downregulated tyrosine hydroxylase (TH) expression in zebrafish and upregulated genes associated with oxidative stress (CAT, GPX1, SOD1/2) and apoptosis (Bcl2b, Caspase 3b, Caspase 8, Caspase 9) in the brain. These findings indicate that $NaClO_3$ exhibits significant neurotoxicity at higher concentrations, particularly affecting iPSC-derived dopaminergic neurons and motor behavior through oxidative stress and apoptosis pathways. Given these neurotoxic effects, high concentrations of $NaClO_3$ in industrial waste and agricultural runoff may pose serious risks to aquatic ecosystems and organisms, highlighting the importance of regulating $NaClO_3$ emissions to prevent environmental and ecological harm.

Funding Source: This work was supported by the National Science and Technology Council [grant numbers: NSTC 112-2314-B-038-043].

T1004

INVESTIGATING THE USE OF ALLELE-SPECIFIC GENOME EDITING IN A RARE AUTOSOMAL DOMINANT FORM OF BLINDNESS

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Best disease is an autosomal dominant inherited macular disease, caused by over 600 individual mutations in the BEST1 gene, leading to degeneration of the Retinal Pigment Epithelium (RPE) and central vision loss. Currently, there are no treatments for Best disease. We explore the therapeutic potential of targeted inactivation of the mutant BEST1 allele in patient-derived induced Pluripotent Stem Cells (iPSCs), using a mutation agnostic CRISPR/Cas9 dual-guide strategy targeting common Single Nucleotide Polymorphism (SNP)-induced Protospacer Adjacent Motif (PAM) sites (SiPAMs) in the dominant allele. A skin biopsy was taken from a patient with Best disease (BEST1, c.37C>T, p.R13C) reprogrammed into iPSCs using episomal vectors. SiPAMs

were identified in the dominant BEST1 allele by PCR amplification and phase sequencing. Synthetic sgRNA guides targeting two SiPAMs and spCas9 were nucleofected into iPSCs as ribonucleoproteins. Editing of the dominant allele was confirmed by PCR and Sanger sequencing. RPE was generated via a directed differentiation protocol. An iPSC line carrying the BEST1 pR13C mutation was generated and pluripotency was confirmed. Immunostaining of BEST1R13C iPSC-derived RPE revealed mislocalisation of BEST1 and flattened cell morphology. Phase sequencing of BEST1 highlighted seven SNPs unique to the dominant allele, of which two generated SiPAM sites that flanked BEST1 start codon. Dual sgRNAs nucleofection of iPSC and clonal expansion revealed clones had the predicted excision of 1672bp in the dominant allele, while the wildtype allele remained unedited. Basolateral Best1 protein expression and polarised RPE cell morphology was restored in the edited iPSC-derived RPE, compared to BEST1R13C cells. We have demonstrated that targeting two SiPAMs specific to the dominant BEST1 allele excises the start codon, inactivating mutant BEST1 expression without affecting the wildtype allele. Our preliminary data shows the restoration of RPE morphology and protein localisation in patient cells, highlighting this approach as a potential therapy for Best disease. We will perform Next Generation Sequencing in our Best disease cohort to identify additional mutant allele-specific SiPAMs, enabling the development of a mutation-agnostic guide library to broaden therapy accessibility.

Funding Source: University College London, Moorfields Eye Charity.

T1006

IPSC MODELS OF ALPK3 CARDIOMYOPATHY REVEAL DYSREGULATION OF PROTEIN QUALITY CONTROL NETWORKS

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The pumping function of the heart relies on the precise regulation of a highly specialised cytoskeleton, known as the sarcomere. These sarcomeres respond to calcium to produce the force required for systemic blood flow. Variants in the genes encoding sarcomeric proteins are strongly linked to a complex group of heart disease, called cardiomyopathy. While the aetiology of cardiomyopathies is diverse, they are commonly characterised by the abnormal structure and function of the heart's muscular walls. Recently, bi-allelic truncating variants in ALPK3 (ALPK3tv) have been associated with severe neonatal-onset cardiomyopathy. Using human pluripotent stem cell (hPSC) models, we defined the pathogenic mechanisms of bi-allelic ALPK3tv-induced cardiomyopathy. Specifically, we discovered that ALPK3 localises to the sarcomere, where it regulates a protein network required for the turnover of sarcomeric proteins. ALPK3 deficiency resulted in impaired protein turnover at the sarcomere, leading to aggregation of contractile proteins and impaired contractile function. Further, autosomal dominant ALPK3tvs have been associated with adult-onset cardiomyopathy, although there is no functional evidence to support

causation. To assess the functional impact of heterozygous ALPK3tvs on heart cell function, we introduced three heterozygous ALPK3tvs into healthy hPSCs. In vascularised cardiac organoids, contractile force and calcium handling was impaired for two of three heterozygous ALPK3tvs investigated, whilst the third showed slowed relaxation time. Aged mice carrying a human heterozygous ALPK3tv showed an increase in ventricular wall thickness and mass, impaired relaxation and myocyte hypertrophy, consistent with clinical reports. Proteomic profiling of cardiac organoids and mouse heart tissue unveiled novel disease mechanisms that were distinct to that of homozygous ALPK3tv. Together, our data indicates that heterozygous ALPK3tv are sufficient to cause cardiomyopathy, both in vitro and in vivo. This suggests that heterozygous ALPK3tvs play a clinically relevant role in the development of adult-onset cardiomyopathy. Strikingly, the disease mechanisms appear distinct from homozygous ALPK3tvs.

T1008

ISCORE-PD: AN ISOGENIC STEM CELL COLLECTION TO RESEARCH PARKINSON'S DISEASE

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Parkinson's disease (PD) is a neurodegenerative disorder caused by complex genetic and environmental factors. Genome-edited human pluripotent stem cells (hPSCs) offer a unique experimental platform to advance our understanding of PD etiology by enabling the generation of

disease-relevant cell-types carrying patient mutations along with isogenic control cells. To facilitate this approach, we generated a collection of over 60 cell lines genetically engineered to harbor high risk or causal variants in genes associated with PD (SNCA A53T, SNCA A30P, PRKN Ex3del, PINK1 Q129X, DJ1/PARK7 Ex1-5del, LRRK2 G2019S, ATP13A2 FS, FBXO7 R498X/FS, DNAJC6 c.801 A>G+FS, SYNJ1 R258Q/FS, VPS13C A444P, VPS13C W395C, GBA1 IVS2+1). All mutations were generated in a fully characterized and sequenced female human embryonic stem cell (hESC) line (WIBR3; NIH approval number NIHhESC-10-0079) using CRISPR/Cas9 or prime editing-based approaches. We implemented rigorous quality controls, including whole genome sequencing of each cell line. Our analysis of the genetic variation in this cell line collection revealed that while genome editing, particularly using CRISPR/Cas9, can introduce rare off-target mutations, the predominant source of genetic variants arises from routine cell culture. While perfect isogeneity remains elusive, the observed genetic variation was minimal compared to those typically found in patient-derived iPSCs, predominantly affecting non-coding regions of the genome. Importantly, the genetic variation can be effectively managed through stringent quality control measures and careful experimental design. This systematic approach ensures the high quality of our stem cell collection, highlights differences between conventional CRISPR/Cas9 and prime editing and provides a roadmap for how to generate gene-edited hPSCs collections at scale in an academic setting. Our iSCORE-PD collection represents an easily accessible and valuable platform to study PD, which can be used by investigators to understand the molecular pathophysiology of PD in a human cellular setting.

T1010

LARGE SCALE ANALYSIS OF LOSS OF CHROMOSOME Y IN HUMAN PLURIPOTENT STEM CELLS: IMPLICATIONS FOR TURNER SYNDROME AND RIBOSOMOPATHIES

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The only viable full monosomy in human pluripotent stem cells (hPSCs) in culture is X0, resulting from the loss of one of the sex chromosomes. Loss of chromosome Y (LOY) occurs in various cancers in males, but the extent and implications of LOY on hPSCs was not thoroughly studied. In this study, we performed a large-scale analysis of RNA-seq data from over 2,700 samples from around 140 independent studies, containing both hPSCs and their differentiated derivatives. We show that 12% of the samples have lost chromosome Y, either heterogeneously or fully, across different studies and cell lines. The LOY samples do not significantly differ from wildtype samples in other technical or genomic irregularities, such as read coverage, autosomal aneuploidies and TP53 mutations. Enrichment differential expression analysis revealed that the undifferentiated LOY samples show downregulated expression of major pluripotent markers, X-degenerate tumor suppressors and, most substantially, ribosomal protein genes. In addition to the downregulation of RPS4Y1, a Y-linked ribosomal protein genes, a significant decrease in expression of the majority of the autosomal ribosomal protein genes, both small- and large-ribosomal subunit proteins, was observed in LOY samples. We performed differential expression analysis on samples of Turner and Diamond-Blackfan anemia Syndromes, known for losing one allele in an autosomal ribosomal gene, and observed similar trend of downregulation in the expression of ribosomal protein genes, supporting our hypothesis of ribosomal haploinsufficiency mechanism. Overall, we present a wide, extensive analysis of LOY in hPSCs, detected samples across different studies and cell lines that have lost their chromosome Y and identified the downstream molecular effects of this

chromosomal aberration, mainly downregulation in pluripotency and in ribosomal protein transcription. Thus, we shed light on the implications of LOY and may provide a possible dosage-dependent explanation for the phenotypes underlying other ribosomal-deficiency diseases.

T1012

LINE-1 MEDIATED NEUROINFLAMMATION IN DIRECTLY CONVERTED ALZHEIMER'S DISEASE NEURONS

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Alzheimer's disease (AD) is a severe neurodegenerative disorder that exclusively effects elderly people. Despite decades of research, AD remains a debilitating, progressive, and ultimately fatal dementia with no disease-modifying treatment options. This is partly due to the lack of human model systems that capture complex human genetics and human biological age. We have previously shown that induced neurons (iNs) directly converted from patient's fibroblast overcome these limitations by retaining neuron-specific hallmarks of aging and reflect unifying sporadic AD-related signatures. Using a multi-omic approach, we showed that AD iNs have an increased population of senescent cells that have impaired electrophysiological activity, metabolic reprogramming, and most critically the gain of inflammatory senescence associated secretory phenotype (SASP) that could activate human glia. Our data indicate that chemical or genetic ablation of this minority population of cells could effectively eliminate the neuroinflammatory signature in AD iNs, highlighting senescence as a functional target for therapeutic interventions in AD. However, it is still unknown through what mechanism neurons, which aren't a pro-inflammatory cell type, could gain this feature during senescence. Here, we provide evidence that late-life activation of the transposon long interspersed nuclear element 1 (LINE-1) underlies the initiation of a SASP in AD neurons. In addition to LINE-1 knockdown, interventions that eliminate LINE-1 reverse transcription reduce SASP expression, suggesting a cytoplasmic DNA sensing mechanism. Our data point to LINE-1 as the mechanism for gain-of-function in inflammation in senescence neurons, and a targetable candidate for reducing late-life neuroinflammation in AD.

T1014

LOCAL HETEROCHROMATIN MITIGATES THE IMPACT OF A TRANSPOSABLE ELEMENT INSERTION CAUSING A NEURODEGENERATIVE DISORDER

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X-Linked Dystonia-Parkinsonism (XDP) is an adult-onset neurodegenerative disorder. Recently, a polymorphic transposable element (TE) insertion in the 32nd intron of the TAF1 gene has been identified as the genetic factor responsible for this disease. The XDP-TE is associated with TAF1 mis-regulation, but the mechanisms behind this phenomenon remain elusive. We hypothesize that repressive epigenetic marks on the XDP-TE are key players in this process. Thus, here we aim to dissect the molecular intricacies that keep the XDP-TE at bay and identify how it triggers aberrant TAF1 expression, ultimately leading to XDP. To do so we used XDP patient-derived iPSCs, neural progenitor cells and post-mortem brain tissue. To understand the epigenetic regulators controlling this insertion we employed CUT&RUN and Oxford Nanopore Sequencing. Moreover, to illuminate what factors establish these marks and their effect on gene expression, we did CRISPR inhibition of various candidate genes coupled with RNA sequencing. Using our patient-derived cellular models we demonstrate that ZNF91a TE-binding KRAB-Zinc Finger Protein - establishes H3K9me3 and DNA methylation over the XDP-TE in a cell type specific manner. Interestingly, removal of these epigenetic repressors severely aggravates the XDP molecular phenotype, causing a reduced TAF1 expression and increased intron retention. In line with this, our preliminary findings from patient-derived post-mortem brain tissues reveal hypomethylation of the XDP-TE in the brain of affected individuals, coupled with high levels of the pathogenic intron retention. Given that XDP is an adult-onset disorder, these results suggest that age-related loss of DNA methylation may play a critical role in driving disease progression. Our study unveils how a polymorphic TE results in XDP and highlights DNA methylation as potential therapeutic target. Moreover, this work underscores the significance of studying polymorphic TEs as disease triggers, with implications for various disorders.

MESENCHYMAL STROMAL CELL THERAPY: A PROMISING APPROACH TO COMBAT AGE-INDUCED SARCOPENIA

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The global population is increasingly ageing, necessitating the development of therapeutic strategies for ageing-related diseases. Sarcopenia, characterised by low muscle strength, low muscle mass, and diminished physical performance, poses a significant risk of falls, loss of autonomy, and increased dependency in the elderly. Systemic immune-inflammation has been implicated in the pathophysiology of sarcopenia, underscoring the need for targeted therapies. Mesenchymal stromal cells (MSCs) have emerged as a promising option due to their potent immunomodulatory properties, which may address both muscle degeneration and associated inflammatory responses. Traditional models for studying sarcopenia, such as hindlimb unloading, immobilisation, and denervation, often fail to accurately replicate the complex, multifactorial nature of the condition in humans. This limitation complicates the translation of preclinical findings to clinical practice. Our study utilised naturally aged mice as a more relevant model to investigate the efficacy of systemic MSC therapy in treating sarcopenia and other age-related symptoms. Our findings indicate that MSC treatment significantly improves muscle function and muscle mass in an age-induced sarcopenia mouse model. Notably, MSC-treated mice exhibited reduced systemic inflammation levels, suggesting a potential link between MSC therapy and immunomodulation. Furthermore, therapeutic benefits persisted for several months post-treatment cessation, indicating long-lasting effects on muscle function and quality. These enduring improvements may be attributed to sustained changes in the inflammatory environment and enhanced muscle repair mechanisms initiated by MSC therapy. This study provides robust support for the continued clinical application of MSC therapy for sarcopenia, aiming to enhance the quality of life for the elderly.

Funding Source: This work was supported by Center for Neuromusculoskeletal Restorative Medicine (Ref. No. CT1.1), Health@InnoHK program, Innovation Technology Commission, Hong Kong SAR, China.

T1020

METABOLIC DYSREGULATION IN BONE MARROW MESENCHYMAL STEM CELLS: IMPLICATIONS FOR OSTEOPOROTIC FRAGILITY IN WOMEN

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Bone marrow mesenchymal stem cells (BM-MSCs) are critical for bone homeostasis, yet aging and osteoporosis (OP) impair their function, contributing to bone fragility and fracture risk. Despite their importance, the metabolic mechanisms underlying BM-MSC dysfunction in aging and OP remain poorly understood. This study investigates the cellular metabolism of BM-MSCs from young healthy (n=6), elderly healthy (n=3), and elderly OP (n=5) women, and correlating seahorse-based quantification of oxidative and glycolytic activities with bone mineral density (BMD) from DEXA scans. Our preliminary results show significant reductions in BMD across different skeletal sites. As expected, BMD declined at the lumbar spine, femoral neck, and total hip in elderly subjects, with BMD reaching T-score below -2.5 in the osteoporotic group. Metabolic profiling revealed altered glycolytic activity in BM-MSCs, with extracellular acidification rate (ECAR) and glycolytic ATP production decreased by 61% in elderly OP women compared to young healthy controls ($P < 0.05$). Elderly healthy women showed trends toward reduced ECAR (35%) and glycolytic ATP (22%), though not significant. Alike the decline in BMD observed from young over elderly to osteoporotic subjects, a stepwise decrease was found in the glycolytic activity of isolated BM-MSCs in vitro. This suggests that the cells exhibit an intrinsic metabolic defect that aligns with the general decline of bone formation observed in elderly and osteoporotic subjects. To explore these findings further, we will implant BM-MSCs from these donors subcutaneously into immunodeficient female mice to assess their capacity to form bone. Additionally, we will use single-cell RNA sequencing to investigate the transcriptional pathways driving metabolic dysregulation in BM-MSCs and their impaired osteogenic potential. These results highlight the critical role of metabolic alterations in BM-MSCs in the pathophysiology of osteoporosis, suggesting that targeting metabolic pathways could offer novel strategies to mitigate bone loss and improve bone regeneration in aging populations.

T1022

METABOLITE-BASED THERAPEUTICS FOR FRAGILE X SYNDROME UTILIZING HUMAN PLURIPOTENT STEM CELLS

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Fragile X syndrome (FXS) is the most prevalent form of inherited intellectual disability and is one of the leading causes of autism spectrum disorders. FXS is caused by a trinucleotide repeat (CGG) expansion located at the 5' untranslated region (5' UTR) of the fragile X messenger ribonucleoprotein 1 (FMR1) gene, leading to its inactivation. To identify genes involved in regulating FMR1 silencing, we have previously conducted a genome-wide screen using human embryonic stem cells. Among the candidate genes identified, SDHAF4 emerged as a key regulator. This gene encodes an assembly factor of the succinate dehydrogenase (SDH) enzyme. Knocking out SDHAF4 reactivated the FMR1 gene in FXS-induced pluripotent stem cells (iPSCs). We further explored small molecule treatments to inhibit screen candidate genes, focusing on oxaloacetic acid (OAA), a known allosteric inhibitor of SDH. Treatment of FXS iPSCs with OAA for 10 days significantly reactivated FMR1 expression. Remarkably, combining OAA with ascorbic acid (vitamin C) resulted in even greater FMR1 reactivation. Furthermore, in pre-clinical experiments with humanized mouse model, human FXS-iPSCs were transplanted into immunodeficient mice. Treatment with intra-peritoneal injection of OAA resulted in significant FMR1 reactivation within the human cells. Further analysis revealed that this treatment caused massive demethylation of the FMR1 promoter region in a subset of the cells. Additional metabolic modulators, including malonate and α-ketoglutarate, also demonstrated strong potential to reactivate FMR1. This study provides novel insights highlighting metabolic pathways as promising therapeutic targets for FXS. These findings open new avenues for the development of effective treatments for this challenging condition.

T1024

MICROGLIA REPLACEMENT EXTENDS SURVIVAL BY REMODELING NEURAL COMMUNICATION NETWORKS IN A MOUSE MODEL OF ALS

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Amyotrophic lateral sclerosis (ALS) is the most common adult-onset neurodegenerative disorder, leading to respiratory failure within 2-5 years of symptom onset. Dysfunctional microglia are believed to drive ALS progression exhibit functional heterogeneity across different disease stages, raising the critical question of whether stage-specific interventions using normal microglia replacement might yield varying therapeutic outcomes. Here, we demonstrate that restoring microglial function through systemic bone marrow transplantation followed by genetically normal microglia replacement reduced microglial activation, delayed symptoms progression, and extended survival in the SOD1G93A mouse model of ALS at both the presymptomatic stage and disease onset. Further analysis revealed this treatment restored crucial microglial-oligodendrocyte/OPCs communication and improved neuronal and oligodendrocyte health. Thus, microglia replacement emerges as a highly effective approach for preventing disease progression, offering potential therapeutic avenues for ALS.

T1026

MICROGLIAL TMEM119 IS INVOLVED IN AGGREGATED A-BETA CLEARANCE IN AMYLOID-BETA DEPOSITING MICE

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The progression of Alzheimer's Disease (AD) involves temporal dynamics of microglial activation. Restoring or maintaining microglial homeostasis has emerged as a promising therapeutic strategy to combat AD. Transmembrane protein 119 (TMEM119), a homeostatic marker of microglia, has not been fully studied under AD pathological conditions. In this study, we observed that Aβ induces a dynamic decrease in TMEM119 in microglia, and TMEM119 is associated with the progression of AD in the 5×FAD mouse model. TMEM119 binds to Aβ oligomers and subsequently recruits LRP1, which in turn degrades TMEM119 itself. Overexpression of TMEM119 in microglia enhances their phagocytic activity and alleviates cognitive deficits in 5xFAD mice. Importantly, the administration of small molecules such as Kartogenin (KGN) and SRI-011381 (SRI), which enhance TMEM119 expression, substantially promotes Aβ clearance and improves cognitive function in AD mice, even during the mid-stage of the disease. Therefore, TMEM119 emerges as a promising therapeutic target for AD.

Funding Source: The National Key Research and Development Program of China (2021YFA1101400); the Strategic Priority Research Program of CAS (XDA 0460205); and the Basic Frontier Science Research Program of CAS (ZDBS-LY-SM024).

T1028

MIXED EFFECTS MODEL TO UNDERSTAND SOURCES OF CONTRACTILE AND MORPHOLOGICAL HETEROGENEITY IN HIPSC-CMS

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The power of stem cells and gene editing is the ability to introduce mutations into isogenic lines; especially for heart disease-linked mutations which contribute to 1 in 5 deaths worldwide. These mutations have measurable effects on the contractility and morphology of hiPSC-CMs. Yet, heterogeneity from line to line and batch to batch remains a challenge and can be exacerbated by gene editing, different media, handling by different researchers, differentiation, etc. We sought to analyze this heterogeneity using mixed effects models. We included known clusters (e.g. researcher or batch) and allowed random slopes and intercepts of those clusters within the model hierarchy. Clustering improves estimates for repeat sampling, unbalanced sampling, and variation. We modeled this heterogeneity in multiple isogenic hiPSC-CMs cell lines from one donor (over 58 batches) and multiple cell lines from donors of different sexes (over 35 batches). We analyzed published Traction Force Microscopy (TFM) data from the diseased cell line collection, generated from cell line GM25256 by the Allen Institute for Cell Science, to model heterogeneity associated with gene editing and researcher (Lee, 2023 and Pardon, 2024). We found robustly similar morphological and contractile phenotypes across users and differentiation batches with small but significant differences from the gene editing process. However, differences between multiple isogenic control lines, undergoing the same editing processes, were dwarfed by the differences caused by the deleterious mutations edited into the disease lines. Thus, we conclude a single isogenic control line can serve to compare the effects of these isogenic mutation lines. We also found mTeSR1 media used on pre-differentiated hiPSCs resulted in larger spread area in hiPSC-CMs when compared to Essential 8 (E8). Additionally, we analyzed published TFM data from multiple male and female donor lines, obtained from the Stanford Cardiovascular Institute, to measure sex differences (Chirikian, 2022). Male cells had 42% higher twitch force ($p = 8.8E-6$) with 25% higher contraction ($p = 1.77E-5$) and relaxation ($p = 0.0006$) velocities. Knowing that deleterious mutations and cellular sex cause larger changes than other sources of heterogeneity of hiPSC-CMs will improve hiPSC-CMs as a cardiac cell model.

Funding Source: This project was supported by the NSF BRITE Fellow award #2227509, NSF Data Driven Biology NRT award #2125644, Connie Frank Biomedical Fellowship, CIRM EDUC4-12821, and the NIH 1RM1GM131981-01.

T1030

MODELING FRAGILE X SYNDROME IN HUMAN HIPPOCAMPAL ORGANOIDS REVEALS ALTERED DEVELOPMENTAL TRAJECTORIES AND FMRP BINDING DYNAMICS

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Fragile X syndrome (FXS), a leading genetic cause of intellectual disability and autism spectrum disorder, is typically caused by CGG trinucleotide repeat expansion in the FMR1 gene that results in gene silencing and complete loss of its protein product, fragile X messenger ribonucleoprotein (FMRP). FMRP is a multifunctional protein that binds to selective mRNAs and regulates their stability, editing, transport, and translation. As a key brain region for learning, memory, and emotional processing – and a major site of FMR1 expression – the human hippocampus is of particular interest, especially in understanding how FMRP loss affects its development. Indeed,

anxiety and aggressive behaviors are common in FXS patients, and hippocampal enlargement has been reported in younger patients, suggesting hippocampal abnormalities. However, the regulatory role of FMRP and the impacts of its loss during human hippocampal development remain unexplored. To address these questions, we generated human hippocampal organoids (HOs) from FXS patient-derived and healthy control-derived iPSCs. Through transcriptomic, cellular, and electrophysiological analyses, we observed altered developmental trajectories, specifically increased neurogenesis and decreased gliogenesis in FXS HOs, which may contribute synergistically to neural network hyperexcitability in them. To investigate potential underlying mechanisms, we performed eCLIP-seq on HOs at the early and late stages and identified stage-specific FMRP targets that correspond to the predominant biological processes at each stage. Particularly, we demonstrated, for the first time, a switch in FMRP binding targets from mRNAs involved in cell cycle and neurogenesis at early stage to those involved in gliogenesis at late stage, highlighting a dynamic temporal regulatory role of FMRP during hippocampal development. Further construction of gene regulatory networks in HOs by single-cell transcriptomic analyses revealed key regulons targeted by FMRP that may drive altered developmental trajectories in FXS HOs. Together, our study delineates the molecular, cellular, and functional impacts of FMRP loss on hippocampal development and provides new insights into its regulatory role and RNA binding dynamics, offering potential avenues for therapeutic advancement.

T1032

MODELING HUMAN HEMATOPOIETIC AND CARDIAC CO-DEVELOPMENT IN HPSC-DERIVED ORGANOIDS

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Deriving hematopoietic stem and progenitor cells (HSCs/HPCs) in vitro remains challenging for both developmental studies and therapeutic applications. This difficulty arises from the limited understanding of the niche-like induction and stabilization of these cells within their proper developmental context, compounded by the ethical and technical constraints of studying human embryogenesis in vivo. Consequently, human in vitro models that recapitulate the complex cellular and molecular mechanisms and dynamics of hematopoietic development in a native-like environment are of significant interest. By modulating our human pluripotent stem cell (hPSC)-based heart-forming organoid (HFO) model via stage-specific supplementation of hemato-endothelial factors, we have recently established the novel, so-called blood-generating HFO (BG-HFO). BG-HFOs comprise functional cardiac tissue with a ventricular-like phenotype, while

featuring distinct endothelial subtypes, including a mesenchyme-embedded endothelial layer that generates hematopoietic cells through endothelial-to-hematopoietic transition. Functional assays demonstrated that BG-HFO-derived hemato-endothelial cells possess erythroid, myeloid and lymphoid potential. The morphological structure of BG-HFOs reflects aspects of both embryonic/primitive and definitive hematopoiesis in vivo. Recent single-cell RNA sequencing data from BG-HFOs, collected at different developmental stages, revealed time-dependent transcriptional patterning consistent with hemogenic endothelial and HSCs/HPCs development and, at later stages, the emergence of hematopoietic derivatives including erythroid, megakaryocytic and myeloid cells. This corroborates our findings that BG-HFOs indeed recapitulate hemogenic endothelial development and both primitive and definitive hematopoiesis known from the native embryo. Taken together, our work introduces the first human in vitro model of self-organized, morphologically structured co-development of cardiac, endothelial, and multipotent hematopoietic tissues. It provides a robust platform for pharmacological testing advancing mechanistic research of hematopoiesis, and overcoming the limitations of studying these processes in human embryos.

T1034

MODELLING DMV FORMATION IN SARS-COV-2-INFECTED AIRWAY EPITHELIAL CELLS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

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The formation of double membrane vesicles (DMVs), a critical step in coronavirus infection to shield viral genomes from innate immune receptors, necessitates the involvement of various host factors. Previous research has identified several host proteins, including TMEM41B, VMP1, and RTN3/4, which facilitate the interaction between viral non-structural proteins Nsp3 and Nsp4, ER zippering and curving, and the fusion of paired ER membranes necessary for DMV biogenesis. Despite these findings, there remains a lack of high-throughput methods for identifying host factors involved in DMV formation. In this study, we employ a genome-wide CRISPR-knockout/activation library screening to identify host factors that either promote or suppress the interaction between Nsp3 and Nsp4. Our validation results indicate that the knockdown or overexpression of specific host factors can modulate the strength of the Nsp3-Nsp4 interaction, influencing DMV formation, as well as viral replication and kinetics in SARS-CoV-2-infected human lung carcinoma cell lines. Having these preliminary findings, we extend our study to human pluripotent stem cell-derived airway epithelium, providing a more physiologically relevant model of DMV biogenesis in true infection scenarios, with implications for antiviral strategies.

T1036

MODELLING PARTICULATE MATTER INTERACTION IN THE ALVEOLAR NICHE USING A CHEMICALLY DEFINED MULTI-CELL TYPE ORGANOID SYSTEM USING HUMAN INDUCED PLURIPOTENT STEM CELLS

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Air pollution was responsible for 8.1 million deaths globally in 2021, according to the State of Global Air Report from 2024. It is the world's largest environmental risk factor for exacerbation and disease development such as COPD, asthma, and lung cancer. Currently, the mouse is the primary model in respiratory inhalation research as it provides whole organ data on disease mechanisms but does not mimic the human physiological response. Human stem cell in vitro models focus only on epithelial cells leaving a significant gap in our understanding of cell-cell interaction and disease mechanisms. Our model consists of human induced pluripotent stem cell-derived alveolar type II cells (ATIIs), macrophages, dendritic cells, fibroblasts, and endothelial cells differentiated using chemically defined conditions. This model allows us to portray the alveolar niche and model particulate matter interaction in a more physiologically relevant way. Each cell type was characterised using flow cytometry, qPCR, bulk RNA sequencing, and cell-specific enrichment analysis. Consequently, organoid models were assembled to further understand cell-cell interaction mechanisms, proliferation, and interaction with particulate matter. Single-cell sequencing reveals the preservation of the initial cell populations, maturation, and transdifferentiation into niche-specific cell types, such as neuroendocrine-like cells and more mature ATII and fibroblast-like cells. Acute injury modelling of particulate matter shows an in vivo-like response of the epithelial population leading to transdifferentiation into basal-like cells showing specific markers (KRT5, KRT17, TP63). Additionally, the epithelial-to-mesenchymal transition is exacerbated upon injury due to maintenance and regeneration of the organoids, increased expression of fibroblast maturation markers differentiating into myofibroblasts, and protein expression shows an increase in inflammatory markers linked with lung homeostasis as well as pro and anti-inflammatory markers linked with an immune-specific response, showing macrophage polarisation into M1 and M2 phenotype. This model allows for human-specific modelling of respiratory injury and may be an important platform to identify biomarkers for lung disease exacerbation and prognosis.

Funding Source: Biotechnology and Biological Sciences Research Council.

T1038

MODULATING THE FETAL ENVIRONMENT WITH EXTRACELLULAR VESICLES: A MINIMALLY INVASIVE APPROACH TO TREATING CONGENITAL DISORDERS

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Congenital malformations affect approximately 1.9 per 1000 live births globally. Prenatal surgical repair using regenerative strategies has shown promise in reducing the severity of these conditions, but mid-pregnancy in utero interventions remain invasive for both the mother and fetus. To address this, we are exploring less invasive approaches by leveraging mesenchymal stem cells derived from amniotic fluid (AF-MSCs) to create a pro-regenerative environment within the amniotic cavity, promote tissue homeostasis, and facilitate repair. We also investigated extracellular vesicles (EVs) as stable, reproducible, and reconfigurable alternatives to cell-based therapies, aiming to overcome the challenges of stem cell treatments while retaining therapeutic efficacy. Biodistribution

studies following intraperitoneal administration of EVs in female mice demonstrated targeted delivery to the uterus and yolk sac, with no observed toxicity in the dams throughout gestation and no significant changes in fetal viability. These findings were observed in a well-established mouse model (SWV/Fnn strain), which is highly susceptible to drug-induced toxicity and neural tube defects. Continuous administration of EVs (10e9/dose) during neurulation (E5.5–E9.5) in a spina bifida model (Fkbp8 knockout) resulted in a significant reduction in lesion size (3mm) compared to PBS-treated controls (5–8mm), along with visible vertebrae closure at the last thoracic and lumbar levels. When used as RNA therapeutics, EVs maintained cargo stability, demonstrating long-term expression of loaded mRNA both in vitro and in an ex vivo whole embryo culture system. This confirms that mRNA functionality is preserved in more complex systems, as EVs successfully cross the yolk sac, reach the embryo, and restore gene function. These findings suggest that AF-MSC-derived EVs represent a promising, minimally invasive and reconfigurable precision medicine approach, with potential for further development in the in utero treatment of congenital malformations.

Funding Source: Research reported in this publication is supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development of the National Institutes of Health under Award Number R01HD113702.

T1040

SSEA3 AND CD105 POSITIVITY ARE ASSOCIATED WITH THE TREATMENT POTENCY OF HUMAN NEURAL CREST-DERIVED NASAL TURBINATE STEM CELLS FOR ALZHEIMER'S DISEASE

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Stem cells have the potential to treat Alzheimer’s disease (AD), but clinical outcomes are unpredictable due to inter-donor differences in stem cell properties. This study aimed to determine whether the pluripotency marker SSEA3 and the mesenchymal marker CD105 positivity are associated with therapeutic efficacy of human neural crest-derived nasal turbinate stem cells (NTSCs) for AD. The therapeutic effects of NTSCs obtained from different donors, each with varying percentages of SSEA3+/CD105+ cells, were assessed by examining multiple neuropathological changes associated with AD, including the expression of beta-amyloid, inflammation, and neuronal survival. These assessments were conducted in 5×FAD transgenic AD model mice and cerebral organoids derived from induced pluripotent stem cells (iPSC) of three AD patients. Their effects on cognitive functions were measured by performance in the Morris water maze (MWM) test. NTSCs from different donors improved cognitive function and AD-related neuropathology to varying degrees, depending on the percentage of SSEA3+/CD105+ cells. Compared with NTSCs with a lower percentage of SSEA3+/CD105+ cells (NTSCs-L), NTSCs with a higher percentage of SSEA3+/CD105+ cells (NTSCs-H) showed a greater in vitro property, including proliferative capacity, multiple differentiation potency, and secretion of neuroprotective

cytokines, which were comparable to pure SSEA3+/CD105+ cells isolated from NTSCs (NTSCs-SC). Both NTSCs-H and NTSCs-SC improved cognitive deficits and reduced cerebral Aβ deposition, inflammation, and neuronal death in AD model mice. Furthermore, NTSCs-H and NTSCs-SC mitigated AD-related pathological features in AD cerebral organoids by decreasing Aβ aggregates, Tau hyperphosphorylation, neuronal death, microglial numbers, and inflammatory cytokine levels. However, there was no significant differences in AD-related pathological changes between NTSCs-H and NTSCs-SC treatment groups. Our findings indicate NTSCs with a high percentage of SSEA3+/CD105+ cells rapidly improve cognitive function and greatly mitigate pathological changes associated with AD, further suggesting that SSEA3/CD105 positivity is a potential marker of NTSCs therapeutic efficacy for the treatment of AD.

Funding Source: This research was supported by the Korea Health Technology Research and Development project through the Korea Health Industry Development Institute (KHIDI) funded by a grant (RS-2024-00397128) from Ministry of Food and Drug Safety in 2024.

T1042

MOUSE LIVER ASSEMBLOIDS RECAPITULATE PERIPORTAL MESOSCALE ARCHITECTURE AND MODEL BILIARY FIBROSIS IN VITRO

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Modelling liver disease has been hampered by the lack of culture systems that represent disease progression in vitro. The current tissue-derived organoid models fail to reproduce the remarkable complexity of the tissue, both at the cellular composition as well as at the tissue architecture level. Here, we describe a next-generation organoid model composed of adult hepatocytes, cholangiocytes and liver mesenchymal cells that reconstruct the architecture of the periportal region of the liver lobule and, when manipulated, model aspects of cholestatic liver injury and biliary fibrosis in vitro. We first generate reproducible hepatocyte organoids (HepOrg) with a physiological and functional bile canaliculi network that retain morphological features of in vivo physiology and cholestatic response. Then, by combining these improved HepOrg models with cholangiocytes and portal fibroblasts, we generate periportal assembloids that recapitulate the architecture and cellular interactions of the liver periportal region. Periportal assembloids are functional as they consistently drain bile from the bile canaliculi into the lumen of bile ducts. Strikingly, the manipulation of the relative number of portal mesenchymal cells is sufficient to induce a fibrotic-like state in the assembloids, independently of an immune compartment. By generating chimeric assembloids from Mdr2-/- mutant and wild-type cells, we demonstrate the utility of our system to investigate cell-autonomous mechanisms in cholestatic injury and biliary fibrosis. Taken together, we demonstrate that periportal liver assembloids represent the first 3D in vitro system suitable to study the mechanisms underlying bile canaliculi formation, bile drainage and the contributions of the different cell types to cholestatic liver disease and biliary fibrosis, in an

all-in-one model. This proof-of-concept study opens an avenue for more complex liver tissue-derived organoid models, which better recapitulate liver complexity, as well as physiological and pathological liver features in vitro.

T1044

MULTI-OMICS ANALYSIS REVEALS ISCHEMIC STROKE-LIKE FEATURES IN MATURED HIPSC-DERIVED NEUROSPHEROIDS AFTER OXYGEN-GLUCOSE DEPRIVATION/REOXYGENATION

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Despite the high global burden of ischemic stroke on patients and society, treatment options are limited and decades of research dedicated to finding new candidate neuroprotective drugs has not led to an effective neuroprotective therapy to date. This is partially due to the lack of appropriate model systems able to recapitulate human ischemic responses in vitro. To address the shortcomings of these models, we developed a 5-month-old matured, bioreactor-based, hiPSC-derived neurospheroid model to more faithfully mimic adult neural tissue and its cellular interactions. Characterization of these neurospheroids showed presence of Tuj1+, MAP2+, NeuN+ neurons, GFAP+, CD49f+, AQP4+, S100β+ astrocytes and spontaneous electrophysiological activity as demonstrated by high-density multi-electrode array recordings and live cell Ca2+-imaging. Notably, culturing these neurospheroids in a bioreactor reduced necrotic core formation typically present in organoids cultured for prolonged periods of time. To mimic ischemic stroke-like conditions, we exposed these neurospheroids to six hours of oxygen-glucose deprivation (OGD), followed by 72 hours of reoxygenation. The release of neurofilament-l, used as a marker for neuronal cell death, significantly increased in the OGD-exposed condition compared to the control in a time-dependent manner. Additionally, analysis of untargeted transcriptomics and proteomics

revealed upregulation of processes related to oxidative stress after 72h of reoxygation. Moreover, alterations in developmental and inflammatory signalling as well as a distortion of cellular metabolism and neurotransmission were detected. This translates to a loss of electrophysiological network activity as demonstrated by live cell Ca2+-imaging. We are currently validating these results by means of immunocytochemistry. Furthermore, we demonstrated the feasibility of incorporating immune cells known to play important roles in ischemic stroke pathophysiology, such as microglia, macrophages and neutrophils into these hiPSC-derived neurospheroids post-OGD. With this, we created a new model system with increased physiological relevance to further investigate the neuroinflammatory cascade following ischemic stroke, which can help identify new targets for neuroprotection or repair.

Funding Source: Fonds Wetenschappelijk Onderzoek (FWO).

T1046

MULTIOMICS STUDY TO ELUCIDATE THE MOLECULAR PATHOGENESIS OF CHOLEDOCHAL CYSTS

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Congenital bile-duct dilatation (CDD) is a rare complex anomaly most commonly found in Asians, which is characterized by abnormal dilatation of the extrahepatic and/or the intrahepatic bile ducts. Despite surgery, many patients still suffer from post-operative complications and an increased risk of hepatobiliary cancers. Our previous genetic study suggested a genetic predisposition in the pathogenesis of the disease. To further elucidate the genetic causes of CDD, we performed whole exome and genome sequencing on 78 trios of type I and IV CDD patients of Chinese and Vietnamese ancestries. De novo analysis identified thirty-seven damaging protein-altering variants, which were enriched in oncogenic MAPK signaling and focal adhesion pathways. Recessive and compound heterozygous damaging mutations were also enriched in associated oncogenic pathways. Spatiotemporal expression of these candidate gene was further evaluated using fetal single cell transcriptomic data and single nuclei RNA sequencing data of CDD extrahepatic bile duct. The findings suggest a link between genetic predisposition, cancer genes, and disease pathogenesis. Unravelling this relationship will help guide the stratification of patients based on the pathological mechanism and to correlate the genetic findings to disease prognosis, particularly the increased risk of malignancy.

Funding Source: The project is supported by General Research Fund 17113320.

T1048

NANOTECHNOLOGIES IN TARGET TREATING PRETERM BIRTH AND PREMATURE OVARIAN FAILURES

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Preterm birth (PTB) and premature ovarian failure (POF) are two severe female reproductive diseases that affects many women at the child-bearing ages. PTB is considered as a live birth before 37 weeks. Nowadays about 15 million babies are experiencing PTB annually, which accounts for 11% of the birth rates globally. Also, PTB is the leading cause among newborns. POF usually refers to women who have experienced amenorrhea before age of 40. POF can also cause severe health issues including psychological distress, infertility, osteoporosis, autoimmune disorders, ischemic heart disease, and increased risk of mortality. In recent years, the development of nanotechnology has provided a new solution for treating these diseases. Nanotechnology involves the engineering of structures on a molecular level. Nanomedicine and nano-delivery systems have been designed to deliver therapeutic agents to a target site or organ in a controlled manner, maximizing efficacy while minimizing off-target effects of the therapeutic agent administered. Our team has been working on using nanorobots and nanoparticles to treat PTB and POF. According to our research results, using a nanorobot which favors the macrophages can help with bring anti-inflammation drugs like metformin and curcumin to the placenta which can further reduce the PTB symptoms. Also, by conjugating a peptide that can specifically target the ovary, nanoparticles can be engineered to specifically deliver drugs to the ovary for further treating POF.

T1050

NEURAL NETWORK DYSREGULATION IN SPORADIC ALZHEIMER'S DISEASE ASTROCYTE ENRICHED BRAIN ORGANOIDS

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Understanding key events of Alzheimer’s disease (AD) etiology resulting in neuronal network dysregulation is essential for illuminating the cause of memory loss in AD. Functional, human derived brain tissues that represent the diverse genetic background and cellular subtypes contributing to sporadic AD (sAD) are limited. Human induced pluripotent stem cell (iPSC) derived brain organoids recapitulate some features of AD-like pathology, providing a tool for investigating the relationship between AD pathology and neural cell dysregulation leading to cognitive decline. However, the percentage of astrocytes is often lower in brain organoids than in human brains. Our lab has developed protocols for the generation of astrocyte-enriched brain organoids (AEOs) containing amounts of astrocytes exceeding those of non-astrocyte enriched brain organoid protocols. We generated AEOs from the iPSCs of sAD patient and non-AD donor controls. AEOs were cultured for 7.5 to 10 months to achieve complex neural network organization comprised of both inhibitory and excitatory neurons. Using Axion Biosystems 16 electrode, multielectrode array (MEA) technology, we explored functional, neuronal network activity patterns of sAD compared to control derived AEOs. sAD AEOs have an impaired ability to organize spiking and bursting activity

into complex patterns of network bursts as demonstrated by a decrease in reverberating burst patterns in sAD AEOs relative to controls. sAD AEOs also show decreased functional connectivity, indicated by using a spike time tiling coefficient that revealed a decrease in correlated electrode activity, when compared to control AEOs. To explore mechanisms that could be contributing to the disruption of complex bursting patterns and decreased functional connectivity of sAD AEOs, we performed pharmacological treatments, single-cell RNA-sequencing (scRNA-seq), and immunohistochemistry. Together, this data suggests a dysregulation in the inhibitory to excitatory ratio of AD AEO neural networks compared to control AEOs.

Funding Source: NIA T32 ADRD Fellowship.

T1052

NEUREXIN1 KNOCKOUT IPSC-DERIVED NEURONS DISPLAY HETEROGENOUS TRANSCRIPTOMIC EFFECTS ACROSS DIFFERENT GENETIC BACKGROUNDS

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Schizophrenia (SCZ) is a severe psychiatric disorder with a high amount of genetic heritability. Neurexin 1 (NRXN1) is a presynaptic cell adhesion gene linked to SCZ; however, individuals with copy number variants in NRXN1 display incredible phenotypic variability from being neurotypical to developing psychiatric or neurodevelopmental disorders, suggesting a contribution of genetic background. To study the influence of the same mutation in different genetic backgrounds, we developed “village editing” (CRISPR/Cas9 gene editing in a cell village format) and generated NRXN1 knockouts (KOs) in induced pluripotent stem cell (iPSC) lines from 15 donors with low, neutral, or high polygenic risk scores for SCZ. Using this method, we achieved high efficiency and recovered untargeted control clones as well as heterozygous (33.1%) and homozygous (28.4%) deletions in NRXN1 for most donors. We differentiated iPSCs to cortical excitatory neurons for 28 days with mouse glia and performed RNA sequencing to determine the effect of NRXN1 KO on

neuron transcriptomes. We found that genetic background deeply influences gene expression changes in NRXN1 KO neurons. Ongoing studies are leveraging these tools to characterize NRXN1 function in neural cells, including examination of synaptic and other cellular phenotypes. In summary, we generated novel tools to examine NRXN1 function, demonstrate the importance of including multiple genetic backgrounds, and provide a framework for rapid and efficient development of similar tools to study gene functions in complex, polygenic disorders.

Funding Source: The Stanley Center for Psychiatric Research, National Institute of Mental Health U01MH115727 (RN), and National Institute on Aging K00AG068523 (RB).

T1054

NEXT-GENERATION ELECTROPHYSIOLOGY FOR FUNCTIONAL CHARACTERIZATION OF HUMAN NEURAL ORGANOIDS AND ASSEMBLOIDS

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Three-dimensional neural models derived from human-induced pluripotent stem cells (hiPSCs), including organoids and assembloids, have emerged as indispensable systems for recapitulating fundamental aspects of human brain development. These models have proven critical for studying neurological disorders like Alzheimer's and Parkinson's disease. To fully understand the intricate dynamics of the neural networks within these self-organizing in-vitro cellular models, there is a need for real-time and label-free electrical activity measurement. High-density microelectrode arrays (HD-MEAs) provide a non-invasive approach to high-content electrical imaging by allowing for real-time electrophysiological recordings from a variety of electrogenic materials, such as neural organoids, assembloids, retinal or brain tissue explants. We utilized the MaxOne and MaxTwo HD-MEA platforms, each equipped with 26'400 electrodes per well, to record extracellular action potentials from various 3D neural models at multiple scales, ranging from network-level activity to single-cell and subcellular analyses. We demonstrated the flexible electrode selection for recording neural activity and how it improves the collected data's statistical power and reproducibility. Key parameters like firing rate, spike amplitude, and network burst profile were extrapolated. We used the AxonTracking Assay to trace action potential propagation along axonal branches, enabling a detailed examination of axon morphology and function, including conduction velocity, latency, axonal length, and branching patterns. This breakthrough assay allows for high-resolution investigation of disease models targeting axon initial segments, axonal branching, development, and conduction. The HD-MEA platforms' capability for targeted electrode selection improves data consistency and enables more comprehensive statistical insights. Furthermore, automated data visualization and metric extraction make these systems a robust and user-friendly choice for in-vitro disease modeling and drug testing in both acute and longitudinal studies.

Funding Source: This work is funded by the HyVIS project, GA 964468, within the H2020 Framework Program of the European Commission.

T1056

OPTIMAL ADMINISTRATION METHOD FOR MRNA-LNP DELIVERY TO THE HEART

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The application of messenger RNA (mRNA) has great potential for the treatment of heart failure. While lipid nanoparticles (LNPs) have proven to be effective vehicles for mRNA delivery, optimal administration methods for targeting the heart remain poorly understood. Here, we investigated the efficacy of transcatheter intracoronary (IC) administration compared to intravenous (IV) and intramyocardial (IM) administration as a method to deliver mRNA-LNPs to the heart. We evaluated these administration methods in both healthy and ischemia-reperfusion (I/R) rabbit hearts using fluorescence (ATTO 700)-labeled LNPs encapsulating Firefly Luciferase (FLuc) mRNA to confirm in vivo distribution. LNP accumulation and FLuc expression in the heart were analyzed by in vivo imaging system (IVIS) 4 hours after administration and followed by immunohistochemical analysis. In the healthy hearts, IVIS spectrum data revealed the highest LNP accumulation in the IM group, followed by the IC and IV groups, respectively. On the other hand, FLuc expression was significantly higher in the IC group than in the IV group and it was comparable to the IM group. Notably, the IC group showed widespread FLuc expression throughout the heart, while the IV group had no expression and the IM group showed strong expression only at the injection sites. Histological analysis revealed that FLuc-expressing cells were observed in cardiomyocytes, endothelial cells, smooth muscle cells, and fibroblasts. In the I/R hearts, IVIS spectrum data showed high FLuc expression in the remote area only in the IC group, although all administration methods resulted in robust LNP accumulation and FLuc expression within the infarcted area. Histological analysis revealed that a large number of cardiomyocytes in the infarcted area expressed FLuc in all administration groups, but only the IC group showed the high FLuc expression in the remote area. These findings demonstrate that transcatheter IC administration effectively delivered mRNA-LNPs not only to the damaged area but also to the non-damaged area in the diseased heart, suggesting that IC administration is a clinically safe and useful method for mRNA-LNPs delivery to a wider range of cardiac tissue.

Funding Source: Japan Society for the Promotion of Science KAKENHI Grant Numbers JP23K08252.

T1058

OVARIAN CANCER PATIENT-DERIVED ORGANOIDS AS A MODEL FOR RECAPITULATING GENETIC PROFILES AND EVALUATING DRUG RESPONSE: A PILOT STUDY

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This study aimed to evaluate the utility of ovarian cancer patient-derived organoids (PDOs) in replicating genetic characteristics and assessing drug responsiveness. PDOs were cultured in Matrigel using a specialized medium, and success rates and proliferation rates were analyzed. Morphology, histology, and immunohistochemistry (IHC) markers (PAX8, P53, and WT1) were employed to identify tumor characteristics. Genetic profiling was conducted through gene sequencing, variant allele frequency (VAF) analysis, and copy number variation assessment. Drug sensitivity testing was performed with carboplatin, paclitaxel, gemcitabine, doxorubicin, and olaparib. Organoids were successfully generated in 54% (7/13) of cases, including 4 high-grade serous carcinomas (HGSC), 1 mucinous carcinoma (MC), 1 clear cell carcinoma (CCC), and 1 carcinosarcoma. Six organoids (3 HGSC, 1 CCC, 1 MC, and 1 carcinosarcoma) were used in subsequent experiments. The organoids exhibited spherical morphology with diameters ranging from 100 to 500 μm. Histological and IHC analyses confirmed that organoids retained characteristics consistent with their corresponding primary tumors. After cryopreservation, organoid proliferation was slower compared to primary cultures (14 days vs. 10 days, $P < 0.01$). Targeted sequencing revealed shared DNA variants between primary tumors and organoids, including mutations in key genes such as BRCA1, PIK3CA, ARID1A, and TP53. VAF analysis demonstrated similarity between organoids and primary tumors, and organoids preserved most copy number alterations. Drug sensitivity tests showed differential responses, with carcinosarcoma organoids exhibiting higher sensitivity to paclitaxel and gemcitabine compared to HGSC organoids. These preliminary findings demonstrate that ovarian cancer PDOs can be successfully established, faithfully replicating histological features, genetic mutations, and copy number variations of primary tumors. Drug testing on PDOs revealed individualized drug responses, underscoring their potential as valuable resources for investigating genomic biomarkers and advancing personalized cancer therapy.

Funding Source: National Science and Technology Council (NSTC 111-2314-B-303-019 and 112-2314-B-303-010-MY3).

T1060

PANCREATIC HYPOPLASIA AND ISLET DYSFUNCTION IN RFX6 MUTATION-ASSOCIATED DIABETES: REVEALING MECHANISMS THROUGH IPSC-DERIVED ISLET ORGANOIDS

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Homozygous mutations in RFX6 lead to neonatal diabetes accompanied by a hypoplastic pancreas, whereas heterozygous mutations cause maturity-onset diabetes of the young (MODY). Recent studies have shown RFX6 variants linkage with type 2 diabetes. Despite RFX6's known

function in islet development, its specific role in diabetes pathogenesis remains unclear. Here, we aimed to understand the mechanisms underlying the impairment of pancreatic islet development and subsequent hypoplasia due to loss-of-function mutations in RFX6. We examined RFX6 expression during hESC differentiation into pancreatic islets and analyzed a single-cell RNA-sequencing dataset to identify RFX6-specific cell populations during islet development. Human iPSC lines lacking RFX6 were generated using CRISPR/Cas9 where various approaches used to study the impact during development. Our immunostaining analysis revealed robust RFX6 expression in PDX1+ cells during posterior foregut (PF) stage. However, at pancreatic progenitor (PP) stage, RFX6 did not exhibit co-expression with PDX1 and NKX6.1, whereas it co-localized with NEUROG3 and NKX2.2 during endocrine progenitor (EP) stage. Single-cell analysis demonstrated elevated RFX6 expression in endocrine clusters across various developmental stages. The absence of RFX6 led to a significant decrease in PDX1 expression at the PF stage but did not impact PPs co-expressing PDX1 and NKX6.1. RNA sequencing unveiled downregulation of crucial genes for pancreatic endocrine differentiation, insulin secretion, and ion transport due to RFX6 deficiency. Moreover, RFX6 deficiency led to smaller islet organoids formation via increased cellular apoptosis, attributed to reduced Catalase (CAT) expression, suggesting a protective role for RFX6 in pancreatic islets. Overexpressing RFX6 reversed defective phenotypes in PPs, EPs, and islets. These results indicate that the pancreatic hypoplasia and reduced islet cell formation linked to RFX6 mutations is not due to alterations in PDX1+/NKX6.1+ PPs. Instead, they result from cellular apoptosis, decreased CAT expression, and a downregulation in the expression of pancreatic endocrine genes. These insights may pave the way for potential therapeutic strategies, such as enhancing catalase levels, to mitigate diabetes related to RFX6 defects.

Funding Source: This work was funded by grants from Qatar Biomedical Research Institute (QBRI) (Grant No. QBRI-HSCI Project 1). The first author, Noura Aldous, is a PhD student with a scholarship funded from QRDI (GSRA9-L-1-0511-22008).

T1062

PATIENT-SPECIFIC IPSC-DERIVED HEPATIC ORGANOIDS AS MODEL FOR MRNA-BASED GENE THERAPY IN FARNESOID-X RECEPTOR (FXR) DEFICIENCIES

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Progressive familial intrahepatic cholestasis (PFIC) represents a group of rare disorders characterized by defective bile formation and account for 10-15% of neonatal cholestasis. The underlying mechanism of intrahepatic cholestasis can be associated with mutations in hepatobiliary transport proteins or in nuclear receptors responsible for proper transcriptional control of these transporters. In our previous work we demonstrated that patient-specific iPS cell lines can be differentiated into hepatic organoids for functional studies of the patients' mutations. In this study, we investigated, if such organoids could serve as model to assess effective mRNA-based gene therapy. First, we studied iPSC-based organoids from two patients suffering from BSEP deficiency

caused by mutations in the farnesoid-X receptor (FXR) gene NR1H4. The NR1H4 gene encodes four different FXR variants whereas the FXR alpha-2 variant is most abundant in the liver. We used one cell line carrying a homozygous variation in exon 7, resulting in a loss of substrate binding of FXR and therefore in a complete abrogation of downstream signal transduction. A second cell line carries a homozygous variation in intron 4 resulting in an exon-skipping which encodes the start codon for both FXR alpha-1 and -2 variants, while transcription of the FXR alpha-3 and -4 variants would be unaffected. We generated lipid nanoparticles (LNP) carrying the mRNA of the four different FXR variants as well as BSEP as a positive control. After treatment of the hepatic organoids with the different LNP, we were able to restore BSEP-mediated hepatobiliary transport with all FXR variants. This indicates that even the gut-associated FXR alpha-3 and -4 variants are able to restore hepatic function and might be targetable to induce BSEP function in FXR alpha-1 or -2 deficient patients. However, as the different FXR variants are involved in several metabolic pathways, the global impact of this alternative activation needs further investigation.

Funding Source: This research was funded by the German Ministry of Education and Research (BMBF) through grants to TC, UB, and VK (HIChol: 01GM2204A, 01GM2204B, 01GM2204C).

T1064

PERSONALIZED IPSC-DERIVED ORGANOIDS FOR HUMAN PANETH CELL MODELING

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Paneth cells (PCs) are specialized secretory epithelial cells crucial for intestinal homeostasis. They produce antimicrobial peptides (AMPs) to protect the intestinal epithelium from pathogens. Studying PCs can be challenging due to their inability to be maintained in long-term ex vivo culture systems and the limited protocols for their enrichment in human intestinal organoids (HIOs). To overcome this, we developed a strategy to enrich PCs in HIOs derived from induced pluripotent stem cells (iPSCs). iPSCs were directed to form HIOs, which were dissociated to generate epithelial-only HIOs (eHIOs). eHIOs were cultured in a proliferation medium containing EGF, Noggin, and CHIR99021, with γ-secretase inhibitor DAPT added to promote PC differentiation. PC enrichment was analyzed using single-cell RNA sequencing (scRNA-seq) to provide an overview of cell diversity and confirm PC enrichment. Quantitative PCR (qPCR), flow cytometry (FC), and immunocytochemistry (ICC) quantified PC numbers and AMP expression. Electron microscopy (EM) evaluated PC granule morphology, and LYZ ELISA assessed antimicrobial secretion. Our scRNA-seq data captured the expression of all well-characterized PC markers, providing a comprehensive transcriptomic profile that confirms the identity and enrichment of PCs in the system. FC showed an increase in the PC marker LYZ, from 1% in proliferation medium to 28% after DAPT treatment. DAPT treatment significantly upregulated PC-associated genes, including LYZ (log2 fold change: +0.83), DEFA5 and DEFA6 (log2 fold change: +18.93 and +7.72), and REG3A (log2 fold change: +11.07). ICC confirmed that DAPT-treated eHIOs were enriched in LYZ-positive granulated cells and expressed additional PC AMPs. EM showed electron-dense granules characteristic of PCs. Stimulation with muramyl dipeptide (MDP) induced lysozyme secretion (log2

fold change: +0.378), confirming functional activity. We developed an effective protocol for enriching PCs in iPSC-derived organoids. This system provides a robust model to study human PCs and enables the generation of patient-specific organoids with genetic variations. The enriched organoids retain functional activity, highlighting their potential for modeling intestinal disease mechanisms and evaluating patient-specific therapeutic strategies.

Funding Source: NIH/1R01DK123511, Mechanisms of TL1A-driven Paneth Cell dysfunction in IBD; California Institute for Regenerative Medicine (CIRM EDUC4-1275).

T1066

PLATELET-RICH-PLASMA IMMUNOMODULATORY EFFECTS ON T CELLS IS DEPENDENT UPON DOSAGE AND TIMING OF INITIAL EXPOSURE

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Platelet-rich-plasma (PRP) are processed blood products containing primarily platelet-enriched portion of the plasma. The enrichment of platelets provides a source for growth factors and molecules with regenerative properties. In many clinical conditions that use PRP as a treatment, such as osteoarthritis, inflammation and immune-mediated events are also present. Therefore, we tested for the immunomodulatory effects that PRP may provide with effector immune cells, including the timing and optimal dosage of PRP treatment using an in vitro assay. We isolated peripheral blood mononuclear cells (PBMCs) of healthy individuals and treated them with PRP derived from allogeneic donors. Our results show that PRP treatment resulted in reduced T cell production of pro-inflammatory cytokines (TNF-alpha, IL-1beta and IL-6) in a dose-dependent manner, yet with a narrow window of concentration to achieve overall immunosuppressive effects. In addition, the timing of PRP exposure to T cells also impacted their level of immunosuppressive capacity. Altogether, our data implies that an optimal condition of dosage and timing of PRP exposure to immune cells plays a role in how effective PRP can mitigate inflammation and potentially impact wound healing in host tissues.

T1068

POPULATION-SPECIFIC TRANSCRIPTOMIC AND MOLECULAR INSIGHTS INTO PRPF31-ASSOCIATED RETINITIS PIGMENTOSA USING RETINAL ORGANOIDS

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Retinitis Pigmentosa (RP), caused by mutations in PRPF31, leads to progressive photoreceptor loss and blindness. This study integrates human-induced pluripotent stem cell (hiPSC)-derived retinal organoids with single-cell RNA sequencing (scRNASeq) to uncover transcriptomic changes and molecular mechanisms underlying RP. Patient-specific iPSCs with a PRPF31 mutation (c.1115_1125 del11) and a control line were differentiated into retinal organoids (ROs) mimicking in vivo development and analyzed at early (d:day85) and late (d285) developmental stages. scRNASeq profiled transcriptomic alterations across retinal populations, including photoreceptors, ganglion cells, and Müller glia. Differentially expressed genes (DEGs) were identified and subjected to pathway enrichment analysis. Key findings were validated through RT-qPCR, immunofluorescence (IF), and functional assessment by multielectrode array (MEA) recordings. In PRPF31-mutant ROs, photoreceptors showed early dysfunction with downregulation of phototransduction and oxidative phosphorylation pathways, progressing to degeneration by d285. Müller glia exhibited upregulation of gliosis and inflammatory markers, while ganglion cells displayed disrupted axon guidance and neuronal differentiation pathways. Temporal comparisons highlighted early photoreceptor dysfunction and late-stage degeneration, linking these changes to aging-related disease processes. Pathway enrichment implicated neurodegenerative and mitochondrial dysfunction as central drivers of RP. Validation through RT-qPCR and IF confirmed those findings. Functional MEA recordings demonstrated diminished electrophysiological activity in PRPF31 ROs. This study shows that PRPF31-mutant ROs recapitulate RP progression, linking late-stage degeneration to aging-related processes. Mitochondrial dysfunction and chronic neuroinflammation emerge as key drivers, offering insights for targeted therapies to preserve photoreceptors and reduce gliosis.

T1070

PRE-CLINICAL IN VITRO EFFICACY OF ENGINEERED MESENCHYMAL STEM/STROMAL CELLS EXPRESSING SOLUBLE ACE2 DECOYS FOR ELIMINATION OF SARS-COV-2 INFECTION

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The emergence of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and its continuously evolving variants has underscored the urgent need for innovative therapeutics to combat severe disease progression. In certain patients, COVID-19 advances to acute respiratory distress syndrome (ARDS), driven by a life-threatening hyper-inflammatory response and multi-organ failure. Mesenchymal stem cell (MSC)-based therapy have shown promising effects in reducing disease severity in ARDS patients and represent a potentially effective option against

severe COVID-19. Whilst a variety of strategies have emerged to optimize MSC-based therapies, genetically modified MSCs for ARDS treatment has emerged as a compelling approach. Our strategy focuses on enhancing the umbilical-cord MSCs (UC-MSCs) by co-expressing various soluble angiotensin converting enzyme 2 (sACE2) decoy receptors and interleukin-37 (IL37). These modifications aim to mitigate the viral infection and attenuate the inflammatory response. In this study, we engineered UC-MSCs by transducing with three variants of sACE2 protein, {sACE2-WT (Wild-type), sACE2-WT-IgG1-Fc and sACE2-v.2.4-IgG}, and further co-transduced them with IL37. Using assays to evaluate protein expression, cell infectivity, and virus neutralization, we investigated the efficacy of UC-MSCs expressing all types of soluble ACE2 to target and destroy the aggressive SARS-CoV-2 Delta variant (B.1.612.2) pseudovirus infection. Upon infection, Calu3 and Caco2 cells exhibited 73.1% and 67.2% infection rates whereas all engineered MSCs displayed negligible infection. Further, in cell-based pseudovirus neutralization assays, indirect co-culture of Calu3 with all engineered MSC-sACE2-IL37 achieved 82.6%, 85.9%, and 90.1% neutralization after 72 hours, while direct co-culture yielded higher rates of 90.3%, 92%, and 93.4%, compared to 50% and 55% in control groups. Comparing the neutralization activities among the three MSC-sACE2-IL37 variants, MSC-sACE2-v.2.4-IgG-IL37 demonstrated robust and consistent neutralization activity, underscoring its superior ability to suppress pseudovirus transduction. Our results highlight the potential of engineered MSC-sACE2-IL37 as an innovative therapeutic strategy for combating severe SARS-CoV-2 infection.

T1072

PROBING THE PATHOLOGY OF AMYOTROPHIC LATERAL SCLEROSIS USING INDUCED NEURAL STEM CELLS OBTAINED BY DIRECT LINEAGE REPROGRAMMING

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Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron disease with no effective treatments. Motor neurons (MNs) derived from ALS patients' induced pluripotent stem cells (iPSCs) can reflect some ALS cellular pathologies. However, during reprogramming, iPSCs are reset to an embryonic state and conceal age-associated pathologies. We developed a method to turn human blood into induced neural stem cells (iNSCs) without going through a pluripotent state. iNSC are amenable for single cell passaging and precise genome editing that allowed us to engineer an TARDBP-Q331K mutation. We have generated iNSCs and isogenic iPSC lines from several donors with different ages, including patients with SOD1 and C9orf72 mutations. I will present comparisons of iNSC with iPSC-derived NSCs using epigenetic clocks and transcriptome analyses and ongoing efforts to model ALS pathogenesis in cell and organoids models. We aim to develop more authentic models to capture ALS pathology to evaluate pharmacological and genetic interventions.

T1074

PT1A AND SORAFENIB COMBINATION TREATMENT EXERTS SYNERGISTIC ANTIHEPATOCELLULAR CARCINOMA EFFECT THROUGH RNA M6A METHYLATION MODIFICATION

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Sorafenib, a multi-target tyrosine kinase inhibitor for advanced hepatocellular carcinoma (HCC), provides only modest survival benefits due to acquired resistance. The novel platinum-based drug, Pt1a, has demonstrated anti-cancer properties by degrading the epithelial-mesenchymal transition marker vimentin, a known mediator of sorafenib resistance. Therefore, we investigated whether Pt1a can sensitize HCC cells to sorafenib. In cultured HCC cells and patient-derived organoids, the combination of sorafenib and Pt1a synergistically reduced cell viability, attenuated self-renewal, and promoted cell apoptosis compared to single-drug treatment. In contrast, this synergistic effect was not observed in the immortalized human hepatocyte cell line MIHA. These findings were further validated in HCC patient-derived xenograft and NRAS/AKT-driven HCC mouse models. Notably, in the NRAS/AKT-driven HCC mouse model, the combination of sorafenib and Pt1a demonstrated superior efficacy compared to anti-VEGFA combined with anti-PD-L1 antibody treatment, which is the current first-line treatment for HCC. Mechanistically, RNA-sequencing and thermal proteome profiling revealed enrichment of m6A-related pathways under combination treatment, with an elevated m6A level confirmed by m6A dot blotting. The m6A writer METTL14 was identified as a potential mediator of the synergistic effect through functional CRISPR-knockout experiments. GLORI-sequencing was performed to identify the downstream m6A target genes of METTL14, and a combined signature of these genes predicted better survival in HCC patients and a negative correlation with cancer stemness. Our findings suggested Pt1a could synergize with sorafenib to inhibit HCC through METTL14-mediated m6A modification. This research provides valuable insights into the potential of Pt1a to overcome sorafenib resistance and opens avenues for novel treatment strategies for HCC.

T1076

RAPID IPSC-DERIVED NEUROMUSCULAR JUNCTION MODEL UNCOVERS MOTOR NEURON DOMINANCE IN AMYOTROPHIC LATERAL SCLEROSIS CYTOPATHY

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The neuromuscular junction (NMJ) is essential for transmitting signals from motor neurons (MNs) to skeletal muscles (SKMs), and its dysfunction can lead to severe motor disorders. However, our understanding of the NMJ is limited by the absence of accurate human models. Although human induced pluripotent stem cell (iPSC)-derived models have advanced NMJ research, their application is constrained by challenges such as limited differentiation efficiency, lengthy generation times, and cryopreservation difficulties. To overcome these limitations, we developed a rapid human NMJ model using cryopreserved MNs and SKMs derived from iPSCs. Within 12 days of coculture, we successfully recreated NMJ-specific connectivity that closely mirrors in vivo synapse formation. Using this model, we investigated amyotrophic lateral sclerosis (ALS) and replicated ALS-specific NMJ cytopathies with SOD1 mutant and corrected isogenic iPSC lines. Quantitative analysis of 3D confocal microscopy images revealed a critical role of MNs in initiating ALS-related NMJ cytopathies, characterized by alterations in the volume, number, intensity, and distribution of acetylcholine receptors, ultimately leading to impaired muscle contractions. Our rapid and precise in vitro NMJ model offers significant potential for advancing research on NMJ physiology and pathology, as well as for developing treatments for NMJ-related diseases.

Funding Source: National Science and Technology Council of Taiwan (MOST 111-2314-B-303-027-MY2 and NSTC 113-2314-B-303-026; Dr. C.-Y.C.) Hualien Tzu Chi Hospital (IMAR-110-01-20; Dr. C.-Y.C.).

T1078

RECIPROCAL ENHANCEMENT OF SARS-COV-2 AND INFLUENZA VIRUS REPLICATION IN HUMAN PLURIPOTENT STEM CELL-DERIVED LUNG ORGANOIDS

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Patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and influenza A virus (FLUAV) coinfections were associated with severe respiratory failure and more deaths. Here,

we developed a model for studying SARS-CoV-2 and FLUAV coinfection using human pluripotent stem cell-induced alveolar type II organoids (hiAT2). hiAT2 organoids were susceptible to infection by both viruses and had features of severe lung damage. A single virus markedly enhanced the susceptibility to other virus infections. SARS-CoV-2 delta variants upregulated α-2-3-linked sialic acid, while FLUAV upregulated angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2). Moreover, coinfection by SARS-CoV-2 and FLUAV caused hyperactivation of proinflammatory and immune-related signaling pathways and cellular damage compared to a respective single virus in hiAT2 organoids. This study provides insight into molecular mechanisms underlying enhanced infectivity and severity in patients with co-infection of SARS-CoV-2 and FLUAV, which may aid in the development of therapeutics for such co-infection cases.

Funding Source: This study was supported by grants from the Korea Disease Control and Prevention Agency, Korea National Institute of Health [grant numbers 2023-NS-001-00, 2020-NI-019-02].

T1080

ROLE OF ZNF808 IN HUMAN PANCREATIC CELL FATE SPECIFICATION

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Human pancreatic development is an intricately coordinated process that generates a gland composed of the endocrine and exocrine cells. Mutations in genes regulating this process result in developmental abnormalities, such as pancreatic agenesis. Our recent work identified loss-of-function mutations in the primate-specific gene ZNF808, leading to pancreatic agenesis, which manifests as neonatal diabetes and exocrine pancreatic insufficiency. ZNF808 belongs to the KRAB zinc finger family and is known to bind MER11 transposable elements and repress their gene regulatory activity. However, the precise mechanism by which ZNF808 regulates human pancreatic development remains unclear. To investigate the mechanism of ZNF808, we generated ZNF808 knockout (KO) in the H1 human embryonic stem cell (hESC) line and differentiated them alongside control H1 hESCs using a stem cell derived islet differentiation model optimized in our lab. Bulk RNAseq analysis revealed an aberrant activation of the hepatic lineage markers and a downregulation in the pancreatic lineage markers at the posterior foregut stage of differentiation. To further understand the regulatory networks disrupted by the lack of ZNF808 we conducted single cell RNAseq and single nuclear ATACseq from day 0 (stem cell stage) until posterior foregut stage. Our preliminary analysis of the single cell genomics data identified an upregulation of BMP-

pathway signaling driven by the derepression of MER11 transposable elements, thereby resulting in increased differentiation into the hepatic lineage. This was accompanied by a downregulation of TGFβ-pathway signaling, disrupting the balance necessary for proper human pancreatic lineage specification. This work identifies a novel mechanism regulating human pancreas specification and highlights the need to study primate-specific regulatory processes controlling development. Additionally, the use of stem cell derived islet differentiation model has shown great potential for disease modelling purposes but also for use as cell therapy in diabetes treatment.

Funding Source: Wellcome Trust Collaborative Funding.

T1082

SEX-SPECIFIC REGULATION OF WNT SIGNALING AND INFLAMMATION IN OSTEOGENIC DIFFERENTIATION OF HUMAN AORTIC VALVE INTERSTITIAL CELLS

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Calcific aortic valve disease (CAVD) is a progressive disorder characterized by pathological calcification of human aortic valve interstitial cells (HAVICs). Despite advances in understanding its pathogenesis, the molecular pathways underlying sex-specific differences in CAVD remain poorly understood. This study aims to explore mechanisms regulating osteogenic differentiation in HAVICs. Clinical assessments revealed that men exhibited a higher calcification burden, with significantly elevated Agatston scores and 18F-NaF uptake, while women demonstrated increased fibrosis, consistent with differences in the OPG/sRANKL axis. To investigate these differences, we isolated HAVICs from men and women and performed transcriptomic and proteomic analyses. These analyses revealed that 1,210 genes and 70 proteins exhibited sex-specific expression during osteogenic differentiation, with Wnt/β-catenin signaling emerging as a key regulatory pathway. To further examine the functional impact of Wnt signaling, we activated the canonical Wnt pathway in HAVIC cultures undergoing osteogenic differentiation. Remarkably, Wnt activation inhibited differentiation, as evidenced by reduced calcium deposition and lower expression of osteogenic markers. Furthermore, Wnt activation decreased caspase-1 activity, suggesting a suppression of inflammation. Transcriptomic analysis of Wnt-activated HAVICs revealed downregulation of FGD4, a gene that was previously upregulated during differentiation. Interestingly, endothelial-associated markers were also upregulated, indicating a potential proangiogenic shift under Wnt activation. This study highlights key sex-specific molecular mechanisms involved in CAVD. Our transcriptomic and proteomic findings identify Wnt/β-catenin signaling as a central pathway regulating osteogenic differentiation in HAVICs. Activation of this pathway not only suppressed osteogenesis but also reduced caspase-1 activity and induced endothelial phenotypic changes, accompanied by decreased FGD4 expression. These results provide new insights into the links between inflammation, endothelial transitions, and calcification, suggesting that targeting Wnt/β-catenin signaling could hold therapeutic potential for developing sex-specific treatments for CAVD.

Funding Source: This study was supported by the Ministry of Science and Higher Education of the Russian Federation (Agreement No. 075-15-2022-301, dated 04/20/2022).

T1084

SINGLE-CELL TRANSCRIPTOMIC ANALYSIS REVEALS DISTINCT CELLULAR RESPONSES AMONG CORTICAL PROGENITORS AND NEURONS IN HUMAN BRAIN ORGANOID MODELING FETAL HYPOXIC BRAIN INJURY

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Fetal hypoxia impacts development and causes 23% of neonatal deaths. The prenatal brain is highly susceptible to hypoxic exposure. Nevertheless, the specific cell types affected and the underlying molecular mechanisms that mediate hypoxic brain injury remain incompletely understood. To address this issue, we developed brain organoids modelling human fetal brain hypoxic injury during the early to mid-gestational period. Utilizing single-cell transcriptome analysis, we identified seven distinct cell types within these brain organoids, representing major cortical progenitors and neurons present in the fetal brain at this developmental stage. Specifically, we observed distinct alterations in mTORC1 signaling, fatty acid synthesis, unfolded protein response, and innate immune responses among these cell types under hypoxic conditions. Notably, unlike cortical progenitors, developing glutamatergic and GABAergic neurons exhibited a pronounced delay in maturation following hypoxic exposure. Furthermore, our analysis identified two distinct GABAergic neuron subtypes that displayed differential sensitivities to hypoxia: type 2 neurons, which are more mature, were found to be more susceptible to hypoxia-induced neural functional impairments compared to type 1 neurons. In summary, our study provides a comprehensive understanding of the distinct cellular responses among cortical progenitors and neurons in fetal hypoxic brain injury, offering potential therapeutic targets for this condition.

Funding Source: This work was supported by grants from the National Natural Science Foundation of China (32300675, 32400598), Sichuan Science and Technology Program (2022YFS0615, 2025ZNSFSC1019).

T1086

SINGLE-CELL TRANSCRIPTOMICS REVEALS REDUCED MOTOR NEURONS AND AXONAL OUTGROWTH IN NEUROMUSCULAR ORGANOIDS GENERATED FROM SPINAL MUSCULAR ATROPHY PATIENTS' URINE DERIVED IPSCS

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Spinal muscular atrophy (SMA) is a neuromuscular disease caused by the loss of the ubiquitously expressed survival motor neuron (SMN) protein, which is essential for the biogenesis of spliceosome complex, resulting in the selective degeneration of spinal motor neurons (MNs). Based on the onset of disease, SMA disease is clarified to type I, II and III, and type I represents the most severe type. While current SMN-enhancing therapies have shown promise in reducing

disease severity, they do not reverse motor axon degeneration, leading to a limited locomotor recovery. Moreover, the mechanism underlying the specific loss of MNs remains unknown. Here, we used single-cell transcriptomics to examine alterations in cellular populations and gene expression in neuromuscular organoids (NMOs) derived from the urine cells of patients with SMA type I, II and III compared to those from healthy individuals. These NMOs self-organize into two distinct compartments comprising neural and muscle lineages. In the mature spinal cord neuron and neural progenitors cluster, there is a significant decrease in pre-mRNA splicing related genes such as HNRNPC, RBM25, SRSF2 and MYEF2, which may be caused by the deficiency of SMN protein. We confirmed a marked reduction in MN formation in neuromuscular organoids (NMOs) derived from SMA patients. When comparing the gene expression of the residual MN cluster in SMA patients to that of healthy individuals, we detected a decreased expression of genes involved in axonal outgrowth and cytoskeletal integrity in axon such as SHTN1, NEFL, NEFM, DCC, DCX, MAPT, suggesting defective axonal projection in SMA patients' MNs. Consistently, these NMOs showed defective formation of neuromuscular junctions (NMJs) and increased cell death as shown by immunofluorescence staining. In summary, our findings provide insights into the cellular and molecular basis that underlie the impaired function of MNs, muscles, and NMJs in SMA patients, paving the way for identifying new therapeutic targets to restore motor axonal outgrowth and NMJ formation.

Funding Source: Research Grants Council and the University Grants Council of HK (GRF_17114619, GRF_17102420, R7018-23F), URC Seed Fund for Collaborative Research 2022/2023.

T1088

SMALL EXTRACELLULAR VESICLES (SEV) FROM GLYCOLYTICALLY ACTIVATED MESENCHYMAL STEM/STROMAL CELLS PROMOTE CHONDROPROTECTION IN VITRO AND CARTILAGE REGENERATION IN VIVO

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Osteoarthritis (OA) affects the articular cartilage causing an increase in oxidative stress leading to cartilage damage. It has been shown that mesenchymal stem/stromal cells (MSCs) therapeutic effects depend on their small extracellular vesicles (sEVs). However, for optimal clinical outcomes there is a need to enhance their therapeutic efficacy and to improve their storage for potential clinical application. We propose that sEVs derived from metabolically reprogrammed glycolytic-MSC (Glyco-sEVs) have a better therapeutic effect that those under basal conditions (Basal-sEVs) and that after a lyophilized process they maintain their phenotype and function against OA progression. Chondrocytes were isolated from the knee of OA patients. Chondroprotective effect of frozen or lyophilized Glyco-sEVs was measured by an apoptotic assay with menadione through

annexin/PI kit and flow cytometry. To determinate the clinical effect of freeze or freeze dried Glyco-sEVs, both were injected intraarticularly in a collagenase murine model of OA (CIOA) and evaluated by MicroCT and histopathology. Our results showed that lyophilized Glyco-sEVs do not change their sEV characteristics, therefore they maintained their chondroprotective effect on chondrocytes when exposed to menadione as a source of oxidative stress. Moreover, CIOA mice treated with both frozen or lyophilized Glyco-sEVs showed a significant reduction in bone mineral density and the clinical damage score obtained from joint histology. Lyophilized Glyco-sEVs maintain their phenotype by expressing CD63, CD81 and CD9 and their size and shape with yield of 86,5%. Moreover, they do not change their chondroprotective effect in vitro since as frozen Glyco-sEVs, they display higher resistance to apoptosis induced by oxidative stress as compared to basal sEVs. Moreover, CIOA mice treated with both Glyco-sEVs showed a regenerative effect with significance evidenced in the histomorphometry parameters and the clinical joint score by histology. These results show that lyophilized Glyco-sEVs represents a potential new strategy for treating OA through their chondroprotective and regenerative properties.

Funding Source: This research was supported by ANID: FONDEF N°21110194; and IMPACT-FB-210024, FONDECYT-Regular N°1211353 and FONDECYT-Iniciación N°11220549.

T1090

SPINAL CORD ORGANOIDS (SCOS) ARE VALUABLE MODELS FOR DEVELOPMENT AND DISEASES. WE PERFORMED SINGLE-CELL RNA-SEQ ON SCOS (D30–120), REVEALING DIVERSE CELL TYPES AND A DETAILED MOLECULAR ATLAS OVER TIME.

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Stem cell-derived organoids are valuable models that recapitulate human physiology in remarkable detail and offer unique opportunities to study central nervous system diseases that are challenging to model using animals or monolayer cultures. Our lab has established a robust protocol for generating spinal cord organoids (SCOs) from induced pluripotent stem cells (iPSCs) to investigate spinal cord development, injury, and neurological disorders. SCOs progress through defined developmental stages: spinal cord progenitor cells (SCPCs) at day 10, motor neurons and interneurons by day 30, and astrocytes and oligodendrocytes by days 60 and 90, as confirmed by immunohistochemistry. However, A comprehensive whole-transcriptome analysis of SCOs at specific time points has been hindered by technical limitations in dissociating organoids into viable single cells. Furthermore, while SCPC transplantation holds promise as a therapeutic approach for spinal cord injury, the differentiation and integration of these cells in vivo remain poorly understood. In this study, we developed a novel protocol to efficiently dissociate SCOs into viable single cells, enabling single-cell RNA sequencing (scRNA-SEQ) from day 30 to day 120. Our data provide a detailed molecular atlas and identify diverse cell types within SCOs at these stages. Additionally, we performed scRNA-SEQ on dissociated rat spinal cords following human SCPC transplantation, obtaining both rat and human cells. By mapping reads to a combined rat-human genome, we observed that cell types derived from day-60 organoids closely resemble human cell types differentiated in vivo after SCPC transplantation. This finding highlights the utility of SCOs as predictive models for the regenerative potential of transplanted cells. Our work establishes a comprehensive molecular and cellular framework for spinal cord organoids, offering valuable

insights into spinal cord biology and advancing their application in regenerative medicine and disease modelling.

T1092

TARGETING PD-1/PD-L1 PATHWAY FOR IMMUNOTHERAPY AGAINST ZIKA VIRUS INFECTION

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Zika virus (ZIKV) has emerged as a significant global health threat in recent years. However, there are currently no approved antiviral drugs or vaccine available for the treatment or prevention of Zika virus infection. The programmed cell death-1 (PD-1)/programmed cell death-ligand 1 (PD-L1) signaling pathway is known to inhibit immune responses against cancers and regulate antiviral immune responses modulated by various viruses. However, its role in ZIKV infection remains unclear. Our study revealed that ZIKV infection upregulates mRNA and protein expression of PD-L1 in SF268 and JEG3 cell lines, as well as human dendritic cells. Furthermore, it is noteworthy that ZIKV infection also enhances PD-L1 expression in human trophoblast stem cells (hTSCs). Screening viral proteins, we identified NS4B as the primary viral protein inducing PD-L1 expression and promoter activities in a dose-dependent manner. In C57BL/6 mice, elevated PD-L1 expression was observed in the brain, testis, and spleen post-infection. Notably, ZIKV infection elevated antigen-specific CD8+ T cells and PD-1+ CD8+ T cells. Blocking PD-L1 effectively inhibited ZIKV infection, reducing virus load in the brain, testis, and spleen of A129 mice. Furthermore, anti-PD-L1 antibody treatment further increased virus-specific CD8+ T cells, KLRG+ CD8+ T cells, and memory CD8+ T cells. PD-L1 blockade also enhanced IFN-gamma, granzyme B, and IL-2 expression in antigen-specific CD8 T cells, indicating improved T cell functionality. These findings suggest that targeting the PD-1/PD-L1 pathway could be a promising immunotherapy strategy against ZIKV infection.

T1094

THE IMPORTANCE OF HIGH-DENSITY MICROELECTRODE ARRAYS FOR RECORDING MULTI-SCALE EXTRACELLULAR POTENTIAL AND LABEL-FREE CHARACTERIZATION OF NETWORK DYNAMICS IN IPSC-DERIVED NEURONS

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Advances in microelectrode array (MEA) technology for in-vitro electrophysiological recordings have made it possible to study neuronal networks across multiple scales, from subcellular properties to network-level dynamics. These devices are essential for exploring the phenotypes of neurological disorders and accelerating drug discovery, offering unique insights into the behaviour of neuronal networks. Key factors such as electrode density, spacing, and size significantly impact signal quality, noise, and sensitivity. To exhaustively characterize neuronal networks, MEAs must combine single-cell and subcellular resolution with high-throughput capabilities, maintaining sensitivity to small extracellular action potentials to capture the full range of network activity. In this study, the MaxOne and MaxTwo high-density (HD) MEA systems (MaxWell Biosystems, Switzerland) were utilized to record activity from induced pluripotent stem cell-derived neurons. These systems, with 26,400 electrodes per well, demonstrated the benefits of increased statistical power in longitudinal data collection. HD-MEA recordings were compared to simulated low-density recordings, where adjacent electrodes on HD-MEAs were clustered to mimic larger, lower-density electrodes. Additionally, the AxonTracking Assay, an automated tool for analysing individual axonal arbours from multiple neurons simultaneously, was used to evaluate axonal structures and network functionality in the recorded cultures. Results showed that higher electrode density and smaller electrode size enhanced sensitivity, allowing for the detection of smaller spikes and capturing the complete spectrum of network dynamics. The high-resolution analysis of network activity, combined with subcellular insights from the AxonTracking Assay, offers a robust platform for drug screening and disease modelling.

Funding Source: This work is funded by the HyVIS project, GA 964468, within the H2020 Framework Program of the European Commission. This work is funded by the NEUREKA project, GA 863245, within the H2020 Framework Program of the European Commission.

T1096

THE RS6903956 GENETIC VARIANT CONTIRBUTES TO ENDOTHELIAL DYSFUNCTION VIA HOX-MEIS COOPERATIVE REGULATION OF PHACTR1 IN CORONARY ARTERY DISEASE

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Cardiovascular diseases, particularly coronary artery disease (CAD), remain leading causes of mortality worldwide. The single nucleotide polymorphism (SNP) rs6903956 on chromosome 6p24.1 has been identified as a susceptibility locus for CAD in East Asian populations through genome-wide association studies. However, its functional role has not been fully elucidated. This study investigates the mechanistic basis of rs6903956 and its contribution to CAD pathogenesis, focusing on endothelial cell dysfunction. Large cohort studies revealed an association between the rs6903956 ‘A’ allele and blood pressure phenotypes, along with impaired endothelial responsiveness indicated by reduced flow-mediated dilation. Single-base editing of patient induced

pluripotent stem cell-derived endothelial cells and expression quantitative trait loci analysis highlighted a cis-acting impact of the 'A' risk allele on PHACTR1 and EDN1 expression, suggesting allele-specific regulatory effects. Using in silico modelling by AlphaFold 3 platform, the 'A' allele exhibited enhanced binding affinity for HOXA4 and MEIS1 transcription factors, forming a stable ternary complex that promoted transcriptional activation of PHACTR1. Functional assays demonstrated the enhancer role of rs6903956 'A' in driving PHACTR1 promoter activity, supporting its locus-specific regulatory function in endothelial cells. Under disturbed flow conditions, endothelial cells harboring the 'A' allele display elevated ICAM-1 expression and increased monocyte adhesion compared to the 'G' allele, indicating allele-specific endothelial inflammatory activation. These findings propose a model in which rs6903956 influences PHACTR1 expression via HOX-MEIS cooperative binding, thereby modulating endothelial function and contributing to CAD susceptibility. This study provides mechanistic insights into the role of rs6903956 in endothelial dysfunction and CAD, informing potential therapeutic targets arising from genetic determinants in cardiovascular pathogenesis.

T1098

THERAPEUTIC EFFICACY OF DANGO TREATMENT ON ALZHEIMER'S DISEASE BRAIN ORGANOIDS DERIVED FROM MULTIPLE PATIENT SOURCES

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Alzheimer's disease (AD) remains one of the most prominent neurodegenerative disorders, with significant advances in understanding its pathophysiology and underlying mechanisms; however, therapeutic progress has been limited. In this study, patient-derived induced pluripotent stem cells (iPSCs) were utilized to generate cerebral brain organoids, which were cultured until they exhibited key AD phenotypes, including the accumulation of amyloid-beta (Aβ) plaques and the formation of hyperphosphorylated tau (pTau)-derived neurofibrillary tangles (NFTs). We aimed to evaluate whether treatment with nano graphene oxide (NGO) could mitigate the expression of these critical AD hallmarks. Although NGO treatment did not alter the levels of pTau, it significantly reduced the accumulation of Aβ plaques in AD organoids. Mechanistically, NGO treatment activated the autophagy pathway via AMP-activated protein kinase (AMPK) signaling and decreased the expression of IFITM3, which correlated with reduced pro-inflammatory cytokine levels. Collectively, the AD brain organoid model effectively recapitulated key disease phenotypes and provides a promising platform for evaluating potential therapeutic interventions in AD.

Funding Source: This research was supported by the Bio and Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MSIT) (No. RS-2023-00266110).

T1100

THERAPEUTIC POTENTIAL OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED MICROGLIA OVEREXPRESSING INTERLEUKIN-10 IN ALZHEIMER'S DISEASE

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Microglia, the primary immune cells of the central nervous system (CNS), play essential roles in immune surveillance, synaptic pruning, and modulation of neuroinflammation. In neurodegenerative diseases such as Alzheimer's disease, microglial dysfunction has been implicated in exacerbating disease progression. Chronic microglial activation results in the release of pro-inflammatory cytokines and neurotoxic factors, leading to neuronal damage and amplifying neuroinflammation. Understanding the dual roles of microglia in maintaining CNS health and contributing to disease processes offers critical insights for therapeutic development. To explore potential therapeutic strategies, we evaluated the functions of several genes involved in the clearance of misfolded proteins, such as amyloid-beta (Aβ) and the regulation of inflammation in induced pluripotent stem cell (iPSC)-derived microglia. Our study revealed that overexpression of interleukin-10 (IL-10), an anti-inflammatory cytokine, in iPSC-derived microglia not only suppressed inflammation but also enhanced Aβ clearance. This dual action represents a key mechanism for addressing neurodegenerative diseases characterized by Aβ accumulation and neuroinflammation. These findings highlight the therapeutic potential of IL-10-overexpressing iPSC-derived microglia as a promising platform for ex vivo gene therapy research targeting Alzheimer's disease, other neurodegenerative conditions, and psychiatric disorders.

Funding Source: This research was supported by AMED under Grant Number JP24bm1323001h0102.

T1102

THREE-DIMENSIONAL ORGANOID MODEL DERIVED FROM STEM CELLS TO STUDY AND DEVELOP TREATMENTS FOR NEUROMUSCULAR DISORDERS, FOCUSING ON SPINAL CORD AND MUSCLE INTEGRATION

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Spinal Muscular Atrophy (SMA) is a severe neurodegenerative disorder caused by autosomal recessive mutations in the SMN1 gene, leading to reduced SMN protein levels. This deficiency results in progressive motor neuron loss, denervation, muscle atrophy, and profound weakness. The most severe form, SMA type I, typically leads to early mortality or requires mechanical ventilation within the first two years of life if untreated. Developing human-derived models is essential for advancing our understanding of SMA and identifying effective therapeutic interventions. In this study, we generated human spinal cord organoids from induced pluripotent stem cells (iPSCs) obtained from three SMA type I patients and three healthy controls. These organoids were used to explore disease-related molecular mechanisms in a 3D model and to test a Risdiplam-like compound (RIS-L), designed to enhance SMN protein expression. We implemented an acute and repeated treatment regimen over a 75–90-day differentiation period, reflecting early human fetal development. Our analyses revealed widespread cellular and molecular defects in

SMA organoids, affecting multiple cell types beyond motor neurons. These findings were supported by bulk transcriptomics, single-cell RNA sequencing, immunophenotyping, and multi-electrode array (MEA) electrophysiology. Notably, RIS-L treatment modulated over 15% of disease-altered genes, restored the full-length SMN2/Δ7 ratio, and reversed hallmark pathological features, underscoring its therapeutic potential. Additionally, molecular profiling revealed altered cilia-associated gene expression, suggesting a broader impact of RIS-L on neurodevelopmental pathways. Using high-density MEAs, we assessed baseline and chemically modulated electrophysiological activity, revealing disease-specific spiking and bursting abnormalities, alongside treatment-driven functional improvements. Our study emphasizes the early developmental origins of SMA and demonstrates the relevance of organoid-based models for evaluating novel treatments and refining therapeutic strategies.

T1104

TRANSFORMER-BASED CELL TYPE PREDICTION INCORPORATING THE BAG STRUCTURE OF GENE EXPRESSION DATA

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Recently, deep learning models for single cell applications have been pre-trained on large-scale scRNA-seq data. These models have been utilized for dissecting disease phenotype, development trajectory, and cellular heterogeneity. However, there remain limitations regarding the model's performance in identifying subpopulations and state-transitions. These limitations hinder our ability to trace cell fate decisions during finer differentiation processes and to comprehensively analyze disease-related cellular behavior within these subtypes. The gene expression data is represented as a bag in computer science. Using the data structure is suitable for analysis, whereas it has been challenging to directly incorporate with bag data in deep learning models. We addressed this issue by developing a new model which can process gene multiplicity and improved the performance of cell type classification. We applied the model to the classification of hepatocytes in liver, since it is crucial for their use in medical examinations. This model demonstrates improved prediction accuracy of the details of cell types compared to existing approaches.

Funding Source: Moonshot Research and Development Program promoted by Japan Science and Technology Agency: Project "Co-evolution of Human and AI-Robots to Expand Science Frontiers".

T1106

TRANSFORMING THE THERAPEUTIC LANDSCAPE OF PANCREATIC CANCER THROUGH LOCAL DELIVERY OF AN IMMUNE-ACTIVATING SMALL MOLECULE AND CYTOKINE-INDUCED KILLER CELL SYNERGY

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Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive cancer with a 5-year survival rate of less than 8%, primarily due to its fibrotic tumor microenvironment and cancer cell-mediated immune checkpoint regulation, which hinder drug penetration and suppress immune responses. This study investigated the potential of the small molecule n-butylidenephthalide (BP), delivered through a thermosensitive sustained-release hydrogel, in combination with cytokine-induced killer (CIK) cells, to enhance the therapeutic efficacy of CIK cells against PDAC. BP treatment significantly activated NK and NKT immune phenotypes ($CD3^+CD56^+$) in CIK cells by downregulating immune checkpoint markers (PD-1, CTLA-4) and the downstream mTOR signaling pathway. These modulations improved CIK-mediated cytotoxicity, demonstrating enhanced therapeutic potential for PDAC treatment. In a murine PDAC xenograft tumor model, the combination of CIK cells with BP-loaded hydrogel further amplified therapeutic efficacy, as evidenced by reduced tumor volume, quantified through fluorescence intensity analysis using IVIS imaging. Elevated Caspase-3 expression, reduced PD-L1 levels in tumor tissues, decreased fibrosis via α-SMA downregulation, and lower expression of pro-carcinogenic factors including Calpain-1 and Vimentin further corroborated these findings. These results highlight the ability of locally delivered BP via a sustained-release hydrogel to modulate the immunosuppressive tumor microenvironment and promote immune-mediated tumor destruction. The study provides compelling evidence supporting the use of BP and CIK cell-based strategies for advancing PDAC therapy and addressing the challenges posed by its highly immunosuppressive microenvironment.

Funding Source: The funding support from National Science and Technology Council, Taiwan (MOST-111-2221-E-259-001-MY3) is greatly appreciated by the authors.

T1108

UBIQUITIN LIGASE RBCK1 MODULATES GLYCOGEN METABOLISM BY ALTERING THE SUBCELLULAR LOCALIZATION OF A GLYCOLYTIC ENZYME

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Glycogen, a primary energy reserve in mammals, depends on precise regulation by metabolic enzymes. While disruptions in canonical glycogen-associated proteins are well-documented causes of glycogen storage diseases (GSDs), the impact of non-glycogen-associated proteins remains underexplored. Here, our study reveals that RBCK1, a ubiquitin ligase, plays a critical role in maintaining glycogen metabolism and cardiac function. We utilized human pluripotent stem cell-derived cardiomyocytes to demonstrate that RBCK1 deficiency causes significant metabolic disturbances, including excessive polyglucosan body formation, cardiomyopathy, and calcium signaling defects. Mechanistically, we found that expression of a RBCK-binding mitochondrial protein was reduced in RBCK1-deficient cells. This reduction promoted trans-localization of a glycolytic enzyme to the cytoplasm, promoting glycogen synthesis. Furthermore, we discovered a small molecule promotes the mitochondrial glycolytic enzyme activity, redirecting glucose towards glycolysis and thereby reducing polyglucosan body accumulation. Thus, deficiency of RBCK1

results in glycogen accumulation, energy imbalance, and severe cardiac phenotypes, including dilated cardiomyopathy (DCM). These findings reveal regulatory mechanisms in glycogen metabolism beyond canonical pathways and highlight potential therapeutic targets for conditions such as cardiomyopathy associated with metabolic disorders.

T1110

UNRAVELING THE ROLE OF WDR19 HYPOMORPHIC MUTATION IN ADULT-ONSET RENAL FAILURE USING HPSC-DERIVED KIDNEY ORGANOIDS

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Variants in the WDR19 gene, encoding IFT144 - a critical component of the intraflagellar transport (IFT) complex A - are associated with renal cystic ciliopathies, a prevalent genetic cause of chronic kidney disease (CKD). In the Arab Druze population, a newly identified hypomorphic WDR19 homozygous missense variant (c.878G>A; p.Cys293Tyr) is the most common genetic mutation associated with kidney failure. To validate the pathogenicity of this hypomorphic variant and explore the molecular mechanisms underlying its effects, we investigated the impact of this mutation on the kidney using kidney organoids derived from CRISPR-Cas9-edited human embryonic stem cells and patient-derived induced pluripotent stem cells (iPSCs). Our findings demonstrate that the WDR19 mutation impairs nephron differentiation from early developmental stages, leading to delayed renal vesicle formation and cystogenesis. At the molecular level, the hypomorphic mutation results in increased activation of the Sonic Hedgehog (Shh) pathway. Notably, elevated Shh signaling was observed even in cells harboring severe nonsense mutations with near-total ciliation absence. This activation correlated with significant downregulation of Fibroblast Growth Factor 8 (FGF8) and transcriptomic alterations in associated pathways, suggesting a potential inverse relationship between these signaling pathways during kidney organoid development. In contrast to the kidney organoids, cerebral organoids derived from the same hPSC lines exhibited no abnormalities, indicating tissue-specific effects of the mutation. Our study validates the pathogenic role of the WDR19 hypomorphic mutation in adult-onset, non-syndromic renal failure and highlights how hypomorphic pathogenic variants disrupt kidney development. These findings emphasize the essential role of cilia in renal development and provide deeper insights into the mechanisms underlying renal ciliopathies. From a broader perspective, our results demonstrate the utility of hPSC-derived kidney organoids as a robust platform for validating the effects of novel candidate pathogenic variants and for investigating adult-onset kidney disorders.

T1112

USE OF ANTIMICROBIAL NANOPARTICLES FOR THE MANAGEMENT OF DENTAL DISEASES

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Dental diseases represent a significant global health concern, with traditional treatment methods often proving costly and lacking in long-term efficacy. Emerging research highlights nanoparticles as a promising, cost-effective therapeutic alternative, owing to their unique properties. This review aims to provide a comprehensive overview of the application of antimicrobial and antioxidant nanoparticles in the management of dental diseases. Silver and gold nanoparticles have shown great potential for inhibiting biofilm formation and thus preventing dental caries, gingivitis, and periodontitis. Various dental products can integrate copper nanoparticles, known for their antimicrobial properties, to combat oral infections. Similarly, zinc oxide nanoparticles enhance the antimicrobial performance of dental materials, including adhesives and cements. Titanium dioxide and cerium oxide nanoparticles possess antimicrobial and photocatalytic properties, rendering them advantageous for dental materials and oral hygiene products. Chitosan nanoparticles are effective in inhibiting oral pathogens and reducing inflammation in periodontal tissues. Additionally, curcumin nanoparticles, with their antimicrobial, anti-inflammatory, and antioxidant properties, can enhance the overall performance of dental materials and oral care products. Incorporating these diverse nanoparticles into dental materials and oral care products holds the potential to significantly reduce the risk of infection, control biofilm formation, and improve overall oral health. This review underscores the importance of continued research and development in this promising field to realize the full potential of nanoparticles in dental care.

Funding Source: The authors would like to express their sincere gratitude to the Health and Medical Research Fund (file no. 09202026) for generously providing financial support.

T1114

USING IPSC-DERIVED NEURAL PROGENITOR CELLS TO MODEL THE DE NOVO MAX P.ARG60GLN VARIANT UNDERLYING MAX-ASSOCIATED GLOBAL DEVELOPMENT DISORDER

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Cyclin D2 (CCND2) stabilization underpins a range of macrocephaly-associated disorders through mutation of CCND2 or activating mutations in upstream genes encoding PI3K-AKT pathway components. Here we present a recurrent de novo c.179G>A (p.Arg60Gln) variant in Myc-associated factor X (MAX) in three individuals with overlapping macrocephaly-associated phenotypes. The variant, located in the b-HLH-LZ domain, causes increased intracellular CCND2 through increased transcription, not stabilization of CCND2 as observed for other macrocephaly-

associated mutations in PI3K-AKT pathway components. We show that the purified b-HLH-LZ domain of MAX-Arg60Gln binds its target E-box sequence with a lower apparent affinity. This leads to a more efficient heterodimerization with c-Myc resulting in an increase in transcriptional activity of c-Myc in individuals carrying this mutation. To model the role of MAX during neurodevelopment, we used CRISPR/Cas9 to introduce the p.Arg60Gln variant into healthy iPSCs and used these to generate iPSC-derived neural progenitor cells (NPCs). We found a reduction in the neuronal marker β-tubulin (TUJ1) and increased expression of the NPC marker TBR2, compared to wildtype, indicating impaired or stalled differentiation of these neuronal precursors. Kinome activity profiling revealed significant upregulation of hepatocyte growth factor receptor (MET) kinase activity in MAX-Arg60Gln NPCs, as well as other receptor tyrosine kinases, such as PDGFRA, linked to cell cycle dysregulation and abnormal cell growth. Of interest, a recurrent phenotype in patients with the MAX R60Q variants is renal agenesis, with previous studies showing a crucial role for MET in kidney formation. Further work to model this mutation in kidney organoids is ongoing. The recent development of Omomyc-CPP, a cell-penetrating b-HLH-LZ-domain c-Myc inhibitor, provides a possible therapeutic option for MAX-Arg60Gln individuals.

Funding Source: UKRI.

T1116

UTILIZING AUTOMATION PLATFORTMS TO ENABLE SCALABLE AND REPRODUCIBLE IPSC GENOME ENGINEERING AND DIFERENTIATION FOR DOWNSTREAM ASSAY DEVELOPMENT AND DISEASE MODELING

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iPSCs provide a unique platform for studying disease through our ability to both genetically engineer and differentiate them into relevant cell types. This singular platform brings with it special challenges in both the time and labor of performing the genetic engineering as well as consistency in the differentiation of iPSCs into the desired cell types. Consistency and reproducibility independent of the user has always been an elusive goal that can be mitigated by moving to automated platforms. One of the major challenges in the automation of engineered iPSCs is generating clean clonal populations that can be expanded, screened, and banked for future applications including differentiation. Using various inhibitors, medias, and extra cellular matrices, we have optimized a protocol for single cell ‘printing’ or sorting of cells post-electroporation into multi well plates with a high rate of clone survival. These clones are then expanded, banked, and screened for clonality before being used for downstream differentiation all within an automated platform. This not only alleviates ergonomic strain on the scientists involved but we also have shown this method reduces overall time and handling of the iPSCs leading to a faster pipeline. The second arm of our automation platforms is differentiation of iPSCs to various cells of interest. As differentiations can be challenging and labor intensive, typically requiring a working group per lineage, automating the differentiation provided us the opportunity to increase the number of differentiations being performed. We have previously adapted several protocols from the literature to go down the mesendodermal pathway to support various groups and allow for downstream assay development and functional characterization. Combining our automation and differentiation protocols with our already existing ability to genetically engineer genetic variants via

CRISPR/Cas9, these platforms could be used to increase the number of generated clones as well as the scale and number of differentiations being performed.

T1118

VALIDATING FUNCTIONAL CARDIOMYOCYTE-SPECIFIC HISTONE-ACETYLATED VARIANT ENHANCERS IN HUMAN ES-DERIVED CARDIOMYOCYTES USING A STARR-SEQ APPROACH

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Genome-wide association studies have identified a large number of SNPs associated with cardiovascular disease, which tend to be located in the Cis-regulatory elements of the non-coding genome such as enhancers, that can modulate the transcriptional activity of its target genes. However, the functional activity of these underlying SNPs in specific cardiac cell types can differ, and biological mechanisms of these genetic variations remain largely unexplored. Previously we have mapped out 47,321 putative human cardiac enhancers and promoters from 70 left ventricular samples and have identified 1680 differential histone acetylome-wide association with its underlying putative SNP based on our histone acetylation quantitative trait locus (haQTL) analysis. Using self-transcribing active regulatory region sequencing (STARR-seq), we have identified 1105/1680 significantly functional cardiac haQTL of varying effect sizes in human H9-hESC derived cardiomyocytes. Additionally, 40/62 unique loci identified by colocalization of haQTLs with subthreshold loci of heart-related GWAS datasets were shown to have regulatory activity in cardiomyocytes. Interestingly, amongst the top STARR-seq hits, the variants rs5758468 and rs11663468 were also identified and validated previously to show disruption to MEF2A binding and the corresponding loss of H3K27ac peak height in cardiomyocytes. Using luciferase assay in H9 ES derived-cardiomyocytes, we have also validated the concomitant reduction in luciferase activity, in the same direction as the STARR-seq assay, but not the non-significant SNPs. Additionally, through overlapping of Hi-C, and chromatin accessibility data, the majority of SNPs within cardiac enhancers validated by STARR-seq are connected to a distal gene by activity-by-contact (ABC) model. Concordant with Posterior inclusion probability (PIP) analysis, our results have also prioritized for functional cardiomyocyte-specific SNPs, that were previously enriched in GWAS studies implicated in cardiomyocyte electrophysiology, such as Atrial Fibrillation. Taken together, our results have adopted an unbiased approach to prioritize for functional cardiomyocyte regulatory elements, which help delineate target genes at cardiovascular GWAS loci implicated in cardiac disease and function.

Funding Source: C.J.M.L supported by grants from the Singapore National Medical Research Council (NMRC) under OF-YIRG24Jul-0014, and intramural fund under Cardiovascular Metabolic Disease Translational Research programme.

T1120

VASCULARIZED SKIN ASSEMBLOIDS INTEGRATING IMMUNE SYSTEM: A HUMAN IPSC-DERIVED PLATFORM TO INVESTIGATE ALLERGEN-INDUCED ATOPIC DERMATITIS

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Atopic dermatitis (AD) is a chronic inflammatory skin disorder characterized by epidermal barrier dysfunction, immune dysregulation, and microbial dysbiosis. Current in vitro and animal models fail to replicate the human-specific pathophysiology of AD, particularly the interactions between immune cells, skin, and vascular components, limiting therapeutic advancements. To address this, we developed vascularized skin assembloids derived from human-induced pluripotent stem cells (hiPSCs), providing a physiologically relevant platform for AD research. This model integrates hiPSC-derived multilayered skin organoids with vascular organoids to replicate the structural and functional complexity of human skin. Incorporating immune cells, including T cells, macrophages, and dendritic cells, it enables precise modeling of immune-vascular interactions. Upon exposure to environmental triggers, such as allergens (e.g., house dust mites) or microbial antigens (e.g., bacterial metabolites), the assembloids faithfully recapitulate hallmark AD phenotypes, including epidermal barrier disruption, elevated Th2 cytokines (IL-4, IL-13), immune cell infiltration, and inflammatory tissue remodeling, closely resembling clinical AD pathology. Histological and molecular analyses revealed bacterial colonization, chronic inflammation, and heightened immune activation, effectively replicating the persistent nature of AD. Therapeutic evaluation demonstrated that Dupilumab and JAK inhibitors significantly reduced Th2-driven inflammation, restored epidermal barrier integrity, and normalized immune responses, validating the platform's translational potential. Beyond AD, this versatile platform holds promise for studying other inflammatory skin diseases and supporting high-throughput drug discovery. By replicating key AD phenotypes and immune-vascular interactions within a human-specific microenvironment, these vascularized skin assembloids establish a new benchmark for preclinical research and accelerate the development of precision therapies.

Funding Source: This work was supported by the Korean Fund for Regenerative Medicine (KFRM) grant (the Ministry of Science and ICT, the Ministry of Health and Welfare) No. 23A0101L1 (2710001356, 00215812) funded by the Korea government.

TRACK: PLURIPOTENCY AND DEVELOPMENT (PD)

Poster Session 2 (EVEN)
5:00 PM – 6:00 PM

T1122

BRIDGING THE GAP IN EMBRYOGENESIS RESEARCH: NHP-DERIVED BLASTOIDS AS MODELS FOR HUMAN DEVELOPMENT

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Embryo development is a complex and dynamic process that remains poorly understood. Integrated stem cell-based embryo models, termed blastoids, may provide valuable information regarding complex spatiotemporal molecular and cellular processes during embryogenesis ex utero due to their resemblance to natural blastocysts. Moreover, the use of such models avoids the bioethical concerns associated with using natural embryos. Mouse and human cell lines were found capable of self-organizing into blastoids. However, limitations hinder their applications as models for human embryogenesis - mouse blastoids are evolutionary distant, while human blastoids raise bioethical concerns. To bridge this gap, we developed a non-human primate (NHP) blastoid model. Given the close phylogenetic relationship between NHPs and humans, NHP-derived blastoids could closely mimic human embryogenesis while avoiding bioethical concerns surrounding human embryo research. We hypothesized that current induced pluripotent stem cell (iPSC) and blastoid technologies used in human models could be successfully adapted to generate NHP blastoids for comparative developmental studies. This project aimed to generate and characterize NHP blastoids and conduct single-cell RNA-sequencing (scRNA-seq) to create a reference database for comparative studies with human models. In this study, we successfully generated blastoids from North Bornean Orangutan (Pongo pygmaeus pygmaeus). These NHP blastoids exhibited morphology, structure organization, and marker profiles similar to those of human blastoids. Furthermore, downstream assays, including stem cell derivation and in vitro attachment culture, confirmed the functionality of these models, demonstrating their potential as a platform for studying early embryonic development in primates.

Funding Source: Temasek Foundation.

T1124

TUMORIGENICITY IN HPSC-DERIVED NEURAL PROGENITOR CELLS: IMPLICATIONS FOR THE SAFETY OF CELL-BASED THERAPIES

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Human pluripotent stem cells (hPSCs) possess the unique ability to proliferate indefinitely and differentiate into various cell types in the human body, making them a promising source for cell-based therapies. However, to ensure their safety as a therapeutic source, it is essential to perform tumorigenicity tests on the differentiated cells derived from hPSCs. In this study, neural progenitor cells derived from seven CMC-hiPSC lines and one H1 hESC line were injected into NOG mice, a severely immunodeficient mouse model, and monitored for 10 months. Remarkably, 6 out of the 8 hPSC lines developed abnormal phenotypes, including brain tumors and choroid plexus tumors, raising concerns about the safety of these cells for therapeutic use. To further investigate the underlying genetic changes, we analyzed gene expression patterns in the neural progenitor cells using transcriptomic data. RNA sequencing (RNA-seq) revealed a significant increase in the expression of genes associated with nervous system development, particularly CNTN2 and CHL1, in the tumor-forming groups compared to the non-tumor groups. Additionally, Gene Set Enrichment Analysis (GSEA) of the RNA-seq data identified a suppression of PD-L1 expression and the PD-1 checkpoint pathway in the tumor-forming groups, both of which are key mechanisms of immune evasion in cancer. The accumulation of mutations during the long-term culture of hPSCs poses significant risks to the safety and efficacy of cell-based therapies. In this context, transcriptomic analysis not only provides gene expression patterns but also helps identify genetic mutations that may compromise the safety of hPSC-based therapies. Accumulating safety data, including information on genetic mutations and gene expression, is crucial for optimizing the clinical application of cell therapies. This will ultimately ensure the safety and efficacy of hPSCs as therapeutic cells, facilitating their safe implementation in clinical settings.

Funding Source: Korea CDC grant (2025-NI-0008-00).

T1126

A NEW CGMP COMPLIANT ADHERENT PSC CULTURE MEDIUM THAT ENABLES ROBUST PERFORMANCE IN CELL THERAPY DEVELOPMENT

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Culture systems for human pluripotent stem cell (PSC) expansion are essential for generating an almost unlimited pool of cells capable of differentiating into all three germ lineages, for cell-based therapies. To transition PSC therapy research from the laboratory to clinical applications, there is a critical need for high-quality materials that comply with GMP standards, undergo rigorous quality and safety testing, ensure raw material traceability, and are supported by comprehensive regulatory documentation. To address these stringent requirements, we have developed Gibco™ Cell Therapy Systems (CTS™) StemFlex™ Medium. CTS™ StemFlex™ Medium is xeno-free and supports the expansion of high-quality PSCs and is designed to support challenging applications within the PSC workflow. Our data demonstrate that PSCs cultured long-term in CTS™ StemFlex™ Medium maintain essential PSC characteristics, including greater than >95% expression of OCT4/NANOG pluripotency markers, normal morphology, trilineage differentiation potential, and normal karyotype. This medium offers a flexible feed schedule including a weekend-free option, and compatibility with multiple defined matrices and passaging reagents. CTS™ StemFlex™ Medium excels in supporting stressful PSC applications, including single-cell passaging, with a consistent cumulative fold expansion over 10 passages. Furthermore, CTS™ StemFlex™ Medium supports PSC recovery and expansion following electroporation-based delivery of the CRISPR Cas9-gRNA complex and facilitates the downstream clonal expansion of edited PSCs. Additionally, adherent PSCs cultured in CTS™ StemFlex™ Medium can be seamlessly transitioned to 3D culture using CTS StemScale™ PSC Suspension Medium, resulting in the formation of high-quality PSC spheroids with a linear fold expansion over multiple passages. In conclusion, CTS™ StemFlex™ Medium delivers robust performance in cell therapy workflows, providing a comprehensive solution to advance PSC research and therapy.

T1128

ADVANCING ALLOGENIC CELL THERAPY WITH AUTOMATED IPSC PROCESSING AND ENGINEERING: BENEFITS OF A CLOSED MODULAR APPROACH

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Induced pluripotent stem cell (iPSC)-derived natural killer (iNK) cells have emerged as a promising platform for next-generation immunotherapy, offering a homogeneous, scalable and versatile approach for consistent large-scale manufacturing of off the shelf allogeneic therapies. This approach involves multiple steps including iPSC culturing and banking followed by gene editing and differentiation to tumor specific iNK cells to enhance cytotoxicity, persistence, and tumor-targeting specificity, while minimizing risks of graft-versus-host disease. Building closed modular automated workflows will help minimize risks associated with manual processes associated with these methods. Through this work we built closed automated cell harvest, cell wash and buffer exchange, gene delivery and editing protocols that can enable iNK-based cell therapy manufacturing. In our current workflow, we cultured and expanded iPSCs up to a billion cells in a

10-layer cell factory system for master cell bank preparation. iPSC manual process and harvest in 10-layer cell factory system is very labor intensive and prone to contamination. Utilizing CTS Rotea counterflow centrifugation system minimized human intervention at multiple stages iPSC large scale process. Using this protocol, we processed the harvesting of entire iPSC culture in 10-layer cell factory system in a single batch to create a master bank for cell therapy development. The iPSCs prepared using this method retain pluripotency characteristics with good viability and expansion rate. We then successfully used these iPSC banks and carried out CRISPR-based non-viral gene editing using Neon NxT or CTS Xenon closed automated electroporation system for gene delivery system. Results show successful generation of engineered CAR-iPSC with reproducible KI efficiency of up to 15%. We established the optimal target gene and promoter combination to stabilize transgene expression during the differentiation of iPSCs to iNK cells. With methods developed through this work, we successfully generated potent CAR iNK cells. Together the workflows described here utilizing the clean room compliant closed automated cell processing and gene delivery platforms and the GMP compatibility iPSC and NK media systems enable clinical scale iNK cell therapy manufacturing.

T1130

ESTABLISHING MOUSE CROSS-LINEAGE TOTIPOTENCY-INDUCING PLATFORM VIA ONE SINGLE CHEMICAL

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Totipotent cells can give rise to all the cell lineages in both the embryonic and the extraembryonic tissues. In the mouse, the zygote and the blastomeres of the early 2-cell embryo are authentic totipotent cells. Despite the importance of totipotency in starting life, how totipotency is established from fusion of the two gametes and subsequently regulated remains fully understood. Derivation and maintenance of stem cells in vitro with totipotency features would offer an invaluable model for studying the molecular mechanism of totipotency. Here, we demonstrate that a single molecule, CBL0137, enables establishment and culturing of totipotent-like stem cells from pluripotent stem cells. These new stem cells generate both embryonic and extraembryonic cell lineages in in vitro differentiation, blastoid generation, and in vivo chimeras. We designate these cells as Z-cells (zygotic genome activation (ZGA)-like cells), which transcriptionally and epigenetically resemble 2-cell embryo cells. Remarkably, CBL0137 can also induce cells in the blastocyst and embryonic fibroblasts to acquire 2C embryo-like molecular features, indicating that CBL0137 re-establishes 2C-specific transcriptome in a lineage-independent manner. Furthermore, CBL0137 can induce MEFs to acquire molecular features of other cell lineages. The CBL0137-induced cell fate transition therefore provides an in vitro system to efficiently generate totipotent-like cells for totipotency dissection and for cell lineage trans-differentiation.

Funding Source: This project is supported by Health@InnoHK, Innovation Technology Commission, HKSAR.

T1132

SUBSTRATE STIFFNESS CONTROLS THE RESPONSE OF HUMAN PLURIPOTENT STEM CELLS TO BMP BY REGULATING EPITHELIAL INTEGRITY AND POLARITY

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Historically, most research on cell fate induction was focused around biochemical signals. It is now, however, clear that mechanical signalling from the extracellular matrix (ECM) also influences fate in various cell types. For example, in human pluripotent stem cells (hPSCs), softening of the substrate increases mesoderm differentiation. Although such phenomena have been observed in different systems, the mechanisms underlying the effect of mechanical properties of the ECM on stem cell fate remain largely unstudied. Here, we investigate such mechanism in hPSCs. We cultured hPSCs on stiff and soft polyacrylamide hydrogels and exposed the cells to the mesoderm-inducing BMP4 signal. On the stiff substrate, hPSCs display BMP activity around the edge of their colony, however, on the soft substrate, they respond within the whole colony and consequently exhibit increased differentiation. We show that the increased response to BMP on the soft substrate stems from the lower activity of the Focal Adhesion Kinase (FAK)-PI3K cascade. The lower FAK-PI3K activity results in morphological changes of the hPSC epithelium. Specifically, substrate softening affects two crucial epithelial properties in hPSCs: epithelial integrity and epithelial polarity. hPSCs on the soft substrate exhibit increased epithelial permeability and loss of apical membrane identity. Both of these changes allow increased accessibility of the BMP receptor to the BMP ligand on the soft substrate, allowing in turn the observed increase in BMP signalling activity. Finally, we show that these changes in epithelial properties on the soft substrate stem from increased retention of endogenous laminin on the apical surface of hPSCs. Together, our work identifies a mechanism through which a change in substrate stiffness affects the response of hPSCs to BMP and the subsequent extent of differentiation. We are currently investigating whether such mechanism is involved in initiation of gastrulation in the epiblast since remodelling of the underlying basement membrane is necessary for the start of gastrulation. More broadly, our findings should impact our understanding of how physiological changes in ECM mechanics impact cellular response to biochemical signals and consequent fate/state transitions during processes like differentiation or metastasis.

Funding Source: This research has been funded by the Wellcome Trust.

T1134

AUTOMATED SYSTEMS FOR MULTIOMIC STUDY OF DIFFERENTIATED STEM CELLS DURING SPACEFLIGHT MISSIONS

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During spaceflight, exposure to microgravity, radiation, and the unique space environment can significantly impact human physiological functions. While studies have explored the effects of spaceflight-induced microgravity on stem cell self-renewal and differentiation, research on its broader effects on lineage differentiation in stem cells remains limited. In particular, the effects of

the space environment on human germ cells remain uninvestigated. To address this gap, we developed automated systems during the Tianzhou-1 and Tianzhou-6 missions to analyze fluorescent cellular morphology and perform multi-omics analysis of differentiated stem cells. These systems enable live-cell imaging, remote control from Earth, and multi-omic analysis of observed cells. The results reveal that spaceflight exposure reduced the number of human germ cells, which exhibited alterations in translational expression—particularly in the cytoskeleton, extracellular matrix (ECM), and several metabolic pathways. However, DNA methylation profiling and whole-exome sequencing showed no significant genomic changes during the same spaceflight period. This study demonstrates that automated systems can assess spaceflight effects on human stem cell differentiation and provides a platform for future research aboard space stations.

T1136

CHIMPANZEE NAÏVE PSCS AS A MODEL TO UNDERSTAND PRIMATE NAÏVE PLURIPOTENCY

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Naive pluripotent stem cells (PSCs) represent the earliest state of pluripotency, akin to the pre-implantation epiblast. While mouse and human naive PSCs exhibit differences in self-renewal requirements and extraembryonic differentiation potential, the conservation of these differences across higher primates remains unclear. To address this, we attempted to establish chimpanzee naive iPSCs (niPSCs). Initial efforts using standard human naive PSC culture conditions resulted in colonies that failed to propagate. However, we discovered that the addition of Activin, IL6, and, crucially, a Polycomb Repressive Complex 2 (PRC2) inhibitor (A6E condition) enabled efficient derivation and stable maintenance of chimpanzee niPSCs. These chimpanzee niPSCs displayed a global transcriptome profile closely resembling human naive PSCs and the pre-implantation epiblast, sharing the expression of key pluripotency transcription factors. Notably, chimpanzee niPSCs demonstrated the ability to differentiate into extraembryonic lineages, including trophectoderm and hypoblast, and formed tri-lineage blastoids, mirroring human blastoids. This highlights their potential as a valuable comparative model for studying pluripotency and early embryogenesis in higher primates. Furthermore, we found that PRC2 inhibition not only overcame the self-renewal barrier in chimpanzee niPSCs but also facilitated feeder-free propagation of human naive PSCs. This suggests that excess H3K27me3 deposition, mediated by PRC2, poses a significant obstacle to naive PSC self-renewal across species. Our findings provide crucial insights into the evolution of pluripotency and offer a promising avenue for generating ethically more acceptable models of early human development using chimpanzee niPSCs.

Funding Source: UKRI/JSPS collaborative award (MR/S020845/1 and JPJSJRP 20181706), ERC (AdG 835312, ERC-CoG-648879), MRC (MR/W025310/1), Leducq Foundation, JSPS (JPMJFR214Y, 22K07886, 20H03170, 23H02401), AMED (JP23bm1123041).

T1138

INO80 IN TRANSCRIPTIONAL REGULATION OF CELL CYCLE, SURVIVAL, AND DIFFERENTIATION IN MOUSE ESCS

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Precise regulation of the embryonic stem cell (ESC) cycle is essential for self-renewal and differentiation. ESCs exhibit a distinct cell cycle structure from that of somatic cells and it varies depending on culture conditions. However, the epigenetic mechanisms governing ESC cell cycle regulation remain unclear. In this study, we demonstrate that the ATP-dependent chromatin remodeler Ino80 regulates cell cycle genes in primed ESCs. Loss of Ino80 significantly prolonged G1-phase in ESCs grown under primed conditions. Mechanistically, Ino80 directly binds to transcription start sites, modulating the expression of cell cycle-related genes. Additionally, Ino80 depletion induced cell apoptosis, highlighting its role in ESC survival. Interestingly, the regulatory function of Ino80 differs in differentiating ESCs, where its loss led to an extended S-phase. RNA-seq analysis revealed persistent dysregulation of genes associated with organ development and the cell cycle in differentiating Ino80 knockout ESCs, suggesting that Ino80's function extends beyond undifferentiated ESCs. Overall, our findings establish Ino80 as a key transcriptional regulator of the ESC cell cycle and suggest its role may be broadly relevant to other cell types.

Funding Source: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (RS-2024-00344340).

T1140

PUMILIO PROTEINS MAINTAIN LINEAGE BALANCE VIA POST-TRANSCRIPTIONAL REPRESSION OF GERMLINE FATE AND PROMOTION OF SOMATIC DIFFERENTIATION

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Embryonic development relies on post-transcriptional regulatory networks, with RNA-binding proteins (RBPs) playing key roles. Among these, PUMILIO (PUM) proteins are essential for early embryogenesis. Mice lacking both PUM1 and PUM2 fail to undergo gastrulation and exhibit embryonic lethality, yet their precise regulatory mechanisms remain unclear. Using embryoid bodies (EBs) as an in vitro model of early embryogenesis, we show that PUM1 and PUM2 coordinate germline suppression, pluripotency regulation, and somatic differentiation to maintain lineage balance. PUM proteins directly repress Prdm1 to prevent premature germline specification while promoting somatic differentiation by modulating pluripotency networks and Wnt signaling. Their loss disrupts germ layer differentiation, impairs anterior-posterior (A-P) and dorsoventral (D-V) patterning, and leads to defective neural differentiation. RIP-seq analyses reveal that PUM1 and PUM2 directly regulate mRNAs associated with these developmental processes, defining a post-transcriptional network that integrates RNA regulation with developmental signaling. These findings establish PUM proteins as essential post-transcriptional regulators of early embryogenesis,

demonstrating how RBPs coordinate cell fate and patterning.

Funding Source: Start-up funding for research groups at ShanghaiTech University.

T1142

THE CORRELATION BETWEEN C-MYC AND KLF2 IN NEURAL STEM CELL CYCLE AND PROLIFERATION

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Neural stem cells (NSCs) are progenitor cells capable of self-renewal and differentiation into various neural lineages. The number of neurons in the brain is largely determined by the proliferative potential of NSCs, which is regulated by the frequency of their cell cycle. Although extensive research has been conducted on NSC proliferation and cell cycle regulation, the precise mechanisms remain unclear. The oncogene c-Myc is well-established as a key regulator of cell proliferation and the cell cycle. However, its role in NSC cell cycle regulation has not yet been fully elucidated. Therefore, this study aimed to investigate the role of c-Myc in NSC cell cycle progression. To assess the effects of c-Myc inhibition on NSC viability, cells were treated with the c-Myc inhibitor (10058-F4), and survival rates were analyzed. While no significant changes were observed in embryonic stem cells, NSCs exhibited a marked decrease in viability. Moreover, when the concentration of the inhibitor exceeded 3 μM, NSC survival was significantly reduced. However, apoptosis analysis revealed that treatment with 3 μM of the c-Myc inhibitor did not induce an increase in NSC apoptosis. Cell cycle analysis demonstrated that treatment with 3 μM of the c-Myc inhibitor led to a decrease in the S phase, accompanied by an increase in the G0/G1 and G2/M phases. Additionally, RNA sequencing analysis identified 930 differentially expressed genes, particularly in gene clusters involved in cell proliferation, cell division, and cell cycle regulation. These findings suggest that c-Myc plays a pivotal role in regulating NSC proliferation and cell cycle progression. To further investigate the correlation between KLF2 and transcription factors downregulated by c-Myc inhibition, we performed KLF2 knockdown using shRNA. As a result, NSC proliferation decreased, and the G2/M phase of the cell cycle increased. These findings suggest that c-Myc may regulate NSC cell cycle progression by modulating KLF2 expression.

Funding Source: This research was supported by Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Education(RS-2024-00410412).

T1144

BASIS FOR NUCLEOSOME BINDING AND OPENING BY THE PIONEER TRANSCRIPTION FACTOR KLF4

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Pioneer transcription factors are master regulators of cell fate and direct the differentiation of the ~200 human cell types during development. For their role in controlling cellular fates, pioneer factors are used to artificially engineer cell states using cellular reprogramming technologies for induced pluripotent stem cells (iPSCs) and induced neural stem cells (iNSCs) for biomedical and clinical applications. Advances in cryogenic electron microscopy (cryo-EM) could provide the first glimpses into how pioneer factors access nucleosome core particles. However, they are largely confined to SOX and POU pioneer factor families bound to peripheral parts of nucleosomes and the underpinnings for the mechanistic basis for other pioneer factors remain obscure. Here we elucidate the basis for the pioneer activity of the multi-domain pioneer factor KLF4—a C2H2 zinc finger protein that plays an indispensable role in stem cell pluripotency and differentiation. We performed quantitative binding assays of different KLF4 mutants to nucleosomes. We could uncover which domains of KLF4 are critical for nucleosome engagement and verify our findings in somatic cell reprogramming to induced pluripotent stem cells (iPSCs). Initial cryo-EM maps reveal that KLF4 exhibits an uncommon binding mode to nucleosomes. Unlike most pioneering factors that bind solely to the DNA, KLF4 binds both DNA and histone components. This suggests mechanism whereby KLF4 destabilizes packing of nucleosomes and triggers DNA unwrapping in a step-wise manner. Through mechanistic insights how pioneer factors resolve chromatin barriers, we design enhanced factors to better control cell fates and boost cellular programming and stem cell differentiation.

T1146

EFFECT OF BISPHENOL A ON BLASTOID FORMATION AND MAINTENANCE IN MICE

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Bisphenol A (BPA), a widely encountered environmental endocrine disruptor, has been reported to affect embryonic development and implantation. However, the effect of BPA on embryogenesis and implantation has not been thoroughly investigated, primarily due to limitations in embryo availability and challenges with in vitro implantation systems. Here, we aimed to develop a novel system for toxicity testing using blastoids, which are derived from mouse induced pluripotent stem cells (iPSCs). To generate totipotent embryos containing both embryonic and extraembryonic cells, iPSCs were transitioned into a chemically induced totipotent stem cell (ciTotiSC) state. The resulting ciToti-blastoids resemble mouse blastocysts and exhibit proper lineage segregation, including the inner cell mass (ICM) and trophectoderm (TE), during blastoid formation. Exposure to

BPA disrupted iPSC aggregation and reduced blastoid formation rates in a dose-dependent manner. Notably, the addition of glutathione, an antioxidant, rescued the detrimental effects of BPA on blastoid formation, suggesting that BPA-induced reactive oxygen species (ROS) generation underlies its toxicity, which can be mitigated by glutathione. Furthermore, glutathione-supplemented medium extended the maintenance of blastoids, highlighting its role in promoting blastoid stability and viability. These findings indicated that the established ciToti-blastoids system can be a reliable platform for studying the impact of environmental toxins such as BPA.

T1148

ESTABLISHING A GASTRULOID-BASED 3D MODEL OF HUMAN HEART DEVELOPMENT TO PROVIDE NOVEL INSIGHTS INTO CARDIAC PROGENITOR SPECIFICATION

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The development of the human heart is complex, requiring the cooperation of multiple progenitors undergoing coordinated morphological events in a time-dependent manner. Abnormalities in cardiogenesis result in congenital heart defects impacting 1 in 100 newborns, highlighting the need for a better understanding of this process. Current human pluripotent stem cell (hPSC)-based models do not fully recapitulate these spatiotemporal events of heart development. We propose that gastruloids, a self-organizing hPSC-based model of gastrulation, can establish a 3D model of human cardiogenesis. We hypothesize that extended culture of gastruloids will allow the formation of a cardiac crescent-like structure with corresponding cardiac progenitors. This will provide a model to dissect the spatiotemporal and morphological processes during cardiac progenitor specification as well as the multilineage cell interactions that are essential for human cardiogenesis. We have generated gastruloids that form anterior-posterior axes based on the polarized expression of GATA6 (anterior) and CDX2 (posterior). The posterior pole contained distinct clusters of cells expressing markers of all three germ layers (TBXT, SOX17, SOX2). After extending the cultures to day 20, spontaneously beating regions were observed in some of the gastruloids. Importantly, 25% of these gastruloids contained cTNT+, suggesting a cardiomyocyte phenotype. We are currently further characterizing these cTNT+ cells. In parallel, we are modulating the gastruloid protocol to improve the efficiency of cardiogenesis. Once optimized, this new model will allow us to dissect the spatiotemporal aspects of early stages of human heart development and thereby advance our understanding of congenital heart defects.

T1150

HUMAN TROPHOBLAST STEM CELLS REVEALING THE MULTIOMICS INSIGHTS INTO MIRNA-RNA TARGETOME IN PLACENTATION AND ITS ASSOCIATION WITH PREECLAMPSIA

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Human placenta villus is composed of cytotrophoblast (CT), syncytiotrophoblast (ST) and extravillous trophoblast (EVT). Impaired placental development and aberrant trophoblast differentiation are major contributors to pregnancy-related complications such as preeclampsia (PE). Human miRNAs are highly expressed in the placenta and exhibit altered expression profiles from patients complicated with PE. However, the molecular mechanisms remain poorly understood. This study aims to construct a comprehensive miRNA-mRNA regulatory network for placental development and pathogenesis of preeclampsia, which may lead to potential diagnosis approaches for pregnancy-related disorders. To investigate trophoblast differentiation, TSCs capable of differentiating into ST and EVT were utilized. miRNA-mRNA were identified using CLEAR-CLIP sequencing in TSC and Day 4 EVT. Bulk RNA sequencing of TSC and EVT at different differentiation stages was conducted. Concurrently, miRNA sequencing of early peripheral blood from normal pregnant (NP) women and women who identified with PE was performed. Additionally, RT-qPCR was used to investigate the change of miRNA and gene expression in TSC and EVT. miRNA FISH was performed to examine the expression of miRNA in the villus. Multi-omics data analysis revealed 12 miRNA-RNA target pairs in EVT vs. TSC, with inverse expression patterns between miRNAs and their target RNAs. Among the 12 pairs of miRNA-RNA targets, miR-455-3p and miR-193a-3p were significantly increased in the early maternal serum of PE patients compared with the NP group. miR-455-3p-SEMA6A and miR-193a-3p-EPCAM are potentially involved in TSC differentiation. The elevated level of miR-193a-3p was found in CVS of PE gourp compared with CVS of NP gorup. Meanwhile, knock-down of miR-193a-3p increases the expression of EPCAM in EVT. Our results revealed the miRNA-mRNA targetome during trophoblast differentiation. Multi-omics analysis showed that the two miRNA-RNA pair were potentially involved in TSC differentiation. Furthermore, serum mir-193a-3p emerged as a potential target for early detection of PE. These insights provide a foundation for understanding the regulatory role of miRNAs in placental development and their contribution to the pathogenesis of PE.

T1152

PLEIOTROPIC ROLES OF PRO-INFLAMMATORY CYTOKINES IN REGULATING EARLY-STAGE AIRWAY DEVELOPMENT

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The development of the human respiratory tract begins at five post-conception weeks (pcw) and continues to form both distal and proximal airways. Previous studies on human foetal airways have revealed the dynamic changes in cell populations during airway development and identified mature myeloid cells and a group of their secreted pro-inflammatory cytokines. Although the roles of these cytokines in host defence systems have been well-documented, their functions in healthy foetal airway epithelium remain poorly understood. To have a better understanding of the interaction between pro-inflammatory cytokines and airway epithelial cells, we used mice as a model and performed single-cell RNA sequencing on prenatal and neonatal airways. Consistent with human single-cell transcriptomic studies, we confirmed that myeloid cells are the primary source of pro-inflammatory cytokines secretion. Our findings further suggest that basal and secretory cells are

the major responders to these inflammatory signals in the murine upper airway. By incorporating cytokines into mouse tracheal epithelial cell (mTEC) cultures, we observed that niche inflammatory signals influence airway morphogenesis by regulating basal cell fate at different developmental stages. Additionally, we tested our hypothesis utilizing human tissue-derived organoids and observed consistent changes in phenotypes and epithelial cell type transitioning. In conclusion, our study highlights the pleiotropic roles of inflammatory cytokines in the regulation of healthy airway development.

T1154

RESOLVING CELL FATE CHOICES DURING MAMMALIAN DEVELOPMENT BY SINGLE CELL MULTI-OMICS LINEAGE TRACING

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Mammalian development requires substantial coordination to translate transcriptional and epigenetic programs into organized cellular behavior such as lineage specification. Using single-cell genomics, the comprehensive molecular landscape of mouse gastrulation and organogenesis has been well described. Because these atlas data lack the lineage information to connect profiled cell states to ultimate cell fates, it remains unclear how these molecular programs control cell fate choices during tissue diversification. The advances in single-cell genetic lineage tracing approaches provides a new lens for parallel assessment of cell fate and gene expression. Recent studies have combined cellular barcoding with single-cell multi-omics approaches to decipher fate-specific epigenome changes. However, it remains a challenge to simultaneously record cell lineage, transcriptomic, and epigenomic information in tissues with a massive number of cells. In this study, we developed a Cre-driven lineage-tracing approach that has high lineage recovery efficiency in single-cell assays and thus enables large-scale single-cell multi-omics lineage tracing in vivo. We validated this approach in embryos and demonstrated the robust capture of lineage barcodes across bulk-seq and scRNA-seq, revealing that epiblasts have heterogeneous lineage contribution to form embryonic tissues. We further leveraged this high-throughput lineage tracing approach by paring it with single-cell multi-omics profiling, to characterize transcriptomic and epigenomic changes in organ progenitors during early organogenesis, particular for the neuro-mesodermal progenitors (NMPs). Due to the addition of epigenomic information to gene expression, this approach allowed us to identify the essential TF in NMP fate decision. We uncovered the transcription factor Cdx2 as a central regulator of mesoderm fate determination, promoting NMP differentiation to mesoderm lineages. Collectively, we demonstrated the ability of in vivo single-cell multi-omics lineage tracing approach to quantify fate contribution of embryonic progenitors, to identify new progenitor subpopulations, as well as to provide new insights into the coordination between the transcriptome and epigenome in cell fate decisions.

T1156

TRANSCRIPTIONAL HETEROGENEITY AND MOLECULAR REGULATION OF TOTIPOTENCY-TO-PLURIPOTENCY TRANSITION IN PORCINE EXPANDED PLURIPOTENCY STEM CELLS

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The molecular characteristics of expanded pluripotent stem cells (EPSCs) have been a subject of debate. Human EPSCs (hEPSCs) are akin to eight-cell and morula-stage embryos, whereas mouse EPSCs (mEPSCs) resemble post-implantation epiblasts. This study investigates the developmental potential and molecular trait of porcine EPSCs (pEPSCs), revealing their unique transcriptional heterogeneity and similarities to both morula and post-implantation epiblast stages. Through single-cell transcriptomic analysis, we identified three distinct subpopulations (C1, C2, and C3) corresponding to totipotency, naïve pluripotency, and primed pluripotency. The use of dual fluorescence reporter system demonstrated transitions between these states, mirroring embryonic development. Mechanistically, we established a framework to understand the conserved roles of OTX2 and LEUTX in regulating transitions between totipotency and pluripotency, both in cells and early embryos. OTX2 promotes the transition to pluripotency by activating pluripotency and lineage priming genes, while LEUTX enhances totipotency and supports blastoid formation. This study bridges gaps in our understanding of mammalian stem cells and underscores the potential of pEPSCs in agricultural and biomedical applications.

T1158

YY1 IS CRITICAL FOR NEURAL STEM CELL MAINTENANCE DURING CEREBELLAR DEVELOPMENT

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Yy1 is integral to several key biological processes, including cell cycle regulation, proliferation, and survival, particularly in stem cell contexts. Patients with Gabriele-De Vries syndrome, caused by dominant mutations in Yy1, exhibit significant developmental defects in the central nervous system (CNS). Despite its importance, the role of Yy1 in mammalian CNS development, especially in cerebellar neural stem cell populations, is not fully understood. To investigate Yy1's role in the development of cerebellar neural stem cell populations, we employed a tissue-specific Cre-LoxP system using two different Cre-expressing mouse lines, Pax7-Cre and En1-Cre. This approach allowed for the selective inactivation of Yy1 in stem cell renewal and specification within the primary neural germinal zones and the developing MHB region, where the isthmus organizer, located at the mid-hindbrain (MHB) boundary, acts as a critical signaling center influencing the early patterning of both the midbrain and cerebellum—areas rich in neural progenitor cells. Our findings reveal distinct morphological phenotypes in the Pax7-Cre and En1-Cre-driven Yy1 knockout mutants. Both YY1 mutant models resulted in severe cerebellar hypoplasia with less foliation. Furthermore, the Wnt/β-catenin signaling pathway may be a key molecular mechanism underlying developmental defects in En1-Cre Yy1 mutant mes/r1 neuroepithelial stem cells. In summary, our research highlights the crucial functions of YY1 in neural stem cell maintenance, renewal, and specification during cerebellar development, as well as in sustaining mouse mes/r1

neuroepithelial stem cell populations. It underscores essential role of Yy1 in the early development of the mid-hindbrain neural tube and provides valuable insights into the roles of YY1 in various neural stem cell populations.

T1160

3D MATERNAL-FETAL ASSEMBLIES RECAPITULATE ACTIVE CO-ADAPTATIONS AND CROSSTALK FOR HUMAN EMBRYO IMPLANTATION

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Human embryo implantation is a highly dynamic and transient process orchestrated by sophisticated interplays between embryo and endometrium. Studying this crucial phase in-vitro has been limited by technical and ethical challenges. Here, we develop a 3D co-culture model by implanting blastoids into endometrial assembloids, which faithfully simulates human in-utero development and enables detailed exploration of maternal-fetal dynamics. Our system unveils implantation-triggered subpopulations, such as pioneering trophoblasts from blastoid derivatives fused with endometrial characteristics, and highly specialized ciliated epithelia within the endometrium that shed epithelial properties. The emergence of these subpopulations and the intense maternal-fetal crosstalk demonstrate active adaptations, responding coordinately to successful embryo implantation from both fetal and maternal perspectives. Additionally, our system highlights the critical roles of LIFR and CSF3 signalings in modulating embryo invasion. Overall, our study offers fresh insights into the mechanisms of embryo development and provides potential targets for reproductive research.

Funding Source: This work was supported by the National Natural Science Foundation of China (NSFC) (Grant Nos.32270850 and 32470843 to Y.W., Grant Nos. 92168205 to S.G., Grant Nos. 32370842 and 32100633 to Y.B.).

T1162

ANALYSIS OF MOUSE PRIMORDIAL GERM CELLS DELAMINATION

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Primordial germ cells (PGCs) are the precursors that develop into sperm and eggs. In mice, around embryonic day (E) 6.25, a small group of epiblast cells adopt germ cell fate and segregate from the epiblast. Studying the segregation of PGCs from the epiblast has been challenging due to technical limitations. In this study, we employed a 3D embryonic stem cell-based in vitro culture system and direct embryo analysis to investigate the morphogenetic changes that drive PGC segregation from the epiblast. We found that the newly specified PGCs undergo dramatic shape changes following their specification at the boundary between the epiblast and extraembryonic ectoderm (ExE). To understand the mechanism behind PGC segregation, we first examined and ruled out the possibility of Epithelial-Mesenchymal Transition (EMT). We then analyzed the morphological

changes in PGCs during early segregation using PGC reporter mouse embryos and the 3D in vitro model in combination with transcriptional analysis. We discovered that as PGCs reacquire naïve pluripotency, they lose epithelial polarity and this allows them to segregate from the epiblast. Additionally, our findings suggest a role of small GTPases downstream of naïve pluripotency factors in orchestrating epithelial morphogenesis during PGC delamination. Altogether, this study provides new insights into the cellular and molecular mechanisms underlying PGC segregation, broadening the fundamental understanding of early germ cell development.

Funding Source: CRC 1348 Dynamic Cellular Interfaces.

T1164

COMPARATION AND CHARACTERIZATION OF PORCINE CEREBRAL ORGANOIDS USING HUMAN-BASED PROTOCOLS FOR TRANSLATIONAL RESEARCH

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Porcine cerebral organoids have multiple important applications in neuroscience research and drug development. Derived from pluripotent stem cells, human cerebral organoids can self-organize into three-dimensional structures that replicate key features of the human brain, such as cortical layers and neurogenesis. This capability allows them to be effectively used to model various neurological disorders. Given the ethical challenges associated with using human tissue for invasive brain development studies, the development of porcine models presents a more effective alternative for investigating disease mechanisms. However, traditional in vivo models, such as transgenic pigs, can be time-consuming and costly to produce. Therefore, the establishment of in vitro models like porcine cerebral organoids may offer a more efficient, ethical, and cost-effective approach to studying human neurobiology and pathology. In this study, by using in vitro fertilization and somatic cell nuclear transfer-derived porcine embryonic stem cells, we primarily used an adapted Lancaster Human Cerebral Organoid Generation Protocol to better suit the development of porcine cerebral organoids. Early porcine cerebral organoids exhibited morphological changes similar to those observed in early human cerebral organoids under bright fields. Immunofluorescence analysis of porcine cerebral organoids confirmed that the organoids were undergoing neural differentiation. At early development, the porcine brain organoids showed strong expression of neural progenitor markers, including PAX6, NESTIN, SOX1, and ZO1. At the midpoint of development, the porcine cerebral organoid exhibited signals indicative of neural progenitor regions and early neurogenesis. They displayed the formation of ventricular-like cavities, with SOX2 (inner layer) and TUJ1(outer layer) staining. By day 50, the porcine cerebral organoid showed positive expression of GFAP around areas expressing NESTIN, and IBA1 showed weak positivity at the margins of the organoid. PAX6 expression was minimal. In conclusion, the establishment of porcine brain organoid models offers an additional and valuable in vitro tool for studying neurological disorders.

Funding Source: This work was granted by the “Technology Innovation Program funded by the MOTIE, Korea (20023068)”, and “IPET in Food, Agriculture, Forestry and Fisheries funded by MAFRA (RS-2024-00398561, RS-2024-00399475)", Republic of Korea.

T1166

EFFECTS OF ERYTHROPOIETIN SUPPLEMENTATION ON NUCLEAR MATURATION AND EARLY EMBRYOGENESIS IN PORCINE OOCYTES DURING IN VITRO MATURATION

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Erythropoietin (EPO), a cytokine secreted by the kidneys, is a key regulator of erythropoiesis; however, its role in female reproduction remains largely unexplored. This study aimed to evaluate the effects of different concentrations of EPO (control, 10 mU/mL, 100 mU/mL, and 1000 mU/mL) on nuclear maturation and early embryogenesis in porcine oocytes during in vitro maturation (IVM). After 42 hours of in vitro maturation, no significant differences in the nuclear maturation rate were observed among oocytes treated with 10, 100, and 1000 mU/mL EPO when compared to the control ($p > 0.05$). However, blastocyst formation rates were significantly higher in the 100 mU/mL EPO-treated group compared to the control ($p < 0.05$), while the 10 mU/mL and 1000 mU/mL groups did not exhibit significant differences. These findings suggest that EPO supplementation, particularly at 100 mU/mL, may enhance early embryogenesis during IVM. Further research is required to elucidate the underlying mechanisms by which EPO influences oocyte maturation and embryonic development.

Funding Source: This work was supported by grants from the “Technology Innovation Program funded by the MOTIE, Korea (20023068)”, and “IPET funded by MAFRA (RS-2024-00398561, RS-2024-00399475)", Republic of Korea.

T1168

ENHANCING OTIC SENSORY EPITHELIUM DEVELOPMENT IN HPSC-DERIVED INNER EAR ORGANOIDS THROUGH STEPWISE BMP4, WNT, AND SHH SIGNALING MODULATIONS

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Human inner ear organoids (IEOs) derived from human embryonic or induced pluripotent stem cells provide a powerful model for studying inner ear development. These in vitro models closely mimic in vivo otic vesicle formation and supporting tissue development. However, the generation of "on-target" otic sensory epithelium remains quantitatively limited and deeply embedded within 3D aggregates, reducing their utility for disease modeling and therapeutic applications. Here, we

systematically optimized human IEO differentiation following key developmental principles. First, we fine-tuned BMP4 concentrations at Day 0 to bias 3D stem cell aggregates toward a preplacodal ectoderm (PPE) fate, a prerequisite for otic lineage commitment. Recent studies have highlighted the critical role of exogenous BMP4 and WNT signaling in otic specification, with early WNT activation shown to improve otic induction efficiency in mouse IEOs. Building on this, we hypothesized that earlier WNT activation in BMP4-optimized human IEOs would enhance otic progenitor formation. To test this, we analyzed morphological changes (Days 0-30) using a newly generated dual-reporter hiPSC line to enable real-time tracking of otic (OC90-mRuby3) and sensory (SOX2-GFP) differentiation. While early WNT signaling (Day 4) significantly promoted otic vesicle formation, it significantly reduced SOX2+ signals in the otic epithelium, suggesting over-dorsalization and disruption of sensory lineage specification. Given that SOX2+ sensory epithelium arises ventrally in the otic vesicle in vivo, requiring both WNT and Shh signaling for proper maturation, we hypothesized that Shh activation following otic vesicle formation could restore sensory lineage development. Using live-cell imaging, we found the treatment with the Shh agonist Purmorphamine successfully restored SOX2+ sensory epithelium. Our findings demonstrate that precise modulation of WNT and Shh signaling can significantly influence the sensory epithelium development in vitro. These optimized human IEOs not only improve differentiation efficiency but also facilitate the isolation and enrichment of sensory otic tissues, paving the way for future applications on inner ear disease modeling, drug screening, and regenerative medicine.

T1170

HUMAN IPSC DERIVED HEPATIC STELLATE CELLS ENHANCED BY LIVER DEVELOPMENTAL MICROENVIRONMENT

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Hepatic stellate cells (HSCs), as liver-specific mesenchymal cells, play pivotal roles in liver development, regeneration, and diverse pathological processes. However, the scarcity of primary HSCs (pHSCs) and suboptimal functionality of induced HSCs (iHSCs) generated by existing methods have limited their application in biomedical modeling. In this study, we developed a de nove in vitro differentiation strategy to successfully generate functionally enhanced iHSCs capable of mimicking the liver microenvironment. Experimental validation demonstrated that these iHSCs not only exhibited core functional characteristics—including α-smooth muscle actin (α-SMA) expression, collagen secretion, and vitamin A storage—but also displayed transcriptomic profiles highly consistent with pHSCs through RNA sequencing. Notably, novel HSC-specific marker genes such as FBLN5, NID2, and SVEP1 were identified. This groundbreaking differentiation strategy

achieves, for the first time, iHSCs that closely approximate pHSCs in both phenotypic and functional aspects, offering new possibilities for accurately modeling the multicellular interactive microenvironment in the liver. The generation of highly functional iHSCs not only provides an innovative platform for unraveling liver physiological regeneration mechanisms and pathological progression but also establishes a critical technical foundation for developing targeted therapeutic strategies for liver diseases.

Funding Source: National Natural Science Foundation of China (82270697), Haihe Laboratory of Cell Ecosystem (HH24KYZX0008), China Foundation For Youth Entrepreneurship and Employment -Incaier Public Welfare Fund (HH25KYHX0003).

T1172

INDUCING LUNG DIFFERENTIATION IN HUMAN HEART-FORMING ORGANOIDS TO FORM COMPLEX LUNG-HFOS

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Heart-forming organoids (HFOs) derived from human pluripotent stem cells (hPSCs) represent a complex, highly structured in vitro model for heart, foregut and vasculature development. Specifically, HFOs are composed of a myocardial layer lined by endocardial-like cells and surrounded by septum-transversum-like anlagen; they further contain spatially and molecularly distinct anterior versus posterior foregut endoderm (AFE versus PFE) tissues and a vascular network. The architecture of HFOs closely resembles aspects of early native heart anlagen prior to heart tube formation, which is known to require an interplay with foregut endoderm development. In the embryo, the AFE gives rise to different organs such as the lung, esophagus and thymus. In HFOs, the AFE is located in the inner core and represents immature tissue expressing progenitor markers of AFE-derived organs as revealed by single-cell RNA sequencing. To further advance the current HFO model towards embryo-like multi-tissue complexity, we here combine our HFO differentiation protocol with directed lung differentiation to induce lung epithelium formation. We show via flow cytometry and immunofluorescence staining that cells expressing the lung progenitor marker NKX2.1 form in the inner core, specifically within the epithelium lining the endodermal cavities. Following maturation, spheres expressing lung epithelium markers emerge from the HFOs. Notably, while both proximal and distal lung epithelial cells form over time, the overall 3D structure and established pattern of HFOs remain intact, despite the increase in tissue complexity. Thus, the first step towards a combined, self-organized heart-lung-vasculature model has been achieved, opening new perspectives for investigating human diseases in vitro, as well as advanced teratogenicity assessment and drug discovery approaches to replace animal experiments efficiently.

Funding Source: This work was funded by Hannover Medical School (HiLF I) and the Ministry of Lower Saxony (Zukunft.Niedersachsen).

T1174

MECHANICAL CONFINEMENT AS A KEY FACTOR IN TRANSPLANTATION-INDUCED PROGENITOR STATE DETERMINES SUCCESSFUL HEPATOCYTE ENGRAFTMENT

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Successful cell engraftment requires navigation through confined microenvironments, yet the underlying adaptive mechanisms remain elusive. Here, we identify a mechano-induced autonomous reprogramming state critical for hepatocyte engraftment during transplantation. Hepatic sinusoidal confinement triggers transplanted hepatocytes (Tx-Heps) to adopt a conserved reprogramming state, characterized by liver progenitor genes activation. This transcriptional reprogramming correlate with stress resistance and enhanced cell fitness, facilitating Tx-Heps to survive distal capillaries entrapment and accelerate engraftment. Lineage tracing reveals most Tx-Heps undergo this reprogramming. Importantly, mechanical confinement emerged as a key inducer of transplantation reprogramming, with Arid1a identified as a downstream regulator. Hepatocyte-specific ablation of Arid1a suppressed reprogramming and led to transplantation failure, highlighting the role of transplantation reprogramming in successful engraftment. Furthermore, we developed a norepinephrine/dopamine-based pharmacological strategy that amplifies confinement-induced reprogramming, achieving a 4.3-fold engraftment improvement. Our findings provide novel insights into the influence of mechanical factors on cell fate reprogramming and suggest strategies for improving transplantation outcomes through mechanopriming and molecular modulation.

Funding Source: This project was supported by the “Strategic Priority Research Program” of the Chinese Academy of Sciences (XDA16020201, XDB38040400), the National Key Research and Development Project (2020YFA0112503, 2022YFA1004800).

T1176

NOTCH INHIBITION IMPAIRS BIDIRECTIONAL COMMUNICATION BETWEEN PORCINE OOCYTES AND CUMULUS CELLS

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Notch signaling plays a crucial role in cell-cell communication, regulating cell proliferation, differentiation, and apoptosis, as well as ovarian follicle development. However, the presence and role of Notch signaling in porcine follicle development remain largely unexplored. Therefore, we aimed to identify the presence of Notch signaling components during folliculogenesis and

investigated the communication between oocytes and cumulus cells. Immunohistochemistry (IHC) and western blot analysis revealed the localization of Notch receptor and ligand in porcine follicular cells across various follicle sizes. Furthermore, we investigated the effects of RO4929097 (RO), a Notch signaling inhibitor, on porcine oocyte maturation by analyzing changes in transzonal projections (TZPs), gap junctions, and metabolite levels, as well as apoptosis rates. During oocyte maturation, RO treatment significantly reduced the intensity and number of TZPs, as well as the levels of gap junctions and metabolites. Moreover, RO treatment notably increased the apoptosis rate in COCs after 42 h of oocyte maturation. We demonstrated for the first time the localization of Notch receptor and ligand in porcine ovarian follicle development. Additionally, these findings suggest that the pivotal role of Notch signaling in mediating oocyte-cumulus cell interactions, as evidenced by the reduction in TZPs, gap junctions, and metabolites, along with the increase in apoptosis rate following RO treatment.

Funding Source: “Technology Innovation Program funded by the MOTIE, Korea (20023068)”, and “IPET in Food, Agriculture, Forestry and Fisheries funded by MAFRA (RS-2024-00398561, RS-2024-00399475)".

T1178

THE NUCLEAR CAP-BINDING COMPLEX SAFEGUARDS STRESS-RESISTANT PROTEIN SYNTHESIS AND PROLIFERATION OF STEM CELLS

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The integrated stress response (ISR) suppresses global translation while allowing the selective translation of key regulatory genes. However, how protein synthesis persists during ISR remains unclear. In eukaryotic cells, the 5′-cap of most mRNAs is bound by either the nuclear cap-binding complex (CBC) or the cytosolic cap-binding protein eIF4E. Our study reveals that under stress conditions, CBC-bound mRNAs utilize eIF2A, an alternative translation initiation factor, to maintain protein synthesis when translation of eIF4E-bound mRNAs is inhibited. Human embryonic stem cells (hESCs), which inherently exhibit ISR, actively proliferate because of a compensatory increase in the eIF2A expression. This increase ensures CBC-dependent translation (CT) and supports the synthesis of essential cell cycle proteins during stress. Notably, YAP, a key proliferation factor, is a critical CT target driving stress-resistant stem cell proliferation. Therefore, our findings highlight CT as a crucial pathway that protects protein synthesis and supports stem cell proliferation during stress.

Funding Source: National Research Foundation (NRF) of Korea grant funded by the Korean government.

T1180

WHARTON'S JELLY MESENCHYMAL STEM CELLS AND MICROGRAVITY: INVESTIGATING STRESS RESPONSE FOLLOWING REAC BIOMODULATION

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Wharton's jelly mesenchymal stem cells (WJ-MSCs) exhibit most of the regenerative and differentiative properties of embryonic stem cells, raising no ethical issues and easily to collect. Under stressing conditions, they can lose these properties, undergoing premature senescence or apoptotic events. Simulated microgravity negatively impacts the physiology of the human body, influencing cell behavior, in growth and differentiation processes. Each cell responds to stress in several ways, generally by activating signaling pathways promoting survival or apoptosis. The kind of response also depends on the type and duration of the stressing event. Radio Electric Asymmetric Conveyer (REAC) biomodulation, as metabolic optimization-microgravity (MO-MG), enhance tissue repair through targeted cellular integrated response. In the present study, WJ-MSCs were exposed for 24h to a simulated microgravity (μg) using a 3D random positioning machine (RPM). The 3D RPM provides a simulated microgravity of less than 10−3 g. Controls were placed in the static bar at 1 g to undergo the same vibration as the sample under μg conditions. At the end of 24h, cells were put in culture and exposed to REAC- MO-MG protocol for 6 days. The cycle consisted of 9 sessions, each lasting approximately 30 minutes. Sessions can be spaced out by a minimum of one hour and with no more than four sessions administered in a single day. We then evaluated cell morphology after REAC-MO-MG and analyzed gene expression of stemness-associated genes (Oct-4, Sox2 and Nanog), and some epigenetic factors, strictly related to stress response (Sirt1, DNMT1 and HSP70). We also evaluate stem cell potency by 21 days of differentiation in the presence of adipogenic or osteogenic conditioned medium. Our results clearly demonstrate a role of REAC MO-MG in restoring WJ-MSC potency, maintaining higher expression levels of the stemness markers and the epigenetic factors. Therefore, REAC- exposed cells are able to modulate stress response, counteracting apoptosis, as compared to WJ-MSCs exposed only to μg. These data demonstrate that MO-MG can act directly modulating cell response, restoring the altered expression of key genes involved in stemness and differentiation induced by microgravity, suggesting the potential use of this treatment in future space missions.

T1182

A CULTURE SYSTEM FOR LONG-TERM MAINTENANCE OF NEURAL CREST STEM CELLS (NCSCS) DERIVED FROM HUMAN IPSCS

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Neural crest stem cells (NCSCs) are a transient population of stem cells that emerge early in vertebrate development; some NCSCs are thought to be multipotent and differentiate into a variety

of cells, including peripheral nerve cells, glial cells, bone cells, chondrocytes and adipocytes. We have developed a culture system that can grow human iPSC-derived NCSCs and maintain expression of the SOX10 gene, a characteristic of pluripotent NCSCs, for more than 6 months. NCSCs maintained for several months were able to differentiate into various cell types such as peripheral neurons, melanocytes, MSCs (mesenchymal stem cells)-mediated osteocytes, chondrocytes, and adipocytes, suggesting that these cultures have a pluripotent stem cell population. The NCSC lines established expressed the major marker genes of neural crest cells and were shown to differentiate into three or more linages. This culture system is expected to be useful not only for basic research on molecular events in pluripotent and differentiated stem cells, but also for applied research such as drug screening.

T1184

A NOTCH-MEDIATED GENE REGULATORY NETWORK OF INTESTINAL STEM CELL FATE DECISION

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The intestinal epithelium's rapid renewal and repetitive structure makes it an excellent model for studying stem cell homeostasis and differentiation. Intestinal stem cells (ISCs) at the crypt base renew the epithelium every 5-7 days, producing secretory and absorptive cells. While ISC regulation is well-studied, little is known about how the cell fate is established at the earliest progenitors (+4/5 cells) in the intestinal epithelium. We hypothesise that this decision is driven by a Notch-mediated gene regulatory network (GRN). By using in vivo lineage tracing, high-throughput sequencing and organoids, we investigate the regulation of this fate decision. Lineage tracing in a mouse model allowed labelling of +4/5 cells. Followed by cell sorting and bulk RNA-seq, we compared the +4/5 cell transcriptome to ISCs and shortlisted +4/5 enriched transcriptional regulators and chromatin organisers. We identified 36 genes, which have not yet been described in ISC regulation and fate decision, alongside Mtg8/16 that have recently been discovered to be co-repressors of secretory fate. The genes were further shortlisted by investigating their expression upon Notch inhibition in organoids and mice as well as RNAscope analysis to prove expression at the +4/5 position. Three candidate genes were higher expressed after Notch inhibition in vitro and in vivo and showed +4/5 specific expression, indicating a role in the Notch-driven fate decision. Knock-out of these genes in mouse intestinal organoids showed significant changes in marker gene expression for all terminally differentiated cell types of the epithelium after Notch inhibition. To further investigate the role of Mtg8 in intestinal homeostasis, we depleted the bona fide ISC marker gene Lgr5 in Mtg8 KO mice, resulting in faster recovery when compared to wild type. At the same time Mtg8 KO mice failed to recover after in vivo Notch inhibition. These findings reveal new players in the GRN driving intestinal fate decision and strengthen the Notch-mediated role of Mtg8. However, specific molecular roles and interactions require further characterisation. Identifying new regulators of intestinal epithelial homeostasis furthers our understanding of the complex GRN behind this process and might also unravel novel mechanisms in intestinal regeneration and disease.

T1186

ADHERENT HIPSC LUMENOIDS: A ROBUST 3D PLATFORM FOR BRIDGING CELLULAR DYNAMICS AND MULTICELLULAR ORGANIZATION

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Over the past decade, the Allen Institute for Cell Science has developed and distributed tools to study human induced pluripotent stem cells (hiPSCs), including fluorescently tagged lines, images of 2D hiPSC colonies, and image analysis workflows. However, 2D cultures cannot replicate the complex multicellular interactions seen in 3D environments. To address this, we present the adherent hiPSC lumenoid model, a simple and tractable 3D system for investigating cellular dynamics in a physiologically relevant context. Lumenoids are hollow, acinar structures with a single central lumen surrounded by a coherent, edgeless, apical-in/basal-out, pluripotent epithelium. Lumenoid formation is initiated by adding dilute extracellular matrix (Matrigel) to 2D colonies. This triggers a cascade of cellular events, including apical contraction, colony edge lifting, and epithelial closure via a purse-string-like mechanism, creating a topologically closed lumen within ~6-24 hours. Once formed, lumenoids grow into large, often spherical 3D structures as the lumen inflates. Lumenoid formation is accompanied by de novo basal secretion of basement membrane (BM) material, and fully formed lumenoids are encased in a BM shell. During an epithelial-to-mesenchymal transition (EMT) induced by WNT activation, lumenoid cells delaminate and migrate through newly formed holes in the BM, revealing key cell-cell and cell-matrix interactions involved in this critical process. The simple geometry of lumenoids, their genetic tractability, and their reproducibility offer significant advantages over other 3D culture models. Importantly, lumenoids remain stably anchored to the coverslip, allowing for long-term live imaging in high throughput. They are also retained through media changes, fixation, and immunolabeling,

enabling seamless downstream multimodal data integration. Using this system and fluorescently tagged lines from the Allen Cell Collection, we have generated hundreds of 60-hour 3D timelapse movies capturing lumenoid formation, growth, and EMT, all publicly available alongside open-source analysis tools. Moving forward, this assay will enable holistic integration of cell dynamics, molecular features, and local context in a reproducible, experimentally tractable, physiologically relevant 3D system.

T1188

AGE-RELATED TRANSCRIPTOMIC ALTERATIONS AT ISOFORM AND GENE LEVELS IN DIFFERENT BRAIN REGIONS

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The accumulation of cellular damage, characterized by aging hallmarks, renders the brain increasingly vulnerable to neurological diseases. This process exhibits region-specificity, as evidenced by the early manifestation of age-related diseases in certain brain regions but not in others. For instance, the cortex and hippocampus show early plaque pathology in Alzheimer's disease. Previous studies have reported both global and cell-type-specific transcriptomic alterations, raising questions about whether transcriptional regulation is disrupted during aging. Here, we constructed a single-nuclei atlas of the cortex, hippocampus, and cerebellum to identify region-specific transcriptional alterations at both the gene and isoform levels. Using an in situ sequencing method, SPRINTseq, we incorporated spatial information on differential gene and isoform expression. Our analysis revealed broad expression changes in histone acetylases and RNA-binding proteins involved in splicing regulation. In neurons, isoform-level analysis from full-length scRNA-seq uncovered isoform usage switches and intron retention in genes involved in critical biological pathways, such as mRNA splicing, synaptic function, and axon projection. Such dysregulation may lead to nonsense-mediated decay of transcripts or the production of truncated proteins, disrupting cellular homeostasis.

T1190

ALPHA-7 NICOTINIC ACETYLCHOLINE RECEPTORS REGULATE RADIAL GLIA FATE IN THE DEVELOPING HUMAN CORTEX

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Exposure to nicotine (NIC) during pregnancy is associated with a reduction in fetal cortical grey matter volume, driven by vulnerable cell types and molecular pathways that are not clearly understood. There is evidence of acetylcholine signaling to fetal cortical germinal centers from cholinergic tracts, but the effect of activation of nicotinic acetylcholine receptors (nAChRs) in progenitor cells and radial glia (RG) of the developing human cortex remains unclear. We find two nAChR subunits, CHRNA7 and the human-specific subunit CHRFAM7A, expressed in SOX2+ progenitors, with CHRFAM7A highly enriched along RG apical endfeet. To explore potential functions of cholinergic signaling, we exposed dissociated primary cortical cultures to nAChR agonists, including NIC, or performed nAChR knockdown followed by bulk and single-cell (sc) RNA-sequencing. ScRNAseq revealed that downstream effects of NIC exposure included upregulation of semaphorin-plexin axon guidance associated genes in ventricular RG, outer Radial Glia (oRG) and excitatory neurons (ENs) at GW15-16. We also observed an upregulation of genes associated with chromatin silencing, DNA methylation, and DNA alkylation changes in RG at GW 19-22. Many genes critical for cortex development including DCHS1, SHANK3, LAMA5, NLGN2, LRP1, KIF1A, were downregulated in ENs following NIC exposure. While some DEGs are downstream of both CHRNA7 and CHRFAM7A, a large fraction of DEGs were unique to each nAChR subunit, suggesting a diversification of the regulatory networks controlled by CHRFAM7A during human cortical evolution. Using immunostaining, we observed that activation of nAChRs through agonists increased the number of SOX2+, HOPX+, and KI67+ RG and decreased NEUN+, deep layer CTIP2+ and upper layer SATB2+ neurons. ShRNA-mediated nAChR knockdown produced inverse phenotypic changes, with a reduction in RG cells and an increase in neuron number. Our results suggest that prenatal NIC exposure is able to change RG cell fate, which we hypothesize may be causing some of the clinical phenotypes observed in the offspring of smokers.

Funding Source: Boehringer Ingelheim Fonds PhD Fellowship R35NS097305-07 Swiss National Science Foundation.

T1192

AN INNOVATIVE MRNA-LNP KIT FOR IPSC REPROGRAMMING FROM PBMC

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The generation of induced pluripotent stem cells (iPSCs) from peripheral blood mononuclear cells (PBMCs) represents a significant advancement for personalized medicine, disease modeling, and drug discovery. Traditional reprogramming methods using plasmids or viral vectors pose risks, including genomic integration and immune activation. To address these challenges, we introduce a novel mRNA-LNP (lipid nanoparticle) kit designed specifically for efficient and safe reprogramming of PBMCs into iPSCs. Our technology leverages optimized lipid nanoparticles to deliver mRNA encoding essential reprogramming factors, providing a non-viral alternative that eliminates genomic integration while enhancing safety and efficacy. The transient nature of mRNA expression ensures rapid degradation of exogenous genetic material, minimizing oncogenic risks and improving compatibility with clinical applications. The LNPs are engineered for high transfection efficiency and minimal cellular toxicity, enabling effective delivery of reprogramming mRNAs into PBMCs. The kit includes a comprehensive set of components, including purified mRNAs for critical reprogramming factors, pre-formulated LNPs, and specialized reagents to maximize delivery efficiency. The ready-to-use mRNA-LNP formulation can be directly applied to cell cultures, eliminating the need for electroporation or supplemental transfection agents. Using this system, PBMCs can be efficiently reprogrammed into iPSC clones that exhibit hallmark pluripotency markers, trilineage differentiation potential, and stable karyotypic integrity. Compared to traditional methods, our approach eliminates genomic integration risks while achieving comparable or superior reprogramming efficiency. This innovation offers significant potential for advancing translational research, including patient-specific disease modeling, high-throughput drug screening, and regenerative medicine therapies. By combining safety, scalability, and user-friendly protocols, the mRNA-LNP platform sets a new standard for iPSC generation in both research and clinical applications.

T1194

ANEUPLOIDY AS A FIRST GENETIC HIT THAT ENHANCES THE TUMORIGENIC CAPACITY OF DIFFERENTIATED HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) are increasingly recognized for their potential in cell-based regenerative therapies for injuries and chronic conditions. However, hPSCs in culture frequently acquire chromosomal abnormalities (CA), raising safety concerns regarding their therapeutic use. Strikingly, these CAs resemble genetic aberrations found in cancers. It remains unclear whether CAs can predispose differentiated cells to oncogenic transformation, a gap stemming from a lack of suitable research models and systematic studies. In this work, we hypothesize that CAs represent a first hit in the oncogenic process, enhancing the potential of differentiated hPSCs to transform upon subsequent oncogenic hits. We used an in vitro organoid-based model of brain tumorigenesis, where we subject set of genetically balanced lines (VUB01, VUB02, VUB03, VUB04, VUB07, VUB14 and VUB19) as controls, and lines with well-characterized recurrent CAs (gains of 1q, 12p, 17q, 20q and losses of 18q), to directed mutagenesis. This approach allowed

investigating genetic defects that could enhance the tumorigenic potential of hPSC-derived cells. Our findings indicate that hESCs with CAs, particularly those with gains on 20q11.21 (commonly found in glioblastomas), 1q24.2, and 17q, are more likely to form tumorous overgrowth in brain organoids (95-100% transformation rate) following mutagenic transformation via cMyc overexpression, compared to hESCcontrol organoids (20-30%). Organoids from hESCs with gains of 12p or losses of 18q exhibited lower transformation rates (20-40%). Notably, organoids with transformed overgrowth retained viability and expanded upon intracranial transplantation in immunodeficient mice, with transformed GFP+ cells proliferating and generating CD99+/SOX2+/GFP+ neoplastic-like regions. Moreover, hESCs with CAs, particularly those with gains on 12p, 20q, and losses on 18q, formed organoids with disorganized shapes compared to control organoids. This suggests that CAs not only enhance the transformation capacity of hPSCs but also impair their ability to develop into normal brain organoids. Our work provides insights into the functional impact of CAs in hPSCs and valuable knowledge for assessing the long-term risks associated with transplanting hPSC-derived cells with genetic defects.

Funding Source: FWO.

T1196

AUTOMATION OF FULL WORKFLOW FOR CARDIAC DIFFERENTIATION AND FORMATION OF 3D CARDIAC ORGANOIDS FROM HUMAN IPSC, AND FUNCTIONAL ANALYSIS OF COMPOUND RESPONSES

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Modeling human tissues using iPSC -derived 3D cardiac organoids is a highly promising technology to facilitate drug development and toxicity assessment. In this study, we developed method to automate the full process for cell differentiation from iPSC and formation and maintenance of 3D cardiac organoids. CellXpress.ai automated cell culture system contains four essential components for automated organoid culture: liquid handler, automated incubator and automated imager, plus scheduling software that that provides automated processing. Cardiac cells were derived from iPSC which were triggered to differentiation with CellXpress.ai instrument. After 36 hours of differentiation, cells were harvested, then cardiac micro-tissues were created in low attachment U-bottom plates. CellXpress.ai instrument automated cell plating and subsequent media exchanges every 2 days and monitoring by imaging every 24h. 3D microtissues were formed within 48 hours and started to contract spontaneously. After additional maturation for 2 weeks, we evaluated the functional activity of cardiac organoids by recording calcium oscillations after addition of calcium-sensitive dye using a fast kinetic fluorescence (FLIPR Penta). Also, we evaluated the morphology of cardiac organoids and confirmed presence of three cell types: cardiomyocytes, fibroblasts and endothelial cells. We tested the responses of the microtissues to 20 compounds including modulators of cardiac activity, blockers of ion channels, and panel of known cardiotoxic compounds (CIPA). Tested compounds, including hERG inhibitors, ion channel blockers and other compounds demonstrated changes in the Ca2+oscillation patterns consistent with expected mode of action of tested compounds or toxicity effect. Waveform analysis of patterns provided multiple read-outs including peak frequency, peak amplitude, peak prolongation,

irregularity, appearance of secondary peaks and other measurements characterizing modulations of oscillation patterns. Additionally, we characterized the morphology and viability of 3D microtissues by image analysis. The data presented highlight the utility and biological relevance of using iPSC-derived cell types in 3D microtissues as promising model for screening potential cardiotoxic effects in human cardiac tissues.

Funding Source: Molecular Devices, LLC.

T1198

CELLULAR MECHANISMS ESTABLISHING EMBRYO-MATERNAL IMMUNE TOLERANCE AT THE IMPANTATION SITE

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Implantation of the blastocyst in the uterine wall is a vital step in mammalian development. At embryonic day four and a half (E4.5) the mouse blastocyst attaches to the uterine wall and initiates implantation. The trophectoderm (TE) cells differentiate into the so-called trophoblast giant cells (TGCs) which penetrate the uterine epithelium and invade the endometrial stroma. In turn, the stromal cells rapidly proliferate to form the decidua, a specialized compartment that engulfs the implanting embryo to support its further development. Three phases with specific inflammatory profiles have been reported during pregnancy. Starting with implantation, early pregnancy is associated with the establishment of a pro-inflammatory environment, which switches to an anti-inflammatory stage during the foetal growth, followed by a final pro-inflammatory phase at the time of parturition (delivery). This suggests a dynamic modulation of the immune response at the maternal-embryo/foetal interface during the progression of pregnancy. Here we examine the first phase aiming to understand the cellular principles governing the maternal immune tolerance to the implanting embryo. Focusing on the natural killer (NK) cells, which are the most abundant decidual leukocytes, our preliminary data show that a “no entry” zone for the NK cells is established around the embryo. Yet, the NK cells exhibited cytotoxicity in vitro eliminating the embryo when placed in close proximity. Thus, we show that the maternal immune system does not “tolerate” the semi-allograph properties of the embryo, but rather the remodelling of the stromal tissue during decidualisation restricts the access of the NK cells to the conceptus.

T1200

PROTEOMIC ANALYSIS REVEALS EJECTED MITOCHONDRIA AS THE MEDIATOR OF OSMOLALITY-INDUCED ADIPOCYTE DEDIFFERENTIATION

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Adipocytes have the potential to dedifferentiate into multipotent mesenchymal cells. Recent studies demonstrated that elevated osmolarity could induce adipocyte dedifferentiation, representing an appealing procedure for the regenerative toolsets. However, it remains elusive about the molecular mechanism that underlies osmotic stress-induced reprogramming of adipocytes. Here we report that high osmolarity prompts the adipocytes to release mitochondrial component, which in turn enhances the secretion of TNF-a as a pro-inflammatory cytokine during the stress response. Importantly, TNF-a is essential for the activation of the Wnt/b- catenin signaling that drives adipocyte dedifferentiation. Despite the generation of multipotent cells, the osmotic stress-induced

adipocyte dedifferentiation is accompanied by a significant level of apoptosis mediated by TNF-a. To circumvent this issue, we show that Wnt agonist 1, a small compound that directly activates the Wnt/b-catenin signaling could effectively induce the multipotent adipocyte dedifferentiation without promoting apoptosis. Our results defined the molecular mechanism of adipocytes reprograming in response to osmotic stress. Furthermore, we provide an efficient approach to induce adipocyte differentiation while mitigating apoptosis.

Funding Source: Shenzhen Science and Technology Innovation Commission; Shenzhen-Hong Kong Science and Technology Innovation Cooperation Zone Shenzhen Park Project

T1202

CHARACTERIZING ELITE CELL TRAJECTORIES IN HUMAN IPSC REPROGRAMMING

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Pluripotent cells, under the correct conditions, can differentiate into any cell type in the human body. By inducing the expression of the Yamanaka Factors - OCT4, SOX2, c-MYC, and KLF4 in any differentiated cell, one can generate induced pluripotent stem cells (iPSCs). However, cell reprogramming has a low efficiency with only a small fraction of the starting population successfully becoming iPSCs. This led us to question whether certain cell states and trajectories that have a greater propensity to reprogram exist; and if they do, when does this advantage arise during the reprogramming timeline. We barcoded human fibroblasts and reprogrammed them while performing single cell RNA-sequencing at different time points. This allowed simultaneous lineage tracing while observing cell fate changes. We identified an intermediate elite cluster enriched with barcodes that could be traced to the final iPSC population. We then characterized the properties of this cluster and identified a molecular profile enriched for cell-survival, proliferation, and a myriad of developmental programs. Using candidate markers we successfully isolated the intermediate population with fluorescence-activated cell sorting (FACS). The sorted elite intermediate cells formed OCT4-, SOX2-, and NANOG-positive PSCs, while the non-elite cells did not. This study provides fresh insights into cell fate trajectories that lead to pluripotency and contribute to our broader understanding of cellular plasticity and cell fate change.

T1204

COMPREHENSIVE MULTI-OMICS ANALYSIS OF PERI-GASTRULOIDS REVEALS KEY REGULATORY DYNAMICS OF HUMAN PERI-GASTRULATION DEVELOPMENT

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Human peri-gastrulation development remains poorly understood, primarily due to limited access to in vivo embryos and ethical constraints. Stem-cell-derived embryo models, such as peri-gastruloids, offer unique opportunities to investigate this critical stage of human development. However, their ability to mimic in vivo development requires further exploration. Here, we present a comprehensive, time-resolved analysis of human peri-gastruloids, integrating single-cell RNA-seq, single-nucleus ATAC-seq, and metabolomics across key stages. We show that human peri-gastruloids recapitulate peri-gastrulation development, from bilaminar to trilaminar disc formation and early organogenesis, at both cellular and molecular levels. Time-resolved transcriptional and chromatin accessibility profiling reveals critical lineage regulators, shedding light on transcription factors driving lineage specification and maintenance. Metabolomic profiling further identifies stage-specific metabolic signatures, while integration with gene expression data uncovers coordinated metabolic and transcriptional regulation during human peri-gastruloid development. Importantly, we demonstrate the utility of peri-gastruloids for teratogenicity test, showing that Methotrexate disrupts critical metabolic pathways such as cholesterol homeostasis and neural ectoderm formation, mirroring clinical observations of its teratogenic effects. Together, these findings establish peri-gastruloids as a robust model for studying human development, with potential applications in disease modeling and drug safety assessment.

Funding Source: New York Stem Cell Foundation (NYSCF); American Society for Reproductive Medicine (ASRM).

T1206

CONTROL OF CELL FATE BY MANIPULATION OF DYNAMICS OF GENE REGULATORY NETWORK

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Transcription factors and co-regulators play a crucial role in determining the functions and properties of cells. Since these factors form complex regulatory networks, it is essential to elucidate the dynamics of the entire regulatory network for understanding of cellular properties both in undifferentiated states and during differentiation processes. Using time-series perturb-seq data, we have previously elucidated the structure of a gene regulatory network involved in maintaining the undifferentiated state of human pluripotent stem cells. In this study, we aim to control the dynamics of the regulatory network by manipulating its core factors, ultimately seeking to efficiently induce differentiation of human pluripotent stem cells into specific cell lineages. To achieve this, we improved conventional CRISPR-a/i transcriptional control systems and established human iPS cells capable of simultaneously perturbing (activating or repressing) multiple factors in various combinations within a single cell. By comparing the previously mentioned perturb-seq data with public scRNA-seq data from early human development, we theoretically predicted which factors should be considered as core of the regulatory network and how these factors should be regulated

to induce differentiation of cells into specific lineages. These predictions were experimentally validated using CRISPR-a/i-modified human iPS cells. As a result, we were able to confirm the expression of marker genes for the predicted cell lineage through the simultaneous manipulation of seven factors. Currently, we are further refining the manipulation system and analyzing the molecular mechanisms underlying this differentiation induction.

T1208

CRISPR-BASED ACTIVATION SCREENING IDENTIFIES NOVEL HUMAN MAJOR ZYGOTIC GENOME ACTIVATION REGULATORS

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The initiation of major zygotic genome activation (ZGA) is crucial for human early embryogenesis. However, the gene regulation network (GRN) behind major ZGA in humans remain largely unknown. Transcription factors (TFs) are master regulators of GRN. To better understand the mechanism behind ZGA, we performed a CRISPR-based activation screen of all the human TFs in human extended pluripotent stem cells (hEPSCs) and identified unreported candidates as major ZGA regulators. Further evaluation of these candidates revealed that over-expression of several targets activated totipotent features that differ from known totipotency and ZGA regulators in hEPSCs. Importantly, knockdown of key candidates arrested human embryo development prior to the 8-cell embryo stage, indicating an unreported mechanism of TFs mediating totipotency in vitro, accompanied by the suppression of a set of 4-cell embryo enriched genes. Our study provided valuable resources for the investigation of totipotency and major ZGA regulation, and brought up new model for TFs regulating ZGA.

Funding Source: The National Key Research and Development Program of China (2021YFA1100300), the National Natural Science Foundation of China (32288102), the Beijing Natural Science Foundation (JQ24042), the National Natural Science Foundation of China (32370843).

T1210

DECIPHERING THE MOLECULAR SIGNATURE OF INDUCED PLURIPOTENT STEM CELL TO T-CELL DIFFERENTIATION USING CRISPR ACTIVATION SCREENS

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Induced pluripotent stem cells (iPSC) are a promising avenue for producing next-generation adoptive cell therapies (ACT). They serve as a potentially unlimited source of cells to differentiate any immune cell of interest. Specifically in T cell-based ACTs, this approach allows the customization and personalization of the final T cell product and the generation of an off-the-shelf, allogeneic product. The differentiation of iPSCs to T cells poses great challenges, mainly associated with the low efficiency of the process and protocol variability. Therefore, decoding the

molecular signature of this process is of significant importance for advancing this type of therapy. In this regard, CRISPR activation (a) or interference (i) screens offer a comprehensive and systematic approach to unravel the complexities of gene function and regulate the differentiation process. We have generated iPS cell lines that constitutively express dead Cas9 (dCas9) fused to either the VP64 transactivating domain or the transinhibiting domain KRAB. We have further optimized the constructs to avoid transgene silencing by incorporating an antisilencing element (UCOE). We have validated the CRISPRa technology in two iPS cell lines, where a robust gene upregulation of CD4, CD8 and CD14 has been achieved. For CRISPRa screens, these iPS cell lines will be transduced with gRNA libraries specifically targeting drugable targets, phosphatases, and signaling pathways. We will then follow the enrichment of gRNAs in differentiated populations to evaluate which genes regulate the differentiation at different stages. We will use our in-house scaffold technology to improve the presentation of the current differentiation factors, as well as implementing the drugable hits from the screen, which allows for a more controlled and localized delivery and improvement of the T-cell yield. We anticipate that the knowledge obtained from the screens will not only enhance the understanding of T cell development but will also provide opportunities for more precise manipulation of gene expression, which will lead to the generation of robust protocols for iPSC-derived T cell products. Moreover, hits from early stages of the differentiation (at HPSC for instance) can also be used to improve the yields of other immune populations of interest such as NK cells.

T1212

DEEP BIOPHYSICAL IMAGING CYTOMETRY FOR IMMUNE-CELL MORPHOLOGICAL PROFILING

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Current state-of-the-art in understanding immunological behavior highly relies on proteins induced and exist within immune cells, such as CD markers, cytokines and gene expressions. To confirm immune cell subtypes within a biopsy, such as blood from clinic, immunostaining techniques were applied while further exploration of their functions are often investigated with gene sequencing technologies in addition to previous procedures. Results were satisfactory in terms of accuracy of diagnosis, yet they require well-trained labor force and expensive bioreagents if single-cell analysis is required in these tests. Recently, morphological profiling of immune cell behaviors under various circumstances is suggested to be another potential aspect to categorize relevant differences. Our study demonstrates how multi-ATOM, a label-free imaging flow cytometry system that captures single-cell images with different contrasts in addition to fluorescence signals, was applied to demonstrate the potential of discovering immune cell subtype behavior differences under different conditions. Morphological profile variations such as sizes and surface texture between human CD4+ and CD8+ T cells were revealed when they were in resting states and exposed to activation antigens (anti-CD2/CD28) in different durations, on the scale of 24 hours intervals across activation groups. Other than that, our in-vitro study also suggested the morphological differences between acute activation of human T cells (within 72 hours), chronic activation of human T cells (exposing T cell towards anti-CD3/CD28 beads for 11 days) and resting T cells (supplemented with Il-2 only). With these evidence, it is suggested label-free imaging techniques can be adopted for fast-

screening of immune cell activation and exhaustion states, which potentially be further applied to study differentiation of hematopoietic stem cell towards immune cells and relevant clinical applications.

T1214

DEFINING SUBPOPULATIONS OF ORAL MESENCHYMAL STEM CELLS

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The aim of this research is to isolate primary oral mesenchymal cell cultures (OMSC) from adult (DPSC) and deciduous tooth pulp (SHED), periodontal ligament (PDL), dental apical papilla (SCAP), gingiva (GMSC) and bone marrow (BMSC). Further more to characterize them for expression of specific mesenchymal stem cell markers and evaluate their ability to differentiate to different cell types. The cells were characterized for expression of stem-cell markers (Nestin, Vimentin, CD146, CD44, CD49f) and markers associated with dental tissues (ALP, COL1A2, COL3A2, DSPP) using immunofluorescence. We made statistical analysis of the expression of the fluorescent markers with In Cell Anazyzer Wokstation 3.7.3. All cell cultures were subdued to osteogenic, adipogenic, chondrogenic and epithelial differentiation conditions. Differentiation was determined by Alizarin Red S, Oil Red O and Alcian Blue and expression of epithelial markers CK10 and p63. We proved that OMSC express characteristic stem cell markers nestin, vimentin, CD44, CD49f, CD146. The statistical analysis revealed the different levels of expression in the studied stem cells cultures. We estimated, also the differences in the ability of OMSC to undergo osteogenic, adipogenic chondrogenic and epithelial differentiation compared with BMSC. Revealing the phenotype of the OMSC and their differentiation readiness gives us invaluable information about the potential of these cells and will help us understand better the regenerative potential of OMSC, compared with BMC.

Funding Source: Bulgarian National Science Fund МП21 / 3.4 / BG-RRP-2.004-0004-C01.

T1216

DERIVATION OF HIGHLY PURE POPULATION OF MESENCHYMAL STEM CELLS (MSCS) DERIVED FROM INDUCED PLURIPOTENT STEM CELLS (IPSCS) USING A XENO-FREE CULTURE SYSTEM

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The application potential of mesenchymal stromal cells (MSCs) for cell therapy products is widely known in regenerative medicine. Although MSCs can be isolated from sources such as bone

marrow, umbilical cord, and adipose tissue, their clinical application is often hindered by scalability challenges due to limited proliferative capacity and donor variability. To overcome these limitations, we developed an alternative source of MSCs by deriving them from induced pluripotent stem cells (iPSCs), which offers a promising solution due to its greater expandability and potentially continuous supply. However, a critical quality concern for iPSC-derived cell therapy products is the presence of residual undifferentiated cells, which pose a risk of tumorigenicity. In this study, we established a xeno-free differentiation protocol to derive pure MSCs from iPSCs. The resulting iMSCs demonstrates stable proliferation over 30 population doublings (PDs) while maintaining consistent expression of MSC-specific markers (CD105, CD73, CD90, CD29, and CD44). Genomic stability was preserved, as confirmed by short tandem repeat (STR) analysis and karyotyping, indicating no genetic alterations during differentiation. Functionally, iMSCs exhibited trilineage differentiation potential into osteocytes, adipocytes, and chondrocytes, comparable to traditional MSCs. To address the concern of impurities undifferentiated cells, we developed a sensitive quantitative real-time polymerase chain reaction (qRT-PCR) assay targeting LIN28A, a pluripotency marker highly expressed in undifferentiated iPSCs. Spike-in experiments determined the assay's limit of detection (LOD) to be < 1 iPSC per 10,000 iMSC cells. This xeno-free protocol efficiently generates a highly pure MSC population and provides an up-scalable source of MSC production. Our studies highlight the potential of iMSCs as a reliable source for cGMP-grade MSC-based therapies, ensuring safety and reliability for future clinical applications.

Funding Source: This work was fully funded and supported by PT. Kalbe Farma Tbk.

T1218

DERIVATION OF LONG-TERM EXPANDABLE HUMAN RETINAL PROGENITOR CELLS

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The irreversible deprivation of retinal neurons leads to a variety of ocular neurodegenerative diseases such as macular degeneration and glaucoma. Retinal progenitor cells have shown their therapeutic potential for these eye diseases. Despite human retinal progenitor cells (hRPCs) in retinal organoids being well-characterized, the long-term maintenance of these cells in vitro is still not feasible. Here, we established long-term expandable hRPCs from human embryonic stem cell (hESC)-derived retinal organoids as three-dimensional spheres. These hRPC-spheres expressed retinal progenitor markers visual system homeobox 2 (VSX2), paired box 6 (PAX6), retina and anterior neural fold homeobox (RAX, also known as RX), and SRY-box transcription factor 2 (SOX2). Also, hRPC-spheres maintained their multipotency properties including both self-renewal ability and differentiation capacities over 15 passages. Therefore, this long-term expanding hRPC-sphere line with lower heterogeneity offers novel insights into the stemness of retinal progenitor cells and a promising source of cell-based therapeutics for alleviating common degenerative eye diseases.

Funding Source: This work was funded by Health@InnoHK funding.

T1220

DIFFERENTIAL METABOLIC PROGRAM REGULATION OF THE HUMAN DORSAL ROOT GANGLION ORGANOIDS DIFFERENTIATION AND MATURATION

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The dorsal root ganglion (DRG), derived from neural crest cells, is composed of sensory neurons and satellite glia, and plays a crucial role in transmitting various sensations such as touch, pain, itch, temperature, and spatial positioning Abnormal DRG development leads to congenital sensory neuropathies, which are frequently with metabolic syndromes, suggesting the potential role of metabolic control in regulating DRG development. Despite this, the interplay between metabolic regulation and the gene regulatory network during DRG development remains poorly understood. To address this, we established human dorsal root ganglion organoids(DRGOs)that recaptured developmental reprograms as observed in vivo, showing the emergence of bipotent progenitors, neuronal-glial lineage segregation, and maturation into various sensory neurons subtypes. Building on SnRNA-seq analysis of human embryonic DRGs and biosensors validation in human DRG organoids, we found that both sensory and glial progenitors exhibit high ATP production and NAD+/NADH ratios, which were maintained in differentiated satellite glia. As sensory neurons matured, there was a metabolic shift from oxidative phosphorylation to glycolysis. Notably, we identified MMD2, a key regulator of mitochondrial oxidative energy metabolism, as a key regulator in this metabolic transition. MMD2 expression is primarily found in early differentiating sensory neuron progenitors and glial lineages. Functional analysis reveals that reduced MMD2 expression severely impairs sensory neuronal differentiation and axonal outgrowth, while overexpression of MMD2 promotes sensory neuronal differentiation with increased axonal density. Additionally, inhibition of glycolysis by 2-DG significantly reduces the population of mature sensory neurons and shortens axon lengths, further implicating glycolytic regulation in promoting sensory neuron maturation. These results provide new insights into the dynamic metabolic programs that contribute to different stages of sensory neurogenesis and gliogenesis, and MMD2 could function as a key regulator in mediating this metabolic switching. These results enhance our understanding of the etiology of sensory neurocristopathies and pave the way for energy/metabolic-based therapies for these disorders.

T1222

DISRUPTIVE EFFECTS OF PERFLUOROOCTANE SULFONIC ACID (PFOS) EXPOSURE ON IN VITRO-DERIVED HUMAN PRIMORDIAL GERM CELLS

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Environmental pollutants, like PFOS, interfere with endocrine and developmental processes, posing significant risks to reproductive health. During early embryogenesis, primordial germ cells, the precursors of sperm and eggs, are specified from epiblast cells, a tightly regulated process essential for gametogenesis. In vitro, pluripotent stem cells can be directed into human primordial germ cell-like cells (hPGCLCs), providing an invaluable model to study early germline development. Although PFOS exposure has been associated with reproductive toxicity, its specific impact on early human germline remains unclear. Addressing this knowledge gap, we evaluated the dose-dependent effects of PFOS (7–700 nM; relevant exposure doses for humans) on the specification of hPGCLCs and embryoid body (EB) development, leveraging two human induced pluripotent stem cells (hiPSCs) lines (CTRL14, female; KICO, male) over a nine-day differentiation protocol. Maintenance and growth of both female and male hiPSC lines were not affected by PFOS exposure when compared with controls. However, a significant reduction in development was observed in EBs generated from the CTRL14 hiPSC line and exposed to PFOS. In contrast, the KICO hiPSC line originated EBs with stable development regardless of PFOS exposure. Moreover, FACS analysis showed an increase in the percentage of hPGCLCs following PFOS treatment in both lines. This increase was more pronounced in the CTRL14 line compared to KICO. Immunofluorescence staining revealed co-expression of hPGCLC markers SOX17, TFAP2C and OCT4, thus validating the specification process. These findings indicate that PFOS exposure alters normal EB development and increases hPGCLC specification, suggesting disruptions in the tightly regulated processes governing early germline formation. Ongoing transcriptomic analyses via bulk RNA sequencing will elucidate the molecular mechanisms underlying these phenotypes, focusing on the transcriptome of the early germline. By demonstrating PFOS's disruptive effects on early germline specification, this study highlights critical reproductive health risks associated with environmental toxicants and emphasizes the urgent need for stricter environmental regulations to mitigate the impact of persistent pollutants on future generations.

Funding Source: This study was supported by the Swedish Research Council, the Birgitta and Carl-Axel Rydbeck Foundation and the Department of Molecular and Developmental Medicine from the University of Siena.

T1224

DORSAL ROOT GANGLIA DIFFERENTIATION FROM AXIAL STEM CELLS THROUGH TIMED NOTCH MANIPULATION

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The dorsal root ganglia (DRG) relays somatosensory information and play a critical role in disorders, such as neuropathic pain, however ex vivo models for the DRG have not yet been established. Peripheral sensory neurons, Schwann cell, and satellite glia (SG) that make up the DRGs, originate from neuromesodermal progenitors (NMPs) giving rise progressively to neural crest progenitors in the formation of 31 pairs of DRGs in the human embryo axis. We derived human axial stem cells (AxSCs) that emulate NMPs from iPS cells and leveraged them to develop novel differentiation protocols for the DRG cells. We found that manipulating Notch signalling governs the bifurcation of sensory- and autonomic-peripheral neurons versus Schwann cells and SG. Moreover, co-culturing these populations improves glial cell maturation markers (e.g. PLP1, MPZ, FABP7), captures nerve-associated glial cell migration, and increases myelination. AxSCs enable faster, more homogeneous differentiation than PSCs, offering a scalable system to model trilineage interactions in the DRG.

T1226

DOT1L REPRESSES HUMAN PSC DIFFERENTIATION INTO DEFINITIVE ENDODERM

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Human induced pluripotent stem cells (hiPSCs) possess the capacity to differentiate into a variety of cell types and hold great promise for clinical applications and disease modeling. However, the efficiency of differentiation into specific lineages, particularly endodermal cells, varies among cell lines, a phenomenon referred to as differentiation propensity. This variability poses a significant challenge for the effective utilization, research, and development of hiPSCs and their derivatives, necessitating the optimization of differentiation methods for each cell line and the selection of cell lines with consistent differentiation capabilities toward desired cell types. Despite these challenges, factors that can overcome the differentiation propensity remain to be comprehensively explored. Using a genome-wide CRISPR-KO screen for definitive endoderm (DE) differentiation and inhibitors targeting hit genes, we discovered that a combinatorial treatment with DOT1L and JNK inhibitors substantially enhanced the efficiency of DE cell differentiation. This improvement was also confirmed in several hiPSC and hESC lines. In addition, DE cells induced by these inhibitors demonstrated the capacity of differentiating into DE derivates, including pancreatic and lung lineages. Mechanistic analysis of DOT1L in DE differentiation revealed that DOT1L suppressed DE differentiation by maintaining the expression of BCOR, which was the 2nd ranked hit gene in our

CRISPR screen. Disruption of BCOR similarly enhanced DE differentiation, achieving levels comparable to those observed in DOT1L-KO hiPSCs. Additionally, we found that the expression level of BCOR negatively correlated with DE differentiation efficiency and that variability in BCOR expression was associated with genetic mutations. In summary, we developed a novel DE differentiation method applicable to a wide range of cell lines by identifying differentiation-blocking genes with a genome-wide CRISPR screen. This approach has the potential to be applied to each differentiation stage across various lineages, leading to the development of more robust differentiation protocols.

T1228

DUAL NUCLEASE SINGLE-CELL LINEAGE TRACING BY CAS9 AND CAS12A

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Single-cell lineage tracing based on CRISPR/Cas9 gene editing enables the simultaneous linkage of cell states and lineage history at a high resolution. Despite its immense potential in resolving the cell fate determination and genealogy within an organism, existing implementations of this technology suffer from limitations in recording capabilities and considerable barcode dropout. Here, we introduce DuTracer, a versatile tool, which utilizes two orthogonal gene editing systems to record cell lineage history at single-cell resolution in an inducible manner. DuTracer shows the ability to enhance lineage recording with minimized target dropouts and potentially deeper tree depths. Applying DuTracer in mouse embryoid bodies and neuromesodermal organoids illustrates the lineage relationship of different cell types and proposes potential lineage-biased molecular drivers, showcased by identifying transcription factor Foxb1 as a modulator in the cell fate determination of neuromesodermal progenitors. Collectively, DuTracer facilitates the precise and regulatory interrogation of cellular lineages of complex biological processes.

T1230

DUAL STATES OF BMI1-EXPRESSING INTESTINAL STEM CELLS DRIVE EPITHELIAL DEVELOPMENT MEDIATED BY NON-CANONICAL WNT SIGNALING

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Intestinal epithelial development and homeostasis critically relies upon balanced stem cell proliferation, involving slow-cycling/label-retaining, and active-cycling/canonical Wnt-dependent intestinal stem cell (ISC) subtypes. However, how different subpopulations may be regulated to maintain homeostasis is not completely understood. Further, ISC regulation during development

remains poorly understood but has important implications for establishing key mechanisms governing tissue maintenance. The developing intestine provides a less complex paradigm to investigate ISC subpopulations, their contribution to the tissue and pathways that regulate their proliferative status. We definitively identified Bmi1+ cells as functional stem cells in early murine intestinal development and determine that they are established prior to the existence of Lgr5-expressing ISCs. Lineage-tracing and single cell RNA-sequencing analyses reveal that Bmi1+ ISC can trace to Lgr5+ ISCs and other differentiated lineages in the developing epithelia. Initially highly proliferative Bmi1+ ISCs transition to slow-cycling states as Lgr5+ ISCs emerge. Omics analyses followed by in vitro functional validation established the non-canonical Wnt signaling pathway as a key regulator of the proliferative Bmi1+ cell state. These novel findings highlight the dynamic interplay between stem cell populations and the opposing Wnt pathways that govern intestinal stem cell proliferation during development. Our findings have implications not only for tissue development, but also for tissue homeostasis, regeneration and tumorigenesis, where these fundamental developmental mechanisms are re-expressed to underlie epithelial proliferation.

Funding Source: NIH/NIDDK, U01 DK085525.

T1232

EARLY DEVELOPMENTAL SHIFTS AND DIVERSE CELL FATE ALTERATIONS DRIVEN BY HNF1A

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HNF1A is a key transcription factor for pancreatic islet development regulating cell identity and function. HNF1A mutations lead to drastic changes in islet architecture, beta-cell dysfunction and MODY diabetes (maturity onset diabetes of the young). While intensively studied in pancreatic progenitors, HNF1A role in earlier human development remains surprisingly underexplored. To investigate this, we differentiated hiPSC bearing a “hot-spot” HNF1A mutation toward definitive endoderm and posterior foregut. At the posterior foregut stage, HNF1A-mutant cells exhibited a significant pancreas-to-intestine cell fate switch. Interestingly, this shift was associated with the deregulation of the Hedgehog signaling identified at protein and transcriptional level. Of note, these observations finally provide a causal explanation for the increased intestine length observed by us and others in mouse models with HNF1A mutation. The in-depth investigation of HNF1A impact during early development (endoderm, primitive gut tube, posterior foregut) also indicated that, at all stages, the HNF1A-mutant cells consistently displayed a shift toward mesodermal cell fate, marked by upregulation of key mesodermal markers. This mesodermal bias aligns with a predisposition for kidney lineage development, consistent with the idiopathic renal phenotypes observed in HNF1A-MODY patients, such as renal cysts and reduced renal function, further demonstrating a wider than expected developmental impact of the HNF1A mutation. These findings provide novel insights into the key role of HNF1A in regulating early lineage decisions and its interaction with the Hedgehog pathway. This work essentially advances our understanding of HNF1A mutations and their broader developmental effects, suggesting a more complex relationship between developmental signaling and disease beyond pancreatic islet dysfunction. Moreover, our observation reconciles the presence of multiple organ phenotypes observed in vivo in HNF1A mutant mice, which were generally underexplored and considered of idiopathic origin.

T1234

EFFECTS OF CO1, AN ACTIVE COMPOUND FROM CHINESE MEDICINE, ON MODULATING NEURAL STEM CELLS FATE DECISION VIA MITOCHONDRIAL METABOLISM

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Numerous researches demonstrate that mitochondria are central regulators of fate decision of neural stem cells (NSCs). NSC are regarded as a promising therapeutic approach to protecting and restoring damaged neurons in neurological disease. However, new research suggests that NSC reprograming is required to make this strategy effective. Here, we found one potential drug CO1, an active compound from Chinese Medicine, could modulate stem cell fate decision through mitochondrial metabolism. We tested the impact of CO1 on embryonic stem cell (ESC) proliferation and mitochondrial structural and function parameters, such as membrane potential and ATP synthesis, as well as oxidative stress indicators. The results indicated that CO1 dose-dependently increases ATP concentration, mitochondrial membrane potential, and ROS synthesis to inhibit the ESC proliferation and promotes it reprogramming into NSC. Therefore, we concluded that CO1 could efficiency develop ESC into NSC via mitochondrial activity promotion.

T1236

EFFICIENT CONSTRUCTION AND OPTIMIZATION OF CYNOMOLGUS MONKEY BLASTOIDS WITH ENHANCED PHENOTYPIC AND MOLECULAR CHARACTERISTICS

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Cynomolgus monkey blastoids have shown similar morphology and lineage composition to primate blastocysts in previous studies. However, their application was limited by low efficiency and undefined cell clusters. Here, we present a novel and efficient protocol for generating cyBlastoids using 4CL naïve cyPSCs. These cyBlastoids exhibit morphology and cell lineage allocation similar to cynomolgus monkey blastocysts. Transcriptomic analysis confirms that the three corresponding cell lineages closely resemble those of natural blastocysts and other cynomolgus monkey blastoids. Furthermore, prolonged in vitro culture of cyBlastoids reveals typical morphology and cell lineage characteristics of post-implantation embryos. This stable and efficient protocol offers a robust model for primate embryo research.

T1238

ELUCIDATING THE ROLE OF GLYCOSYLATION IN EPIDERMAL STEM CELL AGING

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The disruption of communication between epidermal stem cells and their surrounding microenvironment, including adjacent cells and their extracellular matrix (ECM), contributes to the cellular and molecular impairments associated with skin aging. Glycans modify plasma membrane proteins post-translationally and have a role in tissue homeostasis and diseases by regulating cell-cell interactions, ligand-receptor binding, and ECM function. Also, glycans play a crucial role in regulating stem cells by modulating signaling pathways that control their self-renewal and differentiation. Our previous study demonstrated age-related alterations in glycosylation patterns in mouse skin, characterized by an upregulation of sialic acid and a downregulation of mannose in aged epidermal stem cells. However, the functional importance of these alterations of glycans in vivo is still unknown. Therefore, we aim to investigate the function of glycosylation in skin aging at the stem cell level. We show that the elevation of a-2,6 sialylation by overexpressing glycogenes (St6gal1 and Man1a) in vivo exhibits phenotypes associated with skin aging, including hair loss, epidermal thinning, and decreased proliferative capacity of epidermal stem cells. Mass spectrometry, using membrane proteins pulled down by lectin probes, identified several potential core protein candidates modified by a-2,6 sialic acid. These findings indicate that changes in glycosylation regulate epidermal stem cell aging by modifying the membrane proteins. The functional relationship between glycogenes and core proteins in regulating epidermal stem cell function is being further investigated, which may provide a molecular basis and a biomarker for skin aging.

Funding Source: Japan Agency for Medical Research and Development (AMED)/AMED-PRIME (22gm6710003) Grant-in-Aid for Challenging Research (Exploratory) (20K21431).

T1240

ENDOGENOUS PROTEASE ACTIVITY REGULATES METABOLIC AND CELL FATE PATTERNS

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Pattern formation is one of the most important processes during embryogenesis. It is commonly accepted that pattern formation is mainly driven by morphogen gradient. However, heterogeneous pattern still exists in 2D monolayer culture system that does not have obvious growth factor gradient. In this project, we use 2D monolayer human pluripotent stem cell (hESC) culture to study pattern formation in hPSC colonies. We observe that cell fate pattern is determined by the location of individual cells in a colony, and the spatial distribution can be associated with the pattern of metabolic activities in a colony. We show that endogenous protease influences metabolic pattern through the activation of protease-activated receptor (PAR). PAR pathway can further interact with Calcium and metabolism related signaling pathway that is important for fate pattern. Although under 2D monolayer culture condition, metabolic changes caused by PAR itself are not enough to affect or change cell fate, it can still activate a series of signaling and regulate gene expression.

This study highlights novel autocrine feedback important for pattern formation and cell fate determination.

T1242

ENGINEERED PIONEER FACTOR PROTEINS FOR IMPROVED MULTI-SPECIES INDUCED PLURIPOTENT STEM CELL DERIVATION

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Pioneer pluripotency transcription factors OCT4 and SOX2 drive reprogramming of somatic cells into stem cells capable of differentiating into any other type of cell. Recent work has shown that engineering of SOX2 can enhance reprogramming outcomes in multiple species including humans, mice, monkeys, cows, and pigs. Here we demonstrate that more radical engineering of both SOX2 and OCT4 using machine-learning-driven protein engineering approaches can enhance reprogramming outcomes beyond the state of the art. We show that engineering both OCT4 and SOX2 enhance reprogramming of mouse, antelope, and elephant somatic cells and we characterize the improvements in both reprogramming efficiency and reprogramming to a more naïve state. This work is part of a larger effort to design species-agnostic improvements to these proteins to reprogram somatic cells from non-model organisms to a fully naïve state for species conservation and de-extinction.

Funding Source: Colossal Biosciences.

T1244

ENGINEERING A PSC-DERIVED HEPATOBILIARY-GALLBLADDER MODEL BY DICTATING LEFT-RIGHT ASYMMETRIC ORGANOGENESIS

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Hepatobiliary (HB) development requires precise left-right (LR) mesenchymal patterning around the e9.5 liver bud to spatially coordinate hepatic and biliary morphogenesis. This laterality interaction potentially drives divergent growth, with liver progenitor cells, known as hepatoblasts, migrating from the left liver bud to proliferate while the biliary system invaginates on the right to tubularize. Disruptions in LR patterning are associated with congenital malformations of the liver like biliary atresia underscoring the need to uncover the mechanisms guiding LR-directed HB development. Here, we investigated the molecular and cellular roles of HB-associated

mesenchymal populations—Pitx2hi liver fibroblasts and Pitx2lo mesothelial cells— identified through single-cell RNA sequencing and shown to orient in a LR pattern around mouse liver buds by in situ hybridization. Gene set enrichment analysis between these populations revealed >9-fold enrichment of fluid-sensing, cell migration genes, including Postn, in liver fibroblasts that secrete around the left liver bud. POSTN inhibition by neutralizing antibodies disrupted left liver bud development suggesting its critical role in liver morphogenesis. To model mesenchyme-directed left-patterning in vitro, we developed a microfluidic perfusion device that delivers controlled fluid shear stress on HB organoids containing naïve mesenchyme without LR biased signature. Shear stress increased Msx1 expression, a liver fibroblast-specific marker, by 1.8-fold compared to static culture reinforcing leftward mesenchymal patterning in HB organoids. Furthermore, shear stress was sufficient to trigger hepatoblast migration mediated by transcription factor Prox1 activity. Long-term cultures of shear stress-treated organoids showed ~2-fold production of hepatoblasts that adopted a delaminating morphology similar to e10.5 liver buds as compared to static cultures. These findings reveal a unique role for fluid shear stress in modulating mesenchymal LR patterning to prioritize liver morphogenesis over biliary development. Additionally, this work advances in vitro modeling of early liver development by incorporating axial patterning, offering a powerful platform to explore laterality coordination for HB development in health and disease.

Funding Source: This work was supported by the NIH Director's New Innovator Award (DP2 DK128799-01).

T1246

ENHANCING PBMC REPROGRAMMING EFFICIENCY VIA P53 MODULATION AND INSIGHTS FROM DUAL RNA-SEQ ANALYSIS

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Reprogramming peripheral blood mononuclear cells (PBMCs) into induced pluripotent stem cells (iPSCs) is essential for advancing regenerative medicine and disease modeling. However, challenges remain in enhancing efficiency and understanding the molecular mechanisms involved. Synthetic mRNA offers a non-integrative and effective method for reprogramming, yet its full potential has yet to be realized. To improve reprogramming efficiency, we focused on PBMCs and demonstrated that modulation of the p53 pathway significantly enhances reprogramming outcomes by improving cellular survival and reducing stress-induced barriers. This identifies a novel target for optimizing PBMC reprogramming protocols. To further investigate the mechanisms of cellular reprogramming, we utilized human dermal fibroblasts (HDFs) as a model system and implemented an integrative RNA sequencing (RNA-seq) strategy that combines short-read and long-read technologies. Preliminary analyses suggest distinct transcriptional phases during reprogramming,

marked by the activation of pluripotency-associated genes, suppression of somatic gene networks, and potential identification of new isoforms and regulatory elements. These insights, while still ongoing, provide a foundational understanding of transcriptional dynamics and support hypotheses for optimizing reprogramming conditions. Together, these findings not only enhance our mechanistic understanding of reprogramming but also establish a robust platform for improving iPSC generation from PBMCs, paving the way for expanded therapeutic applications.

Funding Source: This study was supported by Core Center for iPS Cell Research, AMED (Japan), the iPS Cell Research Fund (Japan), and World Premier International Research Center Initiative (WPI), MEXT (Japan).

T1248

EVALUATING CORTICAL ORGANOIDS AS A MODEL FOR A-TO-I RNA EDITING DYNAMICS AT SINGLE-CELL RESOLUTION

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The development of the cerebral cortex is one of the most intricate processes in neurobiology. Disruptions to this complex and highly regulated process are central to neurodevelopmental disorders (NDDs), which collectively impact an estimated 317 million individuals globally. Recent work suggests that post-transcriptional modifications, such as A-to-I RNA editing mediated by ADAR enzymes, may contribute to the diverse etiologies of NDDs. Cortical organoids recapitulate key human-specific aspects of corticogenesis and provide a powerful platform to study these modifications in a controlled environment. However, the suitability of organoids as a model for A-to-I RNA editing remains unproven, and current computational approaches cannot fully capture the intricate editing dynamics across diverse cell types in organoid cultures. To this end, we used our recently published algorithm, MARINE (Multi-core Algorithm for Rapid Identification of Nucleotide Edits), to map the A-to-I editome of human fetal brain and cortical organoids at single-cell resolution over an extended developmental time course. This approach enabled a comprehensive analysis of RNA editing dynamics across various stages of corticogenesis. Our findings revealed remarkable concordance between fetal brain and organoid datasets, demonstrating distinct temporal and cell type-specific patterns of A-to-I editing. We also observed significant variation in editing frequency and site specificity across different cell types and developmental timepoints. Additionally, our analysis identified unique expression profiles of co-factors associated with high and low editing activity, suggesting that certain cellular states may predispose cells to enhanced RNA editing. Given the established dysregulation of A-to-I editing in NDDs such as Fragile X syndrome, we leveraged MARINE to compare the A-to-I editome between healthy control cortical organoids and isogenic NDD variant organoids. Despite overall similarities in editing patterns, we noted significant alterations in ADAR expression and editing site variance in FMR1 KO organoids. This study represents the largest investigation of A-to-I RNA editing dynamics in the developing brain and in human-derived model systems of NDD-linked diseases within an isogenic background.

Funding Source: NIH/NIMH Human Isogenic Organoid Models of Genetic Forms of Autism to Identify Convergent and Divergent Patho-mechanisms in Autism (MPI) R01 MH131907.

T1250

FGF8 PROMOTES PORCINE OOCYTE MATURATION AND EMBRYONIC DEVELOPMENT THROUGH FGFR4 ACTIVATION

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Pigs are valuable biomedical models due to their physiological similarities to humans, making them crucial for studying complex diseases and advancing medical research. However, the low efficiency of porcine transgenic model production highlights the need to improve oocyte quality during in vitro maturation (IVM). Fibroblast growth factor 8 (FGF8), a key cytokine in embryogenesis, is known to influence murine oocyte maturation, but its role in porcine oocytes remains unclear. This study investigated the effects of FGF8 supplementation during IVM on nuclear maturation, intracellular glutathione (GSH) levels, reactive oxygen species (ROS) levels, cumulus expansion, and parthenogenetic activation (PA). Immunohistochemistry localized FGF8 and its receptor FGFR4 in porcine follicles, showing FGF8 in cumulus cells and oocytes across all follicular stages, while FGFR4 was detected only in oocytes. Porcine oocytes were matured in vitro with FGF8 concentrations of 0, 1, 10, and 100 ng/mL. After 42 hours, the 100 ng/mL FGF8 group showed significantly ($p < 0.05$) higher nuclear maturation rates, elevated GSH levels indicating improved cytoplasmic maturation, and reduced ROS levels compared to controls. This group also exhibited significantly ($p < 0.05$) enhanced cumulus expansion and higher blastocyst rates in PA. These findings suggest that FGF8 influences both oocytes and cumulus cells, enhancing their maturation and promoting embryonic development. Additionally, co-culture of FGF8 with FGFR4 inhibitor BLU9931 eliminated the beneficial effects observed with FGF8 treatment, confirming that these effects are mediated through the FGFR4 pathway. In further research, we will focus on elucidating the mechanisms by which FGF8 enhances porcine oocyte maturation and embryonic development through comprehensive analysis of signaling pathways and mRNA expression profiles. By providing novel insights into oocyte-cumulus cell interactions, these findings have the potential to optimize IVM protocols, thereby improving the efficiency of porcine IVP and reinforcing their utility as biomedical models.

Funding Source: This work was supported by grants from the “NRF (2021R1C1C2013954)”, “Technology Innovation Program funded by the MOTIE, Korea (20023068)”, and “IPET funded by MAFRA (RS-2024-00398561, RS-2024-00399475)", Republic of Korea.

T1252

FROM RESEARCH TO THERAPEUTICS: THE RELIABILITY OF BFGF IN SUPPORTING IPSCs GROWTH, PLURIPOTENCY, AND DIFFERENTIATION

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Stem cells can self-renew and potentially differentiate into one or more mature cell lineages. The possibilities of stem cells, from basic research to clinical applications, require optimal culture conditions and the use of reagents, such as recombinant proteins. It is essential that these proteins act in a reliable and reproducible manner from the research phase all the way through to clinical applications. Basic fibroblast growth factor (bFGF or FGF-basic), also known as Fibroblast growth factor 2 (FGF2), plays a crucial role in stem cell biology, promoting survival, self-renewal, and pluripotency. Ensuring high-quality and consistency of bFGF is necessary to generate reliable results when working with a stem cell culture workflow; however, the uncertainty about the functional equivalence of different bFGF grades challenges scientists who transition from a research-grade to the clinical application grade. This study addresses this gap by assessing the effect of three Gibco™ PeproTech™ bFGF grades— RUO (Research Use Only; Recombinant Human FGF-basic), AOF (Animal free; Animal-Free Recombinant Human FGF-basic), and GMP (Good Manufacturing Practice; PeproGMP® Recombinant Human FGF-basic) on the growth, pluripotency, and differentiation potential of iPSCs reprogrammed from human dermal fibroblasts. iPSCs were first expanded over multiple passages in basal medium supplemented with one of three bFGF grades, during which growth rate and morphology were observed. Following expansion, iPSCs genetic stability, expression of pluripotency markers, and ability to differentiate into the three primary germ layers was measured. Our results showed that all grades of bFGF supported consistent iPSC growth, morphology, genetic stability, pluripotency, and differentiation potential, thus demonstrating that all three grades are functionally equivalent. Overall, the consistent performance of bFGF grades ensures the smooth transition from discovery to regenerative therapies, without the need for extensive revalidation. This improves operational efficiencies and supports cost-effective workflows, thereby paving the way for advancement of stem cell-based research from basic science to clinical applications.

T1254

GENEA AS A NOVEL TARGET FOR UNDERSTANDING ANTI-AGING MECHANISMS

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Aging is a complex biological process whose underlying mechanisms remain inadequately understood. With the global population of older adults on the rise, it is imperative to deepen our understanding of the aging process. GeneA is a multifunctional protein that serves as a DNA and RNA binding factor, regulating gene expression at the transcriptional, splicing, and translational levels. Previous studies have shown a significant decline in GeneA expression across six mouse tissues with aging; conversely, caloric restriction—a known intervention that mitigates aging—can restore GeneA levels. However, the precise role of GeneA in aging, particularly in humans, remains elusive. In this study, we employed a well-established trophoblast aging model, utilizing the differentiation of trophoblast stem cells (TSCs) into syncytiotrophoblasts (STBs), to elucidate the emerging role of GeneA as a novel anti-aging marker.

T1256

GENERATION OF FUNCTIONAL PERIPHERAL/SENSORY NEURONS FROM CANINE IPS CELLS

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Canine neurological diseases are broadly classified into those originating primarily from the central nervous system and those from the peripheral nervous system. Peripheral nerve diseases, affecting both sensory and motor nerves, are categorized based on their pathogenesis such as traumatic, infectious, inflammatory, neoplastic, or degenerative origins, and result in a variety of clinical signs. In particular, sensory nerve disorders cause abnormal sensations and pain, which are more challenging to diagnose in animals like dogs than in humans due to the difficulty in communicating their subjective symptoms. In terms of treatment, while inflammatory diseases can often be treated aggressively with immunosuppressants, peripheral nerve diseases caused by other factors are primarily managed with physical therapy, as there are few effective medications available other than analgesics. To address these challenges, basic research aimed at developing therapeutic drugs and methods for early diagnosis is crucial. However, the lack of a stable in vitro experimental system using canine peripheral nerves has hindered such research and development. We have attempted to obtain functional canine peripheral nerves derived from iPS cells by combining our proprietary animal iPS cell-technology with an efficient differentiation method for generating peripheral neurons from human iPS cells via neural crest cells. In this presentation, we will share the latest findings, including: 1) Induction of canine iPS cells into peripheral/sensory neurons via neural crest cells, 2) Gene expression patterns during differentiation and maturation, 3) Functional analysis of these peripheral/sensory neurons. Additionally, we propose the potential application of our canine iPSC-derived in vitro peripheral/sensory nerves evaluation system.

T1258

GENERATION OF REGION-SPECIFIC HUMAN IPSC-DERIVED MOTOR NEURONS (IMNS) TO MODEL ALS WITH DIFFERENT SITES OF ONSET

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Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease characterized by the degeneration of motor neurons (MNs), leading to paralysis and death. A defining feature of ALS is the selective vulnerability of MNs in different regions of the nervous system. Whether cervical, lumbar, or hindbrain MNs degenerate first determines the site of disease onset. Contemporary iPSC-derived models (iMNs) predominantly generate hindbrain and cervical MNs. Thus, they fail to capture the full spectrum of regional identities and potentially distinct pathogenic events, particularly those of lumbar MNs. Additionally, the heterogeneous nature of these models,

which often produce mixed populations of MNs, can obscure critical region-specific characteristics and mechanisms of vulnerability, limiting their ability to fully model ALS pathology. To address these limitations, we optimized differentiation protocols by incorporating small molecules to direct the development of hindbrain/cervical and lumbar MN identities within 32 days. These protocols are reproducible across multiple healthy control and ALS cell lines, generating iMNs that closely resemble human spinal motor neurons with distinct segmental identities. Compared to traditional iMN induction methods, these optimized protocols produce iMNs with higher homogeneity and more well-defined hindbrain/cervical or lumbar identities. Notably, the protocols preserve limb-innervating markers, which are critical for accurately modeling the vulnerabilities observed in limb-onset ALS patients. This enhanced consistency and regional specificity provide a robust platform for studying region-specific motor neuron properties and offer new opportunities to explore the diverse roles of spinal motor neurons in health and disease with greater precision.

Funding Source: California Institute for Regenerative Medicine (CIRM): EDUC4-12751.

T1260

GENOME-WIDE GAIN-OF-FUNCTION SCREEN IDENTIFIES NUP133 AS A KEY REGULATOR of the PLURIPOTENT-TO-TOTIPOTENT TRANSITION IN MOUSE EMBRYONIC STEM CELLS

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In mice, totipotency is exclusively observed in zygotes and two-cell (2C) embryos, enabling their differentiation into both embryonic and extra-embryonic lineages. Elucidating the regulatory mechanisms underlying totipotency is essential for the stable in vitro culture of totipotent cells and for advancing our understanding of cellular plasticity during early embryonic development. In this study, we employed piggyBac (PB) transposon-mediated gene activation vectors in conjunction with mouse embryonic stem cells (mESCs) harboring Zscan4::GFP and 2C::tdTomato reporters to conduct a genome-wide gain-of-function forward genetic screen. This screen aimed to identify key factors driving the transition from pluripotency to totipotency. Our findings reveal that the nucleoporin NUP133 plays a pivotal role in facilitating this state transition. Further analysis demonstrated that the totipotent state is associated with a more mature nuclear pore complex (NPC) compared to the pluripotent state, a phenotype similarly induced by Nup133 overexpression in mESCs. These results highlight the critical role of NPCs in the regulation of totipotency and provide new insights into the molecular mechanisms governing cellular plasticity during early development.

Funding Source: The CAMS Innovation Fund for Medical Sciences (2021-I2M-1-024 and 2021-I2M-1-019); National Natural Science Foundation of China (32370898); Beijing Natural Science Foundation (5232024).

T1262

HEMOSTATIC EFFICACY OF PLURIDFDFPOTENT STEM CELL-DERIVED MEGAKARYOCYTES IN A THROMBOCYTOPENIA MOUSE MODEL

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Thrombocytopenia, a hematological disorder marked by a significant reduction in circulating platelets, compromises hemostasis and increases the risk of uncontrolled bleeding. Current therapies, such as platelet transfusions, are limited by short shelf life, donor dependency, and immunological complications. Megakaryocytes (MKs), the precursor cells for platelet production, offer a promising alternative. Here, we evaluated the therapeutic potential of human pluripotent stem cell-derived megakaryocytes (PS-MKs) in restoring platelet function. A thrombocytopenic mouse model was established using NSG immunodeficient mice with busulfan-induced myelosuppression. PS-MKs were differentiated in vitro over five weeks and administered intravenously to evaluate their efficacy in vivo. Transplanted PS-MKs significantly reduced bleeding time, demonstrating effective restoration of hemostasis. Engraftment and platelet release were assessed over 72 hours. Nuclear DNA (GAPDH) from PS-MKs was detected exclusively in lung tissue up to 24 hours post-injection, indicating successful engraftment, with no nuclear DNA detected in the blood. Mitochondrial DNA (mtDNA), however, was detected in both lungs and blood, suggesting sustained platelet release. PKH26-labeled PS-MKs confirmed active lung migration post-injection, supporting their role in platelet production and hemostatic recovery. These results highlight the potential of PS-MKs as a renewable, scalable, and effective alternative to conventional platelet transfusions, offering a novel therapeutic approach for managing thrombocytopenia.

Funding Source: This research was supported by a grant of Korean Cell-Based Artificial Blood Project funded by the Korean government (grant number : RS-2023-KH140925).

T1264

HIGH-RESOLUTION HUMAN FETAL RETINA AND RETINAL ORGANOIDS SINGLE-CELL ATLAS TO GUIDE RETINAL CELL DIFFERENTIATION AND REPLACEMENT EFFORTS

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The complexity of the mammalian retina arises from the unique combination of extrinsic and intrinsic factors in its development. Several single-cell RNA seq datasets have been generated to study retina and retinal organoids. We propose to investigate human retinal development and the questions of cell fate specification, cell class and cell type heterogeneity, and regulation of cell trajectory by intrinsic and extrinsic factors. We start with the scRNA-seq data for human developing healthy retina and retinal organoids. We focus on the methods of data transformation (Harmony,

scVI, ForceAtlas2) and cell-cell interactions analysis (CellChat, Scriabin), cell fate trajectory reconstruction and time transformation (scvelo, scFates, CytoTRACE2). We establish a human fetal retina and human retinal organoids atlas by integrating scRNA-seq datasets into a high-resolution map of retinal development. We use pseudotime with the cell fate trajectory reconstruction, potency and trajectory density methods to deconvolute the transcriptional signal. We demonstrate the application of pseudotime approaches in reconstruction of retinal ganglion cells (RGC) development to unravel the heterogeneity behind cell maturation. We discover hundreds of de novo development-oriented targets from both cell-intrinsic and cell-extrinsic prospectives of retina and RGC maturation. We show how maturation states contribute to forming the environment sufficient for retina development. Our findings confirm developing retina to be a closed and self-supporting system. We demonstrate the comparison of 27 organoids differentiation protocol with such metrics as cell commitment, similarity to native retina, development timepoint correlation, and transplantation probability prediction. We integrate and publish two new tools for the data analysis: a human fetal retina and human retinal organoids atlas. We show how switching from canonical timepoint to pseudotime in studying the cell fate changes the understanding of fate driving genes. Both the atlases can be used as a community tool for analyzing, reference mapping and annotation of the sequencing data. The resulting reference map, publicly available at CellxGene portal, serves as a template for cell differentiation, reprogramming, and transplantation.

Funding Source: NIH/NEI–5U24EY029893-05 (P.B.), NIH/NEI–P30EY003790 (Core Facility Grant), Bright Focus Foundation–G2020231 (P.B.), Gilbert Family Foundation–GFF00 (P.B.), Department of Defense - VRP FTTSA (P.B).

T1266

HUMAN EMBRYONIC STEM CELL-DERIVED NEURAL CREST STEM CELLS DIFFERENTIATED INTO MESENCHYMAL CELLS WITH ODONTOGENIC POTENTIAL

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Odontogenesis begins with the invaginated ectoderm-derived dental epithelium, which is surrounded by condensed neural crest-derived mesenchyme. This process involves sequential and

reciprocal interactions, establishing an excellent system for understanding organogenetic mechanisms. While rodents, particularly mouse models, have provided insights into tooth development and tooth germ organoids, they do not fully represent human odontogenesis. Therefore, establishing human tooth germ organoids is essential for more accurately studying human odontogenesis and tooth regeneration. In early tooth development, odontogenic potential transitions from epithelial cells to mesenchymal cells. Given that dental mesenchyme originates from neural crest mesenchyme, our current experiment utilized human embryonic stem (hES) cells that first differentiated into neural crest stem cells（NCSC）, followed by differentiation into mesenchymal cells. In prior experiments, we identified a medium capable of maintaining the odontogenic potential of isolated mouse primary tooth germ mesenchymal cells in vitro, which we then applied during NCSC differentiation into mesenchyme. Our single-cell RNA sequencing experiments revealed that genes such as Barx1 were specifically expressed in mesenchymal cells after E12.5 days. These genes were also detected in hES-derived mesenchymal cells, indicating that these mesenchymal cells may possess odontogenic potential. By recombining ES-derived NCSC and mesenchymal cells with oral epithelial cells, we observed the formation of tooth germ-like structures following in vitro culture. Our findings provide valuable in vitro tools for investigating human tooth development and tooth-related diseases, enhancing research efficiency, and reducing the number of animals needed for in vivo studies.

T1268

IDENTIFICATION OF A MYF5-INDEPENDENT PATHWAY FOR HUMAN BROWN ADIPOCYTE DEVELOPMENT

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Brown adipocytes (BAs) and skeletal muscle in the trunk arise from a common PAX3+ progenitor cell derived from the paraxial mesoderm. PAX3+ cells transition through a MYF5+ state before developing into thermogenic brown adipocytes (BAs) but alternate pathways of BA development have not been described. Using single-cell transcriptomic profiles of human pluripotent stem cell (hPSC)-derived trunk organoids we identify a second, MYF5-independent, pathway for BA development that originates from the same PAX3+ progenitor previously shown to be important for BA development. The alternate BA pathway involves the transition from a PAX3+ state to one marked by EBF2, TGFBI and MEF2C. Guided by this analysis, two distinct pathways for BA development were reproduced by 2-dimensional, lineage-specific differentiation of hPSC-derived PAX3+ progenitors. Time-course, single cell RNA sequencing and velocity analysis revealed that although both EBF2+ and MYF5+ BA progenitors differentiate efficiently into BAs, they exhibit different capacities for producing vascular endothelial and skeletal muscle derivatives, respectively. Furthermore, single-cell transcriptomic comparison with existing dataset from Carnegie Stage 10-16 human embryos confirms these findings. This work highlights the utility of using organoids to understand human BA development.

Funding Source: The Hong Kong Jockey Club Charities Trust.

T1270

IDENTIFYING GENES THAT DRIVE DEMETHYLATION IN HUMAN PRIMORDIAL GERM CELL-LIKE CELLS

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Germline cells are responsible for passing genetic information from parent to offspring in sexually reproducing organisms. During mammalian embryonic development, molecular signals orchestrate the development of primordial germ cells (PGCs), the precursors to germline cells. As these PGCs mature, they undergo dramatic epigenetic remodeling, including genome-wide demethylation, that is central to their normal development towards gametes. Efforts to generate PGC-like cells (PGCLCs) in vitro from mouse pluripotent stem cells have been successful in replicating in vivo epigenetic remodeling, while human PGCLCs show more limited reprogramming, their dynamics are slower than in vivo, and the molecular signals necessary to induce the reprogramming are still unclear. To identify factors that trigger epigenetic remodeling in human PGCLC development, we conducted single-cell methylome and transcriptome sequencing on PGCLCs in extended culture for up to 21 days. From these results, we identified several candidate genes that were differentially expressed in PGCLCs with decreased methylation levels. Additional work is focused on validating candidate genes, DND1 and SOX15, as well as identifying additional candidate molecular epigenetic triggers. Gaining insight on mechanisms that govern epigenetic remodeling in PGCLCs will enable better in vitro models which are a valuable resource to study some forms of infertility.

Funding Source: NIH R01HD099517 NIH R01HG011013 NSF 2339849 NSF 2139319 NSF 2125644.

T1272

IMPACT OF PLURIPOTENT STEM CELL SOURCE ON NATURAL KILLER CELL DIFFERENTIATION AND ITS IMPLICATIONS FOR IMMUNOTHERAPEUTIC STRATEGIES

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Natural killer (NK) cells are pivotal in immune surveillance and hold significant potential for cancer immunotherapy. Although pluripotent stem cells (PSCs), including induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs), are emerging as promising sources for NK cell generation, the influence of PSC origin on NK cell differentiation and function remains poorly understood. Given the variability in differentiation efficiency and functional properties of PSC-derived NK cells, this study aimed to compare NK cell differentiation from various PSC sources and evaluate their cytotoxic potential for immunotherapeutic applications. We derived NK cells from

iPSCs generated from CD34+, CD56+, and CD56- cells isolated from human umbilical cord blood, as well as from ESCs. Our results demonstrated that NK cells from CD34+ iPSCs (CD34+ iPSC-NK cells) exhibited superior expansion and cytotoxicity compared to those derived from CD56+ or CD56- iPSCs. ESC-derived NK cells (ESC-NK cells) displayed higher cytotoxic activity than CD34+ iPSC-derived NK cells, despite sharing similar NK receptor profiles. Further, we enhanced ESC-NK cells by introducing a chimeric antigen receptor (CAR) targeting HER2, a tumor-associated antigen. CAR ESC-NK cells significantly outperformed wild-type ESC-NK cells in cytotoxicity against HER2-positive cancer cells. Our findings highlight that ESCs represent a robust source for generating functional NK cells with enhanced cytotoxicity, especially when engineered with CAR constructs. However, due to variability observed between different PSC origins, our study emphasizes the importance of screening PSC lines to optimize NK cell differentiation and function prior to clinical application in immunotherapy.

Funding Source: This research was supported by a grant of Korean Cell-Based Artificial Blood Project funded by the Korean government (grant number : RS-2023-KH140925).

T1274

IN VITRO HUMAN PLURIPOTENCY AT THE EXPANDED STATE HARBOURS BI-LINEAGE DIFFERENTIATION POTENTIAL INTO EXTRA-EMBRYONIC LINEAGES AND MOLECULAR CHARACTERISTICS DISTINCT FROM PRIMED PLURIPOTENCY

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The elucidation of the molecular machinery orchestrating human embryogenesis has been limited by both technical and ethical challenges. The establishment of human pluripotent stem cells (hPSCs) has offered valuable in vitro models for the scalable and perturbable dissection of the diverse genetic mechanisms underlying human embryo development. We have previously developed human expanded potential stem cells (hEPSCs), which exhibit molecular signatures resembling human pre-blastocyst embryos, and have the ability to differentiate into both embryonic as well as extra-embryonic cell types. In this study, we first validated the expanded developmental potency of hEPSCs for differentiation into the AME-like and trophoblastic lineages using single-cell transcriptomics. From this platform, we further identified the transposable element (TE) HERVE-int as a novel marker of AME cells. Furthermore, a systematic analysis of the molecular characteristics of hEPSCs was performed, which identified that hEPSCs were substantially different from primed hPSCs but share remarkably similarities with pre-implantation human embryos. These include but are not limited to their transcriptomic signatures like relatively higher HERVK-int/LTR5_Hs and DNA damage repair including homologous recombination gene expression; lower glycolytic activity; mitochondrial morphology as well as low H3K27me3 levels, which were also exclusively observed in morula-stage human embryos. Distinct from naïve hPSCs but similar to primed hPSCs, hEPSCs exhibited appropriate DNA methylation patterns and therefore maintained genomic imprinting. Additionally, the various molecular signatures of hEPSCs were evolutionarily conserved in porcine and bovine EPSCs. In conclusion, hEPSCs exhibit a distinctive dual extra-embryonic differentiation potential with certain molecular characteristics resembling those of the pre-blastocyst human embryos. Hence, hEPSCs hold the potential for investigating human cell fate commitment and developing stem cell-based therapies for regenerative medicine purposes.

Funding Source: This project is supported by Health@InnoHK, Innovation Technology Commission, HKSAR.

T1276

INDUSTRIALIZATION OF CULTURE MEDIA FOR IPS CELLS: EFFORTS FROM BENCH TO COMMERCIAL MANUFACTURING

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While the clinical development of cell therapy products using human stem cells is progressing, in their manufacturing, materials such as culture media and growth factors, in addition to the manufacturing process, significantly contribute to manufacturing efficiency and product quality. With the improvement of culturing methods, animal origin free culture media for iPS cells have been developed. However, the manufacturing methods often follow laboratory procedures, and in the case of culture media products, not only the composition but also the form, supply, and storage of the products remain unchanged, leading to cases where they are unsuitable for large-scale manufacturing of cell therapy products. In this presentation, we will discuss the advantages of optimizing not only the composition but also the form of culture media products, which have been optimized in the laboratory, for large-scale manufacturing of clinical cell therapy products.

T1278

INVESTIGATING NANOG GENE FUNCTION IN HUMAN EMBRYOGENESIS BY BASE EDITING

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The molecular mechanisms regulating the establishment of the human epiblast are poorly understood in human embryogenesis. To date, a small number of studies have used CRISPR/Cas9 mutagenesis to investigate gene function during pre-implantation development across species, including human embryos. These studies have recurrently identified loss of heterozygosity (LOH), genomic instability and complex chromosomal rearrangements following CRISPR/Cas9-mediated double stranded breaks (DSBs). To identify alternative methods to study gene function in human embryogenesis we adapt the adenine base editing (ABE) system and

targeted the transcription factor NANOG as a proof of principle. The ABE system allows single base A>G edits that can be used to ablate the eukaryotic exon/intron splice motif, resulting in nonsense transcript production and loss of gene expression. We find efficient loss of NANOG protein following base editing in human embryonic stem cells. We demonstrate highly efficacious editing rates (>70%) in mouse and human pre-implantation embryos. We further observe the absence of any LOH events at the on-target locus in both species of embryos. Using single cell RNA sequencing, we identified that NANOG null mutations led to the misexpression of molecular markers of the epiblast and yolk sac and suggest that the inner cell mass is stuck in an unspecified state in the mouse. We are currently analysing single cell RNA sequencing of NANOG targeted human pre-implantation embryos and will be interested to compare this phenotype to the mouse to determine conserved and divergent mechanisms of NANOG function. Overall, we demonstrate that adenine base editing is a powerful tool to perform precise single base pair changes to perturb and study gene function in human embryogenesis.

T1280

INVESTIGATING THE ROLE OF METABOLISM IN REGULATING FOXG1 PROTEIN STABILITY

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Forkhead box G1 (FOXG1) is a crucial transcription factor for neural stem cell (NSC) proliferation during cortical expansion. As NSCs transition from proliferation to differentiation, FOXG1 levels decrease, coinciding with a metabolic shift from glycolysis to oxidative phosphorylation. This metabolic reprogramming is increasingly recognized as a driver of differentiation. Moreover, evidence suggests that FOXG1 may localize to mitochondria and influence bioenergetic state of NSCs. Given the intricate relationship between proliferation, differentiation, and metabolic state, we aim to investigate the potential link between metabolic state and FOXG1 stability. To accomplish this, we first determined a baseline stability of FOXG1 in two cell types: a HEK293 testing model, and three iPSC-derived cortical NSC lines. We then perturbed various metabolic pathways in a pharmacological screen in HEK293 cells, before validating in iPSC-derived NSCs. We identified several promising candidates during the initial screening, many with >2-fold change on FOXG1 levels. However, these effects were not consistently seen in all three of our NSC models. These results suggest FOXG1 may be regulated by metabolic state of the cell, but that the proper coordination of this would be highly complex and require very specific intracellular conditions. To characterize these conditions, we will be conducting a systematic analysis of metabolic activity within our NSC cell lines during proliferation and during the onset of differentiation to inform what metabolic changes best promote loss of FOXG1 and neuronal differentiation. This research would establish a mechanistic link for the onset of differentiation in cortical NSCs and be the first to potentially identify FOXG1 as a master metabolic regulator of this event.

T1282

LINEAGE ANALYSIS OF THE HUMAN ESC-DERIVED LIVER ORGANOID

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Human liver organoids (HLOs) derived from human pluripotent stem cells (hPSCs) hold a promise for modeling liver development and disease. However, HLOs generated using current protocols lack physiological complexity and cellular diversity, with hepatocytes exhibiting a fetal-like phenotype, limiting their utility for advanced applications. This study aimed to investigate into the underlying mechanism to enhance the functionality of HLOs. In this study, we optimized multiple conditions to generate HLOs from H9 human embryonic stem cells (hESCs). RNA sequencing (RNA-seq) and immunostaining were used to characterize the lineage composition of the HLOs, while functional assays evaluated key liver-specific functions, such as urea synthesis and albumin production. To further explore the mechanisms underlying hepatocyte maturation, HepaRG cells were co-cultured with HUVECs (human umbilical vein endothelial cells) and HHSECs (human hepatic sinusoidal endothelial cells). We successfully generated improved HLOs from H9 hESCs. RNA-seq analysis revealed the expression of hepatocyte markers alongside non-parenchymal cell markers. Immunostaining confirmed the presence of hepatocyte-specific HNF4A and stellate cell-specific vimentin in distinct regions of the organoids. Notably, the mutually exclusive expression patterns of HNF4A and CK19 (cholangiocyte marker) highlighted the spatial organization of these cells within the organoids. The HLOs expressed key genes essential for liver function, including those involved in cholesterol and fat metabolism, drug detoxification, and hepatic-specific transcriptional regulation. Furthermore, co-culturing HepaRG cells with HUVECs and HHSECs significantly enhanced hepatocyte proliferation and maturation compared to monoculture, supporting the hypothesis that multi-lineage composition enhances HLO functionality. In summary, this study optimized multiple factors to generate HLOs with improved lineage composition and functionality. Future work will focus on characterizing the responses of these organoids to liver injury factors and further investigating the roles of specific cell types, particularly endothelial cells, in promoting hepatocyte maturation.

T1286

MESODERMAL DIFFERENTIATION OF PIG EMBRYONIC STEM CELLS FOR DERIVATION OF MESENCHYMAL STEM CELLS

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Lineage specification during early embryo development results in the formation of the pluripotent cell population, known as inner cell mass (ICM), and the ICM has the potential to generate three germ layers of endoderm, mesoderm, and ectoderm during gastrulation. Embryonic stem cells (ESCs) can recapitulate early embryogenesis in vitro and differentiate into various cell types derived from all three germ layers, making them widely used in developmental biology research. The generation of multiple mesodermal derivatives including cardiovascular, hematopoietic, and mesenchymal cells from ESCs is well demonstrated in human and mouse. However, since ESCs of pig have been established relatively recently, studies of their direct differentiation through the modulation of signaling pathways are limited. Therefore, we elucidated the signaling pathways involved in the mesodermal differentiation of pig ESCs and optimized the differentiation protocol for inducing pig ESCs into the mesodermal lineage. Transcriptional expression levels of mesodermal markers were significantly upregulated in pig ESCs treated with a combination of signaling molecules, compared with non-treated pig ESCs. In addition, this treatment did not enhance the transcript levels of endodermal and ectodermal markers. Interestingly, a certain signaling molecule reported to be associated with mesodermal differentiation of human ESCs had no effect on pig ESCs, indicating species-specific characteristics of pigs. Taken together, we efficiently derived mesodermal progenitors from pig ESCs by modulating signaling pathways related to mesodermal lineage development. Because ESCs are capable of unlimited self-renewal, these progenitors can be employed as a stable cell source for the production of various mesodermal derivatives. Especially, ESCs are known to pass through intermediate stages such as the mesoderm during

differentiation into mesenchymal stem cells (MSCs), and thus these progenitors can be utilized for establishing MSCs derived from ESCs. Therefore, as part of further studies, we are preparing to induce mesodermal progenitors into MSCs and dissect the mechanisms thereof.

Funding Source: This work was supported by the BK21 Four program, the Korea Evaluation Institute of Industrial Technology (KEIT) [20012411]; and the National Research Foundation of Korea (NRF) grant [2023R1A2C1005026].

T1288

METTL3-DEPENDENT RNA METHYLATION SAFEGUARDS MITOSIS FIDELITY AND GENOME STABILITY IN EARLY HUMAN DEVELOPMENT

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Genomic integrity is critical for early embryonic development. Severe genomic instability, including chromosome missegregation and aneuploidy, can lead to miscarriage and developmental abnormalities in humans. However, the molecular mechanisms underlying mitotic defects in early embryos remain poorly understood. Here, we reveal a novel role for METTL3-dependent N6-methyladenosine (m6A) modification in safeguarding DNA replication and cell division, supported by evidence from both pluripotent and trophoblast stem cells, and, importantly, human embryos. METTL3 depletion leads to pervasive polyploidy, cell cycle delay, and compromised proliferation. Mechanistically, m6A modification on centromeric satellite repeats suppresses their transcription and stabilizes CENPA loading during DNA replication. These findings establish the METTL3-satellite repeats-CENPA nexus as a critical regulator of centromeric stability in early human development, providing new insights into the epi-transcriptional control of mitotic fidelity and potential therapeutic avenues for pregnancy complications.

Funding Source: This project is supported by Health@InnoHK, Innovation Technology Commission, HKSAR.

T1290

MITOCHONDRIA-TARGETING, SINGLE-ATOM NANOZYME PROMOTES OSTEOGENIC DIFFERENTIATION OF SKELETAL STEM/PROGENITOR CELLS AND ACCELERATES CRITICAL-SIZED BONE DEFECT REPAIR

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Bone defects, including structural and functional abnormalities affecting the integrity of bones, represent a significant global health concern. These defects are a key factor in the elevated rates of disability observed globally, underscoring their significant impact on global health. Critical-sized bone defects (CSBDs) are defined as bone defects that are not expected to heal without secondary (surgical) intervention. Skeletal stem/progenitor cells (SSCs) play essential roles in the regeneration of CSBDs and have been enthusiastically pursued in bone tissue engineering. Modulating the metabolic pathway represents an effective strategy to enhance the osteogenic differentiation of SSCs. However, this process is hindered at bone defect sites. The objective of this study was to develop a mitochondria-targeting, single-atom nanozyme to promote the osteogenic differentiation C3H/10T1/2 SSCs. Our mesoporous silica nanoparticle (MSN)-based nanozyme significantly enhanced the osteogenic marker gene expression and mineral deposition by the SSCs. Seahorse assay revealed a higher level of oxidative phosphorylation for the nanozyme group. In a CSBD model in rat femur, we found there was more bone regeneration in the MSN-based nanozyme group based on the μCT analysis. This innovative approach opens a new avenue for accelerating bone tissue regeneration via regulating the metabolic pathways of SSCs with mitochondrial-targeting nanozymes.

Funding Source: CUHK's Vice-Chancellor Early Career Professorship Scheme and the Shun Hing Institute of Advanced Engineering, CUHK (#BME-p2-24).

T1292

MODELING OF ENDODERM DEVELOPMENT WITH HUMAN PLURIPOTENT STEM CELL-DERIVED GASTRULOIDS

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Diabetes mellitus is a chronic condition affecting over 500 million people globally, with profound health and socioeconomic implications. Advances in stem cell-derived islet cell therapies offer hope for restoring glucose regulation but are hindered by the immaturity and heterogeneity of these cells. Current differentiation protocols condense weeks of human pancreatic development into days, bypassing crucial steps in endoderm specification and patterning that are potentially important to properly achieve mature pancreatic cell types. To address this gap, we are establishing 3D gastruloid systems with human pluripotent stem cells that mimic the early stages of human endoderm formation. These models aim to recapitulate the spatial and temporal dynamics of gastrulation, enabling the study of endodermal cell fate decisions and patterning toward pancreatic development. For this purpose, we are utilizing SOX17 and PDX1 reporter stem cell lines that enable agile screening of culture conditions that robustly yield definitive endoderm and pancreatic endoderm, respectively. Using single-cell omics, we are dissecting the genome regulatory mechanisms behind pancreatic endoderm development. By overcoming the current limitations of existing stem cell models based on directed differentiation, self-organizing gastruloid systems advance our understanding of the early stages of human pancreas development, paving the way for improved strategies to treat diabetes using stem cell-derived cellular replacement.

T1294

MULTI-ANCESTRY GENOME-WIDE ASSOCIATION META-ANALYSIS REVEALS NEW HIRSCHSPRUNG'S DISEASE GENES

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Hirschsprung's disease (HSCR) is a rare, congenital disorder of the colon in children. Abnormal development of the enteric nervous system (ENS) is known to be involved in its pathogenesis. The disease is caused by multiple genetic factors, but only a few causal genes are reported until now. In this study, we performed the largest multi-ancestry meta-analysis of genome-wide association study (GWAS) involving 1,250 HSCR cases and 7,140 controls and identified four novel HSCR-susceptibility loci, with three loci (JAG1, HAND2 and ZNF25) reaching genome-wide significance and one putative locus (UNC5C) prioritized by functional relevance. Single-cell transcriptomic data and immunofluorescence staining results suggested that four novel candidate genes were expressed in migratory enteric neural crest cells (ENCCs) and/or neurons. In vitro, disruption of HAND2 and ZNF25 in the SK-N-SH cell line caused abnormal migration behaviors. In vivo, CRISPR/Cas9-mediated candidate genes knockout in zebrafish displayed abnormal ENS development. Conclusively, our study showed that JAG1, HAND2, ZNF25 and UNC5C are four novel risk genes for HSCR, and genetic dysregulation of these candidate genes may disrupt ENCCs migration and impair ENS development.

Funding Source: Thanks to Theme-based Research scheme [grant number T12C-714/14-R], FDCT [grant number 0097/2022/A2], REACH research grant, Health Medical Research Fund (HMRF) [grant numbers 06171636, 08193446, PR-HKU-1 and 08191496].

T1296

MYO-INOSITOL SUPPLEMENTATION IMPROVES IN VITRO MATURATION AND DEVELOPMENTAL POTENTIAL OF PORCINE OOCYTES FOLLOWING PARTHENOGENETIC ACTIVATION

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The most abundant form of inositol, myo-inositol (Myo-Ins), is an antioxidant and an important molecule for mammalian embryonic development. This study aimed to examine the effect of Myo-Ins supplementation during in vitro maturation (IVM) of porcine oocytes and its impact on oocyte development after parthenogenetic activation (PA). Key parameters, including nuclear maturation, cumulus cell expansion, levels of reactive oxygen species (ROS), intracellular glutathione (GSH)

levels and developmental competence were evaluated. IVM medium was supplemented with Myo-Ins at concentrations of 0, 5, 10, and 20 mM for 42 hours. Although Myo-Ins treatment had no effect on nuclear maturation rates, cumulus cell expansion was significantly enhanced ($p < 0.05$) in treatment groups compared to the control group. Among the treatments, the 5 mM Myo-Ins displayed a significant increase ($p < 0.05$) in blastocyst formation rate compared to the control group. Additionally, oocytes treated with 5 mM Myo-Ins group showed significantly lower ($p < 0.05$) intracellular ROS levels compared to the control group. Furthermore, intracellular GSH levels were significantly increased ($p < 0.05$) in 5 and 20 mM groups compared to the control group. In summary, Myo-Ins supplementation during IVM improved the developmental potential of porcine embryos by mitigating the oxidative stress.

Funding Source: This work was supported by the NRF (2020R1A2C2008276, 2021R1C1C2013954), MOTIE (20023068), and MAFRA (RS-2024-00398561, RS-2024-00399475), Republic of Korea.

T1298

NOVEL STRATEGY FOR IMPROVING IPSC DIFFERENTIATION STABILITY BY SYNCHRONIZING BEHAVIOR-DRIVEN MECHANICAL MEMORY USING BOTULINUM HEMAGGLUTININ

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To industrialize induced pluripotent stem cell (iPSC)-derived cell therapy, it is necessary to develop robust and highly efficient iPSC differentiation strategies. The cell behavior has been shown to be an important factor regulating cell fate and yield during differentiation. Thus, the spatial variation in cell density during early differentiation may lead to heterogeneity of differentiation. In this study, we explored the concept of “synchronization” in the context of cell behavior-driven mechanical memory in iPSC culture using botulinum hemagglutinin (HA), an E-cadherin function-blocking agent. We showed that temporal disruption of cell-cell interactions by HA results in suppression of spatial heterogeneity of cells within the culture. HA resulted in mechanical memory synchronization with Yes-associated protein (YAP), which increased pluripotent cell homogeneity. Synchronized iPSCs were applied to hepatocyte differentiation medium to test the functional role of synchronized mechanical memory with YAP in regulating cell differentiation. The synchronized iPSCs have higher capability to differentiate into functional hepatocytes than unsynchronized iPSCs, resulting in improved efficiency and robustness of iPSC differentiation. Furthermore, we demonstrated that the synchronization of cell behavior-driven mechanical memory using HA can be used to improve the robustness of differentiation process of various differentiated cell types such as pancreatic progenitor, neuron, and retinal pigment epithelial cells. Our results from the synchronization of mechanical memory using HA confirmed that iPSC differentiation improve the yield and quality of various differentiated cell types, providing a valid tool for various disease modelling and drug discovery applications.

T1300

OPEN DIGITAL TOOLS AND RESOURCES FOR QUANTITATIVE IMAGE ANALYSIS, DATA VISUALIZATION, AND DATA ORGANIZATION

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The Allen Institute for Cell Science aims to elucidate how human induced pluripotent stem cells (hiPSCs) establish and maintain dynamic cellular structures and transition between states during differentiation and disease. A crucial step towards this goal is being able to quantify changes accurately and reproducibly in the size and shape of the cell and subcellular organelles. We took advantage of high-resolution 3D microscopy images of fluorescently tagged human induced pluripotent stem cells (hiPSCs) from the Allen Cell Collection to develop a suite of image-analysis tools. This includes the Allen Cell and Structure Segmenter, a Python-based open-source toolkit for 3D segmentation of fluorescence microscope images, which we have updated and made more accessible by developing napari plugins for each the Classic Image Segmentation workflow and the Iterative Deep Learning Workflow. The latter is powered by a comprehensive deep learning tool for streamlining microscopy image transformations called CytoDL which allows integration of multiple workflows. Already integrated is MegaSeg, a 3D convolutional neural network segmentation model trained on a large dataset of high-quality 3D fluorescent images and corresponding segmentations for 13 intracellular structures with distinct morphologies. MegaSeg enables robust, morphology-agnostic deep feature extraction that generalizes across various intracellular structures and permits their accurate 3D segmentations. To visualize these data, we recently released the Timelapse Feature Explorer, a web-based application designed for the interactive visualization and analysis of segmented time-series microscopy data. Finally, to address the challenges associated with handling these large datasets, we developed BioFile Finder, an advanced, web-based application designed to make data easy to search, aggregate, and share in ways that promote novel insights and reproducible science while reducing redundant pixel storage. This open-source tool provides a robust search engine that allows users to quickly locate specific images from user-supplied or publicly available datasets, accelerating data analysis, management, and discovery. These tools, resources, and data and more can be found at allencell.org.

T1302

PANCREATIC ALPHA AND BETA CELL FATE CHOICE IS DIRECTED BY APICAL-BASAL POLARITY DYNAMICS

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A central question in cell and developmental biology is how extracellular cues control the differentiation of multipotent progenitors in a dynamically changing niche. Here, we identify apical-

basal polarity as the main regulator of the differentiation of multipotent pancreatic Neurogenin3+ endocrine progenitors (EPs) into the beta or alpha cell fates. We show that human EPs dynamically change their apical-basal polarity status. Whereas polarized EPs are predisposed to differentiate into beta cells rather than alpha cells, inhibiting apical-basal polarity selectively suppresses beta cell differentiation. Single-cell RNA sequencing and complementary mechanistic data demonstrate that apical-basal polarity in human EPs promotes beta cell specification via cAMP/PKA-CREB-EGR1-mediated inhibition of ARX expression, while reduced cAMP levels in non-polarized human EPs maintain expression of ARX leading to alpha cell differentiation. These findings identify the apical-basal polarity status of multipotent EPs as a critical epithelial feature that determines their fate into the alpha or beta cell lineages.

Funding Source: The European Union's Horizon 2020 research and innovation program (ISLET, no. 874839); The Novo Nordisk Foundation Center for Stem Cell Biology (DanStem) (NNF grant, NNF17CC0027852); The Helmholtz Zentrum München.

T1304

PERLECAN-CONJUGATED LAMININ FRAGMENT AS A NEXT-GENERATION CULTURE SUBSTRATE FOR HUMAN PLURIPOTENT STEM CELLS

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Laminin and perlecan are key components of basement membranes, providing essential cues for stem cell maintenance and function. Laminin binds integrins to regulate cell survival, proliferation, and differentiation, while perlecan captures growth factors (GFs) via heparan sulfate (HS) chains attached to its N-terminal PDI domain, modulating GF availability to cell surface receptors. Given the cooperative signaling between integrins and GF receptors, a culture substrate integrating both laminin's adhesive properties and perlecan's GF-binding capacity is highly desirable. We engineered a recombinant laminin fragment conjugated with perlecan's HS-rich PDI domain, termed PerLam. PerLam was produced in cGMP-banked CHO-S cells and purified via chromatographic steps. The purified protein carries HS chains and binds bFGF in an HS-dependent manner. When used as a coating, PerLam exhibits potent cell-adhesive activity and robustly supports human iPSC (hiPSC) proliferation. hiPSCs maintained on PerLam remain undifferentiated for over 10 passages. Notably, PerLam eliminates the need for precoating, as hiPSCs can be passaged directly onto fresh plates with PerLam-supplemented medium. Beyond expansion, PerLam promotes directed hiPSC differentiation into mesodermal lineages, including skeletal muscle progenitors, and enhances dopaminergic progenitor maturation in rodent Parkinson's disease models. By combining laminin's integrin-binding activity with perlecan's HS chains, PerLam offers a promising next-generation culture substrate for hiPSC expansion and differentiation in regenerative medicine.

Funding Source: Supported in part by a grant from AMED (Japan Agency for Medical Research and Development; JP20bm0804025).

T1306

PHYSIOXIA-DRIVEN MATURATION OF IPSC-DERIVED HEPATOCYTES IN 2D CULTURES

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Atmospheric oxygen levels differ significantly from tissue-specific physioxia; for example, the liver consists of hepatic lobules exhibiting physiological oxygen gradients that greatly influence cellular functionality. Our study aims to emulate the hepatic microenvironment and enhance the maturation of induced pluripotent stem cell-derived hepatocyte-like cells (iPSC-HLCs) by culturing the cells under varied oxygen concentrations. To expose the differentiating cells to specific oxygen concentrations within a physiologically relevant range, we culture them in 1-well culture chambers (BioGenium Microsystems), which are connected to a gas supply. The culture chambers are coated with an oxygen-sensing film, allowing quantitative assessment of the oxygen microenvironment during the culture period. Initially, we introduced oxygen stimulation on day 12 of iPSC-HLC differentiation. In subsequent experiments, we progressively adjusted the start of physioxic oxygen exposure, starting with day 8 and later including even earlier time points. Our analyses indicate enhanced functionality of the iPSC-derived hepatocytes, demonstrated by increased secretion of liver-specific factors such as albumin and urea, alongside upregulated expression of key hepatic genes, even in the absence of media perfusion or 3D culture conditions. The timing of oxygen stimulation initiation appears to have a greater impact on, for example, albumin secretion, compared to the tested range of oxygen concentrations. Additionally, ratiometric oxygen imaging reveals that the oxygen concentration experienced by the cells differs from the set gas level, emphasising the importance of oxygen monitoring during the culture period. Our research highlights how altering a single physical parameter, such as oxygen levels, can significantly enhance the functionality of iPSC-derived cells. Our simple, yet impactful approach ultimately aims to contribute to a more accurate representation of human liver physiology.

T1308

PLURIPOTENCYSCREEN – A NEW EPIGENETIC FRAMEWORK TO QUANTIFY EARLY DIFFERENTIATION OF PLURIPOTENT CELLS

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Characterization of induced pluripotent stem cells (iPSC) and quantification of early differentiation events pose significant challenges to reliably determine differentiation potential. Here, we describe PluripotencyScreen – a combination of cell-type specific DNA methylation signatures to distinguish pluripotent, endoderm, mesoderm, neuronal and non-neuronal ectoderm cells. Furthermore, these signatures can be used for deconvolution to estimate the composition of cell types.

PluripotencyScreen was established with the computational framework CimpleG to derive epigenetic signatures, consisting of a few CG dinucleotides per cell-type, enabling targeted analysis with digital PCR or pyrosequencing. We employed six defined differentiation conditions and used publicly available trilineage differentiation kits to generate genome wide DNA methylation profiles of defined cell states. Based on these datasets, we determined a pluripotency score to monitor reprogramming and to characterize pluripotent state of iPSCs, and lineage-specific scores to monitor either directed differentiation of iPSC or undirected multilineage differentiation in embryoid bodies. These scores have been validated through pyrosequencing and digital PCR for iPSC lines of various laboratories. The signatures have also revealed differentiation impairments, e.g. after genetic knockout of PRDM8 and YAP1. We demonstrate optimization and validation steps of our methodology to develop this approach in an easily applicable tool for the scientific community. Taken together, the cell-type specific DNA methylation changes underlying PluripotencyScreen facilitate reliable and reproducible quality control of iPSC and their derivates.

Funding Source: BMBF.

T1310

PORCINE NAÏVE-LIKE PLURIPOTENT STEM CELLS

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The pig, as a biological analog of human in multiple biomedical research due to its more conserved physiological, metabolic, and genetic characteristics to human than lower mammals such as rodents, has long been considered as a useful model for studying mammalian embryonic development and human disease. However, it has been challenging to establish pluripotent stem cells from pig preimplantation embryos. Here we report the derivation of Naïve-Like Porcine Pluripotent Stem Cell (N-L pPSC) line from porcine Expanded Potential Stem Cell (pEPSC) and pre-implantation embryo through the optimization of culture condition with PD0325901 included, the key inhibitor for capturing and maintenance of human and mouse naïve pluripotent stem cells. Our newly established N-L pPSCs are robust in culture with high expression of typical pluripotency factors such as OCT4, SOX2 and NANOG. We have also observed remarkable upregulation of the naïve pluripotency markers including TFAP2C and KLF4 compared to pEPSCs indicating the naïve state transition. Moreover, N-L pPSCs appear to be highly efficient in deriving porcine trophoblast stem cells (pTSCs) and porcine blastocyst-like structures (blastoids) indicating retaining an extraembryonic lineage differentiation potential. In vitro differentiation assays confirmed that N-L pPSCs formed three-dimensional embryoid bodies, spontaneously differentiated into cell types from all three germ layers as well as trophoblasts. The high differentiation potency and molecular features indicate our derivation of porcine naïve-like pluripotent stem cells.

Funding Source: This project is supported by Health@InnoHK, Innovation Technology Commission, HKSAR.

T1312

QUANTIFICATION OF EMERGENT NEURON NETWORK MORPHOLOGY IN VITRO

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Throughout brain development, neural stem cells self-organise into refined networks, establishing the infrastructure for efficient neuronal communication. Here, we established an in vitro platform to study the formation and perturbation of these networks using a live-cell imaging protocol and bioimage analysis pipeline. Cortical neurons, derived from human embryonic stem cells via Neurogenin-2 and green fluorescent protein (GFP) viral transduction, were imaged over 33 days. To minimise phototoxic stress under repeated fluorescence imaging, we optimised culture conditions by testing three factors: extracellular matrix (human- or murine-derived laminin), culture media (Neurobasal or Brainphys Imaging media), and seeding density (1×105 or 2×105 cells per cm2). Brainphys Imaging medium was observed to support cell survival to a greater extent than Neurobasal medium with either laminin type, as quantified by PrestoBlue viability assay ($p = 0.0052$) and neurite outgrowth analysis ($p < 0.0001$). However, a combination of Neurobasal medium and human laminin detrimentally impacted neurite outgrowth ($p < 0.0001$). Using our optimised protocol, we captured timeseries microscopy images and built a computational pipeline to evaluate multiscale network features. At global resolution, we introduced two measures of population-wide somata clustering and neurite fasciculation. At local resolution, we developed a tool that generates spatially-embedded models of neuron networks, representing cell bodies as points (network nodes) and neurite connections as links between these points (network edges). Application of this pipeline to pharmacologically-induced disease models of schizophrenia (treated with MK-801) and epilepsy (treated with kainic acid) revealed distinct topological profiles. Graph theoretical analysis demonstrated significantly reduced clustering coefficients ($p = 0.0398$) and small-world indices ($p = 0.0268$) in schizophrenia relative to epilepsy models. These analytical tools reveal, for the first time, disrupted anatomical connectivity across neuronal networks, extending beyond standard neurite outgrowth measures to capture precise motifs in neuron organisation.

T1314

RBP9 REGULATES GERMLINE CELL DIFFERENTIATION AS A TRANSLATIONAL REGULATOR IN THE DROSOPHILA OVARY

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Germline cell development is a critical biological process that facilitates the generation of healthy gametes necessary for species reproduction. This process encompasses the self-renewal of germline stem cells (GSCs) and the differentiation of cysts. A comprehensive understanding of the

intrinsic and extrinsic factors governing germline cell development is essential for elucidating mechanisms of stem cell maintenance and differentiation in adult tissues, and it may provide insights for advancements in regenerative medicine. RNA-binding protein 9 (Rbp9), a highly conserved member of the Elav/Hu family, has been previously implicated in promoting cystocyte differentiation in the Drosophila ovary. However, the molecular mechanisms underlying its function remain largely unexplored. In this study, we demonstrate that Rbp9 plays a pivotal role in regulating early GSC differentiation by directly binding to the 3' untranslated region of key differentiation factors, including bam, sxl, mei-P26, and modulating their translation. Furthermore, our findings reveal that Rbp9 interacts directly with pAbp and eIF4E1, suggesting its potential role as a translational regulator. Collectively, our data indicate that Rbp9 regulates early germline cell differentiation in Drosophila through the translational regulation of its mRNA targets, rather than influencing mRNA stability as a post-transcriptional regulator.

Funding Source: This work was funded by the Hong Kong Research Grants Council (RGC) Theme-based Research Scheme grant, T13-602/21N.

T1316

REGULATORY MICRORNAS IN GABAERGIC INTERNEURON MIGRATION

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In the intricate landscape of brain development, clusters of interneurons, originating from specific progenitor domains, undergo long-distance migration within the developing telencephalon to establish neural connections and form the central inhibitory system of the brain. A disturbance in this balance, often resulting from anomalies in migration, has been implicated as the pathogenesis of several neurodevelopmental disorders. This underscores the critical significance of neuronal migration in shaping the cellular functions and neuronal circuitry of the neocortex. Here, we aimed to elucidate the role of molecular regulators guiding the interneuron migration through human pluripotent stem cell-derived medial ganglionic eminence cells through a 25-day directed differentiation protocol. A detailed radial migration assay was performed to identify the temporal migration of interneuron progenitors. Subsequently, mRNA and miRNA sequencing at two critical time points identified from the migration assay was performed to decipher the genes involved in human interneuron migration. The results of next-generation sequencing of enriched microRNAs from two distinct groups- one displaying minimal migration potential and another with maximum migration potential unveiled a total of 419 known and novel miRNAs, of which 138 miRNAs were differentially expressed between groups. In addition, 31 mature microRNAs were expressed exclusively on attaining maximum migratory potential. Further extensive analysis identified that several of these microRNAs control mRNAs that are involved in interneuron migration. Thus, the identified microRNAs contribute to the evolving understanding of the regulatory role of miRNAs in inhibitory GABAergic interneuron development. Furthermore, it explores the implications of their dysregulation in the emergence of numerous neurodevelopmental and neuropsychiatric disorders.

Funding Source: Science and Engineering Research Board, Government of India.

T1318

REWIRING OF SINE-MIR ENHANCER TOPOLOGY AND ESRRB MODULATION IN EXPANDED VERSUS NAIVE PLURIPOTENT STATES

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Despite extensive study of gene regulation in mouse embryonic stem cells, the role of 3D genomic interactions and transposable elements (TEs) in pluripotent state-specific gene expression remains unclear. Understanding this is crucial for fully harnessing the differentiation potential of each state. Here, we investigate how TE-derived enhancer interactions differ between the conventional naïve state and the expanded state with extraembryonic differentiation capacity. Using integrative HiC, ChIP, and H3K27ac HiChIP analyses, we identified mammalian-wide interspersed repeat (MIR), a short interspersed nuclear element (SINE) family member, as highly associated with naïve-specific genomic interactions. In the naïve state, Esrrb co-opts MIR enhancers to regulate the naïve-specific gene expression program, forming enhancer and super-enhancer loops. We demonstrated that the loss of an Esrrb-bound MIR enhancer impairs self-renewal, underscoring its importance. Additionally, Esrrb co-binds with Yy1, a structural protein complex, at MIR enhancers, highlighting its topological role. Altogether, our study reveals the significance of Esrrb at MIR-derived enhancers in shaping the naïve potency state. This finding advances the recognition of TEs as an element of gene regulation.

T1320

SAGA COMPLEX REGULATES PLURIPOTENCY OF MOUSE EMBRYONIC STEM CELLS

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The pluripotency state of embryonic stem cells (ESCs) is determined by both their epigenome and transcriptome. The transcriptional co-activator complex SAGA plays a critical role in a wide range of biological processes. We observed that SAGA complex is stably expressed in various types of high-potency stem cells, including spermatogonial stem cells, haematopoietic stem cells,

mesenchymal stem cells, and embryonic stem cells (ESCs). However, whether and how SAGA mediates epigenetic modifications and pluripotent gene transcription is not fully understood. Sgf29, a subunit of the transcriptional co-activator complex SAGA, plays a critical role in a wide range of biological processes. Overexpress Sgf29 in dox-induced Flag tagged Sgf29 mESC line did not affect the morphology, embryoid body (EB) information and the pluripotent markers Oct4 and Nanog expression. Induced knockout of Sgf29 was achieved by inducing Cas9 protein expression in the presence of Sgf29-targeted gRNAs. A significant increase in differentiated ESC colonies was observed after 72 h of iKO. It was found that Sgf29 KO significantly reduced Oct4, Nanog, and Sox2. RNA-seq was used to assess the gene expression profile of WT and Sgf29 KO mESCs. Gene ontology (GO) analysis revealed that the major pathways enriched for down-regulated genes were pattern specification process, embryonic organ morphogenesis, cell fate commitment and cell fate specification. Down-regulated genes included pluripotency-associated factors such as Pou5f1 (Oct4), Nanog. Additionally, Sgf29 KO significantly inhibited EB growth and proliferation. Finally, we found that dis-functional SAGA induced the differentiation of mESCs by reducing H3K9ac and chromatin accessibility at the promoters and enhancers of key pluripotency genes. These results suggest that SAGA plays a critical role in maintaining the pluripotency and differentiation fate of mESCs.

Funding Source: This study was funded by the National Natural Science Foundation of China (82271688), the Guangdong Province Excellent Youth Team Project (2024B1515040009).

T1322

SENESCENCE MARKERS IN PERIODONTAL LIGAMENT STEM CELLS AFTER LONG TERM CULTIVATION

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The aim of the present study is to investigate the markers of cell cycle arrest and senescence, i.e. betagalactosidase and telomerase activity, after long term cultivation (up to 16 passages) of periodontal ligament cells. Periodontal ligament stem cells were isolated from routinely extracted third molars via enzymatic digestion (3 mg/mL collagenase type I and 4 mg/mL dispase) and cultured continuously up to passage 16. Cell count and population doubling were evaluated at each passage. The enzymatic activity of telomerase and betagalactosidase were assessed, as these are wellknown markers for cell senescence and aging. The following kits were used for these purpose, following the manufacturer`s instructions: Human TERT / Telomerase Reverse Transcriptase ELISA Kit (ELISAGenie, Dublin, Ireland) and ELISA Kit for Galactosidase Beta (GLb) (Cloud Clone Corp, Katy, TX, USA). Total protein amount was previously identified in all samples using Nanodrop 1000 (Thermo Scientific). The results demonstrate 2 peaks with significantly increased cellular proliferation rate at passage 6 and passage 12. Slight differences in the proliferative ability were identified between cells from 1 st and 16 th passages without any statistical significance. Significant decrease in telomerase activity was observed starting after the first passage. Betagalactosidase activity was found to be uninterrupted following longterm in vitro cultivation. Our study indicates that PDL stem cells do not enter cell proliferation arrest phase after longterm in vitro cultivation (16 passages – about 40 doublings), as the PDL stem cells did not show significant decrease in the proliferation ability. The telomerase activity is known to be quite typical for neoplastic cells. Therefore, our data suggest lack of tumorigenic potential in human PDL stem cell culture as we revealed suppression of telomerase activity following continuous cultivation.

Funding Source: Bulgarian National Science Fund КП-06-Н73/12 15.12.2023.

T1324

SEQUENCE YOUR CELL AND KEEP IT ALIVE: CYTOPLASMIC LIVE-CELL BIOPSIES FOR TEMPORAL SINGLE CELL PROFILING

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The advent of single-cell transcriptomics (scRNA-seq) has revolutionized biological and biomedical research by enabling the study of cellular diversity and states within a population. However, standard approaches often fail to capture lowly expressed genes and subtle signatures from rare cells, while also losing spatial and phenotypic information during library preparation. Chen et al. (2022) demonstrated a novel approach to single-cell transcriptome profiling by employing a biopsy technology that retrieves cytoplasmic material from live cells using FluidFM® (Fluidic Force Microscopy). Since these single-cell biopsies are not destructive to the cell, it becomes possible to detect the transcriptome while keeping the characterized cell alive, opening the possibility to temporal transcriptomics for the examination of long-term transcriptomic changes in single-cells. One such process is the differentiation of adipocyte stem and progenitor cells, which was successfully characterized with live-seq. Here, we introduce a streamlined workflow using the FluidFM OMNIUM platform to collect cytoplasmic biopsies from Panc-1 cells and generate transcriptomes from less than a cell's worth of RNA. Utilizing the ultra-low RNA input LUTHOR™ HD library preparation kit, we show that transcriptomes can be prepared and sequenced from as little as 0.15 pg of RNA—equivalent to less than 1% of a Panc-1 cell. This advancement highlights the ability of the FluidFM OMNIUM platform, combined with LUTHOR HD, to enable high-resolution single-cell RNA profiling from live-cell biopsies, opening new avenues for investigating cellular heterogeneity and dynamics.

T1326

SETD2 ENHANCES SOMATIC CELL REPROGRAMMING BY ALLEVIATING EXCESSIVE H3K4ME3/H3K27ME3 BIVALENCY

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Achieving successful cloning via somatic cell nuclear transfer (SCNT) encounters substantial hurdles due to epigenetic impediments. Emerging research has pinpointed the roles of H3K4me3

and H3K27me3 as likely contributors to these challenges. Nonetheless, the precise mechanisms behind these obstacles remain largely enigmatic. In this study, we characterized the landscapes of H3K4me3 and H3K27me3 during pre-implantation development of SCNT embryos. Comparative analysis with naturally fertilized (NF) embryos uncovered aberrant overabundance of both H3K4me3 and H3K27me3 in NT embryos at 2-cell stage, leading to severe expression defects in ZGA genes with a bivalent state. By contrast, H3K36me3, which is mutually exclusive with H3K4me3/H3K27me3, showed deficient establishment in NT 2-cell embryos. Importantly, overexpression of Setd2, a methyltransferase of H3K36me3, effectively rescued the expression of ZGA genes in NT embryos and benefited embryo development. Mechanistically, SETD2 facilitated these genes escape from H3K4me3/H3K27me3 bivalency, by augmenting H3K36me3 marks at gene bodies and further squeezing H3K27me3 out of promoters. In summary, our data underscore the excessive bivalent state at gene promoters as a formidable impediment during somatic cell reprogramming. The removal of such barriers is a promising strategy for advancing cloning efficiency.

T1328

SIGNALING MEDIATES HIGH-FIDELITY HUMAN POST-IMPLANTATION EMBRYO MODELLING

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Embryo models play a role as surrogates for unraveling human early development. However, existing models show low efficiency when modelling post-implantation stages. Here, we manage to reprogram human pluripotent stem cells (hPSCs) into early embryo fates in 60-120 hours by recreating signaling environment of the blastocyst stage. Upon aggregation, the reprogrammed hPSCs self-assembly to develop into post-implantation embryo-like structures, containing all embryonic and extraembryonic lineages. These structures are morphologically replica of Carnegie Stage (CS) 5-7 embryos, exhibiting a bilaminar disc-like structure, including epiblast and hypoblast discs, yolk-sac and amniotic cavities, mesenchyme tissue, chorionic cavity, and trophoblast at the correct locations. Immunostaining and single-cell RNA sequencing results at different time points reveal that our model also recapitulates key developmental events, including gastrulation, germ cell specification, chorion development, and primary to secondary yolk sac transition. Collectively, we demonstrate that by modulating key signaling of the early embryo allows us to efficiently generate a high-fidelity model to mimic development up to a gastrulating embryo both morphologically and molecularly. Our findings offer a state-of-the-art strategy for advancing our understanding of post-implantation human embryogenesis.

T1330

SINGLE-CELL TRANSCRIPTOMICS OF GENETICALLY-MATCHED IN VIVO AND IN VITRO-DERIVED HUMAN BLOOD STEM/PROGENITOR CELLS REVEALS NOVEL ROLE OF MICRORNA-MODULATED REGULATORY PATHWAY GENES

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Prominent researchers throughout the world have been striving to develop HSCs in vitro that could be used to reconstitute the entire human hematopoietic system. Although there have been recent impactful advances towards this goal, improvements are still needed to obtain and optimize fully functional HSCs in vitro that have the robust long-term secondary hematopoietic transplantation capability that is required for their use in a clinical setting. What molecular differences distinguish fully functional human HSCs obtained in vivo from their in vitro iPSC-derived counterparts? While comparative transcriptomic studies of in vivo HSCs and in vitro iPSC-derived populations have led to significant insights as to the differences between these populations, they have also been hindered by a number of potentially confounding effects. The inclusion of non-functional cells due to the previous absence of a pure HSC cell surface marker profile, and the effect of individual genetic background variation on differential gene expression in genetically-diverse samples, might all lead to the detection of differentially-expressed genes that may not be affecting cell function, or the masking of more subtle changes that are critical for cell function. We hypothesized that a more focused view of molecular variation underlying functional differences could be achieved by performing comparative analysis on genetically matched cells. To accomplish this goal, we performed the first single-cell transcriptomic analysis of in vivo pure HSCs and their genetically matched in vitro iPSC-derived HSPC counterparts. By eliminating genetic background as a source of variability, we discovered novel differences in regulatory pathways modulated by miRNAs that were only seen when genetic background was taken into account. Furthermore, when the identified miRNAs were modulated during differentiation, this led to a significant increase in both overall hematopoietic differentiation efficiency, as well as an increase in several reported key HSC genes. Further analyses are ongoing, but we propose that modulation of these identified key miRNAs during hematopoietic differentiation provides a novel potential strategy towards the successful and reliable derivation of clinically viable HSCs in vitro from human pluripotent stem cells.

Funding Source: This research was funded in part by the National Institute on Aging, National Institutes of Health, grant number R21AG078872-01A1 (to ADP).

T1332

SPATIOTEMPORAL MAPPING OF MORPHOGENESIS AND DIFFERENTIATION IN PANCREAS DEVELOPMENT BY SPATIAL TRANSCRIPTOMICS

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Single-cell RNA sequencing has been a prevailing method to identify and discover cell types in an organ and has been generated a vast amount of data across tissues and species. However, some caveats, such as loss of positional arrangements of cells and under- and/or over-representation of certain cell types, are inevitable due to cell dissociation. Spatial transcriptomics is an emerging, cutting-edge technology enabling not only transcriptional profiling but also spatial mapping of cells in an organ. Recent advancement of this method has resulted in data visualization in single-cell resolution. Pancreas is an organ consisting of exocrine and endocrine cells, which arise from

pancreatic progenitor cells during development. Architectural organization of branched pancreas with terminal acini connected to ductal network and nearby islets of Langerhans coincides with cell differentiation in embryos. Although snapshots of different cell type emergence in pancreas have been shown by multiple techniques and animal models, spatial transcriptomics in high resolution has not yet been tested. Here, we use Visium HD (10X Genomics) with formalin-fixed paraffin-embedded sections of embryonic pancreata (E12.5, E14.5, E16.5, E18.5) for spatiotemporal mapping of morphogenesis and differentiation in development. We are in the process of cell type analysis and mapping after clustering of transcriptomics data based on cell segmentation in hematoxylin-and-eosin stained images. Our preliminary data confirm lineage progression of bi-potent progenitors, early acinar progenitors, early and late islets, and surrounding mesenchyme. One of technical difficulties is to prepare samples with a good RNA quality, especially later stages of pancreas at E16.5 and E18.5, due to the prevalent expression of RNases in acinar cells and slow penetration of fixative. We are seeking enhanced sample preparation procedures for the late stages of pancreas samples. When samples are ready at different stages, spatial transcriptomics will be an excellent benchmark for unbiased spatiotemporal development of the organ.

T1334

STAGEWISE INDUCTION OF RETINAL PIGMENT EPITHELIUM FROM HUMAN PLURIPOTENT STEM CELLS IN CHEMICALLY DEFINED CONDITIONS

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Age-related macular degeneration (AMD) impairs central vision in almost 9% of people globally. During the development of AMD, the degeneration of retinal pigment epithelium (RPE) leads to the loss or dysfunction of photoreceptors in retina, resulting in vision loss. RPE transplantation is a promising approach to treat AMD, however most RPE induction methods utilize spontaneous differentiation in culture systems supplemented with animal or serum products. In this report, we develop a chemically defined culture system to stagewisely induce RPE differentiation. The new platform accelerates the differentiation process, and allows RPE induction without colony isolation. The cell product demonstrates typical RPE phenotypes and functions. This method will be beneficial to the development AMD treatment with iPSC-derived RPE in the near future.

Funding Source: This work was supported by the University of Macau (File No. MYRG-GRG2024-00192-FHS) and by the Science and Technology Development Fund, Macau SAR (File No.0010/2023/AKP and 0002/2023/RIB1).

T1336

STEMBROIDS - PLURIPOTENT STEM CELL DIFFERENTIATION PLATFORM AS A NOVEL SOURCE FOR PATIENT SPECIFIC TRANSPLANTABLE CELLS

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Since the discovery of induced pluripotent stem cells (iPSCs) in 2006 and their first clinical application in 2014, progress in the development of iPSC-based therapeutic agents has been relatively modest, with iPSC-derived products only being administered to humans for approximately 7–8 years, yet any of them are clinically approved. This limited advancement is largely attributed to challenges in directed in vitro differentiation protocols, genomic instability, vague target cell identity, and poor engraftment. RenewalBios' platform utilizes innovative 3D human developmental models, termed "stembroids," derived from iPSCs. This groundbreaking approach is based on three patented technologies: First, a unique naïve media composition enabling enhanced differentiation plasticity, allowing iPSCs to generate not only the three germ layers but also extraembryonic tissues. Second, the stembroid generation process creates developmental models by aggregating iPSCs, their derivative placental, and yolk sac precursor cells, providing optimal conditions for proper differentiation while avoiding ethical obstacles associated with real embryos. Third, a specialized ex-utero culture device supports natural development and differentiation of genuine cell types by providing the optimal physiological and mechanical conditions. Together, these technologies establish a robust and unique platform for generating authentic, and functional cells for various therapeutic applications. The stembroid platform represents a holistic and multifaceted approach to cell therapy. Unlike traditional unilateral methods, a single stembroid protocol can yield various cell types, significantly enhancing cost-effectiveness and enabling the efficient production of multiple target cell types. This versatility, combined with improved authenticity and functionality, positions RenewalBios' platform as a superior alternative to conventional in vitro differentiation methods.

T1340

THE EFFECT OF GRAPHENE OXIDE SCAFFOLDS ON YAP1-MEDIATED CYTOSKELETAL REMODELING, MITOCHONDRIAL DYNAMICS, AND ADHESION IN INDUCED PLURIPOTENT STEM CELL-DERIVED CORNEAL ENDOTHELIAL CELLS

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Graphene oxide (GO) scaffolds are promising for supporting cell adhesion, survival, and growth. This study investigates GO's effect on iPSC-derived corneal endothelial cells (CECs), which are difficult to proliferate in vitro, focusing on the YAP mechanotransduction pathway and its role in mitochondrial function and actin cytoskeletal remodeling. Two types of CECs—an immortalized cell line and iPSC-derived CECs—were cultured on 20 nm thick GO-coated surfaces for up to 5 days. Western blotting, immunocytochemistry (ICC), RT-PCR, and RNA interference (RNAi) assays were used to explore YAP signaling and mitochondrial interaction. Antibodies specific to YAP1, TAZ, F-actin, and mitochondrial marker ATP5A were used to analyze protein expression, while qPCR assessed gene changes. GO scaffolds were non-cytotoxic and promoted CEC proliferation, as demonstrated by increased expression of Ki-67, phospho-histone H3, and phosphorylated ERK. GO enhanced cell adhesion through upregulation of N-cadherin and Focal Adhesion Kinase while preserving the intrinsic characteristics of CECs. YAP signaling was significantly activated in the GO group. YAP1-RNAi analysis confirmed that GO scaffolds regulate mitochondrial dynamics, cytoskeletal remodeling, and adhesion via YAP1 signaling, emphasizing their mechanotransduction effects on iPSC-derived CECs. Furthermore, a reduction in ATP5A expression suggests that GO-YAP1 interactions influence mitochondrial functionality, linking YAP signaling to cellular energy dynamics and highlighting the role of GO scaffolds in supporting iPSC-derived CECs growth through mechanotransduction pathways. This study confirms that GO scaffolds improve iPSC-derived CECs adhesion and proliferation by activating YAP signaling in

response to mechanical cues, influencing mechanotransduction, cytoskeletal remodeling, and mitochondrial dynamics to regulate iPSC-derived CECs growth.

Funding Source: This work was supported by a grant of the Korea Health Technology Research and Development Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health and Welfare, Republic of Korea (RS-2024-00438366).

T1342

THE POTENTIAL ROLE OF CDH1 AND CTNND1 IN ENDOMETRIAL RECEPTIVITY

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Infertility is a major health care issue and many infertile women require assisted reproduction technology to get pregnant. Despite improvements in in vitro fertilization, repeated implantation failure (RIF) remains a significant challenge for couples. One factor contributing to this failure is inadequate endometrial receptivity. Our previous study demonstrated the important role of endometrial E-cadherin (CDH1) during embryo implantation. With the use of an embryo mimic derived from human expanded potential stem cell (hEPSC), this study aimed at investigating the roles of CDH1 and its stabilizer p120ctn (CTNND1) in endometrial receptivity. The functional role of CDH1 in endometrial receptivity was studied by overexpression of CDH1 in non-receptive endometrial epithelial HEC-1B cell line. hEPSC derived trophoblastic spheroid was cocultured with CDH1 overexpressing HEC-1B cells to test the receptivity. CDH1 protein induction was confirmed in HEC-1B. However, CDH1 overexpression had no improvement in the attachment rate as compared with receptive endometrial epithelial Ishikawa cells. In conclusion, overexpression of CDH1 in non-receptive HEC-1B cell was not sufficient to induce the receptivity of the cells, indicating the involvement of multiple molecules which warrant future investigation.

T1344

TOTIPOTENT STEM CELL REVEALS TRANSCRIPTIONAL REGULATORY ACTIVITY OF TRANSPOSABLE ELEMENTS

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Zygotic genome activation (ZGA) is a significant event of mammalian pre-implantation embryo development, standing for the initiation of oocyte-to-embryo transition with the ebullition of transcription activity. Transposable elements (TEs) are short and repetitive sequences that composite the majority of mammalian genome with retrotransposition ability. Multiple TEs are highly transcribed in this stage. Moreover, instead of being a selfish byproduct in this awakening transcription, a growing body of evidence suggests these elements acquire an essential role and can contribute to ZGA. Nevertheless, the role of various classes of TEs and their mechanism of activation in embryo remains enigmatic. With the use of totipotent stem cells, we exploit the

potential of how TEs participate in the access of totipotency in in vitro models. Our data evaluates the contribution of different class of TEs and disclose their potential regulatory mechanisms. Comprehensively, the advancement of totipotent stem cells provides an invaluable system with their distinguishing resemblance to cleavage state embryo, enabling the acceleration of the research progress of TEs in ZGA.

Funding Source: This project is supported by Health@InnoHK, Innovation Technology Commission, HKSAR.

T1346

TRACING THE DEVELOPMENTAL PROGRESSION OF HUMAN HAEMATOPOIETIC STEM CELLS BETWEEN HUMAN FOETAL LIVER AND FOETAL BONE MARROW: A SNAPSHOT

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Haematopoietic stem cells (HSCs) are multipotent cells characterised by self-renewal, multilineage differentiation and engraftment ability. Human HSCs that first emerge in the aorta-gonad-mesonephros, transition to foetal liver (FL) where they undergo expansion and functional maturation. From mid second trimester onwards, HSCs start relocating to bone marrow (BM), the niche where they will reside throughout life to support haematopoiesis. However, the mechanisms underlying the HSCs transition from FL to BM in humans are poorly understood. To understand the factors driving the relocation of HSCs to BM and their molecular implications, we investigated the dynamic changes in FL and BM HSCs derived from the same embryos at 19 weeks post conception, using a single-nucleus multiome assay, which simultaneously profiles gene expression and chromatin accessibility. We analysed the cell-intrinsic differences, comparing highly-purified HSCs isolated from both niches, and the cell-extrinsic cues of FL and BM environments, which contain the niche cells providing essential signals to HSCs. Transcriptome data shows that, while the expression levels of genes related to HSC maturity is comparable, the expression of HSC signature genes such as RUNX1, MLLT3, and HLF is increased in BM HSCs. In addition, we observe downregulation of the translational machinery in BM HSCs, suggesting the progression towards quiescence. Upregulation of genes linked to the granulocytic lineage points to an expansion of the multilineage potential of the HSCs in the bone marrow. Overall, chromatin accessibility exhibits minor changes at this stage. Aside from the intrinsic HSC changes, we also evidence various differences in ligand-receptor signalling from the niche to HSCs: cytokines and chemokines such as KITL and CXCL12, feature different expression levels and connectivity between FL and BM stromal cell populations, supporting an evolving role of the niche signalling within same-age HSCs. These findings contribute to an advanced understanding of the development of HSCs in humans, and highlight the need for suitable niche-derived support for functional HSC generation in vitro.

T1348

ULTRASLICE™ MRNA INCORPORATING 5-METHOXYURIDINE EFFICIENTLY INSERTS TRANSGENE SEQUENCES IN IPSCS WITH HIGH VIABILITY AND MITIGATES DSRNA PRODUCTION

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mRNA-mediated gene-editing allows for transient expression of editing proteins, reducing risks of insertional mutagenesis. However, mRNA can contain double-stranded RNA (dsRNA) byproducts of in vitro transcription (iVT). Previous research on mutant T7 RNA polymerases or modified nucleotides (NTs) to reduce immunogenicity has not focused on gene-editing. Since gene-editing-mediated insertion of transgenic sequences relies on cellular mechanisms of homology-directed repair, dsRNA-induced immune responses may limit editing efficiency. To address this challenge, we synthesized UltraSlice™ gene-editing mRNA with mutant T7 RNA polymerases and modified NTs to explore immunogenicity and editing efficiency in induced pluripotent stem cells (iPSCs). GFP-encoding mRNA was synthesized to analyze protein expression, dsRNA, and immunogenicity. RT-PCR of innate immune markers and dsRNA dot blot showed that the standard T7/canonical (AUGC) NT sample produced the greatest amount of dsRNA and induced a significantly higher immune response ($p < 0.01$) compared to samples synthesized with a mutant T7 and/or 5-methoxyuridine (5moU). This effect was consistent in iPSCs and mesenchymal stem cells (MSCs). Moreover, the standard T7/canonical NT condition produced 10-fold greater dsRNA than samples made with a mutant T7 and canonical NTs. While all synthesis conditions resulted in similar percentages of GFP-expressing cells when electroporated, median fluorescence intensity doubled in MSCs and iPSCs electroporated with mRNA synthesized with a mutant T7 or 5moU. Similar results were observed with UltraSlice™ gene-editing mRNA. iPSCs were co-electroporated with UltraSlice™-encoding mRNA and a GFP template for targeted insertion into TRAC. Mutant T7- or 5moU-containing mRNA maintained efficient editing when compared to mRNA made using standard conditions and outperformed mRNA synthesized with both a mutant T7 and 5moU by 4-fold. The iPSCs electroporated with these mRNA also maintained high viability (>85%) 48 hours post-electroporation. These results suggest that incorporation of 5moU alone is sufficient to reduce the generation of immunogenic dsRNA byproducts during iVT and enable efficient targeted insertion of transgene sequences in cells with high viability.

T1350

UNDERSTANDING TRANSCRIPTIONAL REGULATION IN TOTIPOTENCY-TO-PLURIPOTENCY TRANSITION FROM IN VITRO TO IN VIVO

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Mouse embryonic stem cells (ESCs) cycle in and out of 2-cell-like (2C-like) state in culture. The molecular mechanism governing the exit of 2C-like state remains obscure, partly due to the lack of a reporter system that can genetically mark intermediate states. Here, we identify an intermediate state that is marked by the co-expression of MERVL::tdTomato and OCT4-GFP (MERLOT) during 2C-like-to-pluripotent state transition. Using this reporter cell line, we systematically identified

transcription factors that regulate the transition process from totipotency to pluripotency. Our study identifies a genetically traceable intermediate state during 2C-like-to-pluripotent state transition and provides a valuable tool to study molecular mechanisms regulating the totipotency-to-pluripotency transition in vitro and in vivo.

T1352

UNVEILING THE ROLE OF M6A MODIFICATION IN HUMAN PLURIPOTENT STATE TRANSITION

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Human naïve pluripotent stem cells (PSCs), mirroring the in-vivo ground pluripotent state, exhibit plasticity and unbiased differentiation potential, capable of being induced into primed state PSCs or 8-cell(8c)-like cells. The N6-methyladenosine(m6A) modification has been identified as a pivotal regulator of cell fate, closely associated with the transcriptional programs governing totipotency in mouse embryonic stem cells. However, its specific impact on human pluripotency remains elusive. In this study we demonstrate that disrupting the function of METTL3 in human naïve PSCs, by knocking out or inhibiting METTL3, impedes the transition from the naïve state to the primed state, while fostering a shift towards an 8c-like state. METTL3 deficiency significantly boosts the expression and transcriptional activity of 8c-specific transcripts, elevates the proportion of 8c-like cells, and enhances their interspecies chimeric competency and their totipotency developmental potential. Additionally, METTL3 inhibition leads to a pronounced and rapid reduction in m6A levels. Interestingly, this reduction is not primarily observed in differentially expressed genes. Instead, it occurs predominantly in regulatory RNAs, including enhancer RNAs (eRNAs) and repeats, leading to increased chromatin accessibility. Overall, our findings suggest the potential regulatory role of m6A in the transcriptional programs transition between pluripotency and totipotency in human PSCs, underscoring the nuanced role of the epitranscriptome in human cell fate determination.

T1354

COMPLETE SUSPENSION CULTURE OF HUMAN INDUCED PLURIPOTENT STEM CELLS SUPPLEMENTED WITH SUPPRESSORS OF SPONTANEOUS DIFFERENTIATION

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Human induced pluripotent stem cells (hiPSCs) are promising resources for producing various types of tissues in regenerative medicine; however, the improvement in a scalable culture system that can precisely control the cellular status of hiPSCs is needed. Utilizing suspension culture without microcarriers or special materials allows for massive production, automation, cost-effectiveness, and safety assurance in industrialized regenerative medicine. Although many attempts have been made to develop suspension culture technologies enabling rapid and large-scale preparation of hiPSCs, completed processes from clonal hiPSC generation to mass production of hiPSCs based on the precise control of cell status have not yet been achieved. we have investigated what hampers the stable maintenance of undifferentiated cell states in suspension conditions. hiPSCs cultured in suspension conditions with continuous agitation without microcarriers or extracellular matrix (ECM) components were more prone to spontaneous differentiation than those cultured in conventional adherent conditions. From screening candidate molecules to suppress the spontaneous differentiation of hiPSCs, we have identified that inhibitors of PKCβ and Wnt signaling pathways suppress their differentiation into ectoderm and mesendoderm, respectively. In these conditions, we successfully completed the culture processes of hiPSCs, including the generation of hiPSCs from peripheral blood mononuclear cells (PBMCs) with the expansion of bulk population and single-cell sorted clones, long-term culture with robust self-renewal characteristics, single-cell cloning, direct cryopreservation from suspension culture and their successful recovery, and efficient mass production of a clinical-grade hiPSC line. Our methods are validated in several conventional culture media and many hiPSC lines. Thus, our findings show that suspension culture conditions with Wnt and PKCβ inhibitors can precisely control cell conditions and are comparable to conventional adhesion cultures regarding cellular function and proliferation. Our results demonstrate that precise control of the cellular status in suspension culture conditions paves the way for their stable and automated clinical application.

T1356

SIGNAL-INDUCED EMERGENCE ENHANCERS SAFEGUARD EMBRYONIC STEM CELL DIFFERENTIATION BY ORCHESTRATING CHROMATIN ARCHITECTURE-DEPENDENT CLUSTER GENE EXPRESSION

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Embryonic stem cells (ESCs) undergo fate transitions in response to external signaling stimuli, a process dependent on precise expression of the specific genes; however, the intrinsic regulatory mechanisms involved in this process remain largely unknown. Here, we integrated bulk RNA sequencing, single-cell RNA sequencing (scRNA-seq), 4D-SmartDIA quantitative proteomics and retinoic acid (RA) receptor deletion modeling to demonstrate that RA signaling-induced early ESC differentiation is characterized by a multi-lineage process, with Cyp26 and Hoxb cluster genes identified as its key targets. RA signaling activation leads to the emergence of RA-induced enhancers (RAiEs) in proximal/distal regions of the cluster gene loci, while inhibiting RA signaling markedly decreases the activity and chromatin accessibility of these RAiEs. Mechanistically, we found that RAiEs form specific chromatin loops with cluster gene loci upon RA induction, whereas these chromatin interactions are significantly reduced in RARs knockout cells. Deletion of proximal/distal RAiEs suppressed cluster gene expression, thereby disrupting ESC differentiation.

Our results reveal a critical mechanism that highlights the importance of the specific 3D chromatin structure of RAiEs in regulating cluster gene expression and ESC fate transitions.

T1358

INTEGRATING SINGLE-CELL RNA-SEQ AND SPATIAL TRANSCRIPTOMICS REVEALS THE ROLE OF MMP14 AS AN IMMUNE-RELATED REGULATOR FOR ENDOCHONDRAL BONE INTEGRITY

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Hypertrophic chondrocytes (HCs) in the growth plate contribute to endochondral bone growth by continuing to differentiate, giving rise to osteoblasts in trabecular bone and adipocytes in the bone marrow. Precise regulation of the HC differentiation program is critical for their proper lineage continuum. In this study, we found that the loss of MMP14 in HCs and its descendants led to a decrease in trabecular bone in adult mice, while cortical bone and bone marrow adipose tissue were unaffected. Further analysis revealed a decrease in HC-derived osteoblasts in Mmp14 cKO mice, suggesting that Mmp14 is necessary for maintaining chondrocyte-osteoblast lineage continuum and skeletal stem and progenitor cells (SSPCs) in adult mice. Single-cell RNA sequencing, spatial transcriptome analysis and protein omics analysis identified alterations in major signaling pathways involved in bone development, including extracellular matrix organization, TGF-β signaling, and TNF signaling. The TGF-β signaling pathway was found to be disrupted, with decreased expression of its target genes. Additionally, an increase in neutrophils was observed in Mmp14 cKO mice, which was further confirmed by spatial transcriptomics. The activation of neutrophils was detected in the hypertrophic zone-trabecular region and bone marrow of Mmp14 cKO mice. Cell-cell interactions between immature neutrophils and osteogenic cells were identified, suggesting a potential role of these interactions in bone loss. Overall, this study provides insights into the role of Mmp14 in maintaining bone homeostasis and highlights the importance of HC-derived cells in bone formation and immune regulation.

Funding Source: HMRF07183766 and HMRF09202246

T1360

LOSS OF UBIQUITIN-SPECIFIC PROTEASE 11 ALLEVIATES PULMONARY FIBROSIS IN HUMAN PLURIPOTENT STEM CELL-DERIVED ALVEOLAR ORGANOIDS

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Idiopathic pulmonary fibrosis (IPF) is a fatal chronic interstitial lung disease (ILD) of unknown etiology and mechanism that causes pathological lung scarring leading to lung stiffness, impaired gas exchange and ultimately premature death. Deubiquitinase (DUB) is an important protease that regulates the degradation of target proteins by modulating ubiquitin signaling and has been implicated in several diseases, including pulmonary fibrosis. USP11 has been reported to promote fibrogenesis by stabilizing the pro-fibrotic protein GREM1, and its significant increase in IPF patient tissues suggests that USP11 could be a key therapeutic target for IPF treatment. However, the role and regulatory mechanisms of USP11 in IPF have not been reported. Here, we aimed to investigate the role of USP11 in the progression of pulmonary fibrosis using human induced pluripotent stem cell (hiPSC)-derived alveolar organoids (AOs). To this end, we generated hiPSC-USP11KO using the CRISPR/Cas9 system and confirmed that the pluripotency status of hiPSC-USP11KO was not altered by genome editing. We also found that the expression of alveolar type 2 (AT2) markers (SFTPC and ABCA3) and alveolar epithelial progenitor cell marker NKX2.1 were decreased corresponding to the decrease of USP11 in hiPSC-USP11KO-derived AOs. Interestingly, the depletion of USP11 resulted in decreased collagen deposition and reduced fibrosis markers both at the protein and mRNA levels following TGF-β treatment to characterize pulmonary fibrosis. Collectively, our study suggests that USP11 might be a potential therapeutic target to mitigate pulmonary fibrosis.

Funding Source: This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MSIT) (RS-2022-NR067319).

T1362

MTOR and TGFB SIGNALING REGULATE IN VITRO EMBRYO LINEAGE DEVELOPEMNT FOLLOWING ATTACHMENT TO ENDOMETRIAL ASSEMBLOIDS

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Ethical reasons restrict our ability to study human embryo implantation in vitro. For this vital reason, our lab established an endometrial assembloid model which can be used in co-culture studies with human blastocysts. For our in vitro implantation assay, endometrial assembloids (n=12 biopsies) were hormonally stimulated with 8-bromo-cAMP, estradiol, and a progestin to induce decidualisation. Human 5 days post fertilisation (dpf), hatched blastocysts (n=78) were positioned on the surface epithelium of assembloids and cultured at 37°C, 6% CO2 and 5% O2.

The average percentage of successful embryo attachment was 70%, ranging from 83.3 to 58.3%. We observed that early invasion of human blastocysts coincides with migration of surface epithelial cells away from the attachment site. We further showed that the described co-culture conditions promoted trophectoderm differentiation while the development of ICM lineages was suboptimal at 8 dpf. We set out to optimise culture conditions with emphasis on growth factor supplementation. Human blastocysts at 5 dpf (n=17) were co-cultured with decidualised assembloids (n=4 biopsies) at 5% O2 until 7 dpf and 20% O2 until 8 dpf. The basal co-culture medium was supplemented with IGF1 (50ng/µl) and/or ActivinA (100ng/µl). Attachment of embryos was assessed upon overnight co-culture by liquid disturbance. Embryo development and presence of embryonic lineages was confirmed by immunofluorescence antibody labelling for NANOG (epiblast), GATA4 (primitive endoderm) and GATA3 (trophectoderm), and 3D fluorescent visualization was performed using confocal microscopy. IGF1 supplementation resulted to a 3-fold increase in total ICM cell numbers. Supplementation of both IGF1 and ActivinA resulted to a 6-fold and 2-fold increase in epiblast and primitive endoderm average cell numbers, respectively. Moreover, post-implantation embryos were larger in diameter, from average 379µm to 578µm. Our findings signify that mTOR (IGF1) and TGFβ (ActivinA) signalling pathways ameliorate in vitro embryonic development at post-implantation stages. The ability to establish co-cultures using endometrial models and blastocysts is poised to lead to major advances in our understanding of mechanisms of reproductive success and failure, nonetheless, a plethora of technical hurdles remain.

Funding Source: EUTOPIA funding between the University of Warwick and VUB for PhD project and Wetenschappelijk Fonds Willy Gepts (WFWG) funding by UZ Brussel for work at VUB

T1364

AI-ENABLED AUTOMATION OF 3D ORGANOID CULTURE

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3D organoid models are increasingly important for biological research and drug development, however because of complexity of steps those processes are difficult to automate. To enable automated control of cell culture, we developed the automation solution CellXpress.ai. CellXpress.ai contains four essential components for automated organoid culture: liquid handler, automated incubator and imager, plus integrated AI-powered software that that provides automated processing of complex protocols. Automated 3D organoid culture includes processes of plating organoid domes, periodic media exchanges, and periodic monitoring by imaging and analysis. It provides automated passaging of organoids, which can be triggered by AI-based organoid classification based on organoid phenotypes. We developed AI-based protocols and present results from the automation of three different organoid types: mouse intestinal organoids, human intestinal organoids, and patient-derived colorectal tumor organoids. 3D organoid cultures were started from seeding organoids into matrigel domes in 24 well plate format. We automated organoid culture protocols using media kits for mouse and human organoids recommended by STEMCELL Technologies. Media exchanges were done automatically every 24 hours. Passaging organoids were also performed automatically using liquid handling, but timing for passaging depends on maturation of organoids, which requires decision action done either by scientists of by

software. Cultures were monitored by imaging every 12 hours with transmitted light and 4X magnification by integrated imager. Then machine-learning based image analysis allowed to detect organoids and provide analysis of organoid objects to measure variety of phenotypic read-outs including size, density, and texture measurements. Phenotypic classification of organoids for mature and immature phenotypes was done by pre-trained model that combined un-supervised and supervised machine learning. We created models for three tested organoid types. Passaging steps were then triggered automatically by a user-defined percentage and number of mature organoids in the culture, typically >50%. The AI-based classification allowed to fully automate 3D culture and expansion of organoids, also to increase productivity and throughput.

Friday, 13 June 2025

TRACK: ORGAN GENERATION AND REGENERATION (OGR)

Poster Session 3 (ODD)
4:00 PM – 5:00 PM

F1001

ENGINEERED EXOSOMES FOR EFFICIENT DELIVERY OF REJUVENATING FACTORS FOR TISSUE REPAIR AND REGENERATION

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Research in fetal development and adult tissue regeneration has uncovered multiple conserved signaling pathways regulating organ morphogenesis and progenitor cell activation in adult tissue. This knowledge has helped us understand the complexities of organ and tissue morphogenesis and, more importantly, enabled multiple research breakthroughs. These include isolating and expanding adult stem cells from healthy and diseased tissues and generating organoid cultures that mimic human organs. Amongst these highly conserved pathways, WNT signaling is reportedly active in multiple organs during development, regeneration, and disease. The loss of WNT activity in rare genetic conditions resulted in pleiotropic multi-organ phenotypes, highlighting the importance of this pathway in humans. This central and conserved regulatory role makes activating the WNT signaling pathway an enticing candidate for developing therapeutics to achieve multiple organ regeneration. Herein, we report the efficient loading of all 19 WNT ligands on engineered exosomes. We demonstrate that cell lines could be engineered to produce exosomes enriched with functional WNT ligands that could be delivered long-range to target organs. RSPONDINs, a key modulator of the WNT signaling pathway, could also be loaded onto exosomes and exhibit synergistic activity. To assess the therapeutic potential of these exosomes, we systematically evaluated the effects of exosome treatment in mice with various liver conditions. We discovered the remarkable capacity of the exosome to efficiently rescue mice from acute injury and reverse chronic phenotypes in the liver. This panel of WNT signaling-inducing exosomes opens up a novel avenue for evaluating WNT ligand activity in various biological systems and offers an alternative strategy for inducing adult tissue regeneration.

Funding Source: Guangzhou laboratory.

F1003

CLEAR DISTINCTIONS BETWEEN MESENCHYMAL STROMAL CELLS AND PERICYTES DERIVED FROM MOUSE LUNG TISSUE REVEALED BY A SINGLE CELL TRANSCRIPTOMIC ANALYSIS

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The distinct identity of mesenchymal stromal cells (MSCs) versus pericytes remains unknown, but both are reported to be associated with endothelial cells, regulating the permeability of capillary vascular system. The present study was undertaken to unveil the distinctions between MSCs and pericytes by a single-cell transcriptomic analysis. Mouse lung tissue was obtained via thoracotomy, and the bulk of all different cells from the entire tissue was subjected to scRNA-seq analysis. Single-cell functional gene expression was analyzed to identify MSCs by Tmem119, Fbln5, Kcnk2, Cldn11, and Dkk1, and pericytes by Pdgfrb, Cspg4, Cox4i2, Kcnk3 and Ndufa4l2. There were about 0.01% of MSCs and 1% of pericytes in the overall lung tissue cell populations. After these cells were cultured for 3 passages, the MSCs were increased to 5% of the total populations and the pericytes remained the same proportion. There were about 80% of MSCs expressing genes enriched in mitosis and cell cycle regulation pathways, and 20% of MSCs sharing a global gene expression profile with that of pericytes, with genes enriched in the PDGF signaling and extracellular matrix degradation pathways. Single-cell trajectory analysis revealed that MSCs and pericytes trailed different developmental lineages reaching at distinct terminal stages of differentiation, indicating a parallel rather than sequential differentiation paths between MSCs and pericytes. In addition, MSCs displayed a farther developmental cliff than that of pericytes from embryonic stem cells. Cell–cell communication analysis based on single-cell transcriptomic enrichment identified that cultured MSCs secreted FBLN5, CLDN11, and TMEM119, stimulating pericytes to release tissue repair and angiogenesis signals, including TIMP, PDGF, Cxcl12, and VEGFA. This study thus unveils clear distinctions between MSCs and pericytes, and in the lung, pericytes would be the majority of cells associated with endothelial cells for the regulation of vascular permeability. However, MSCs would play a stimulatory action on pericytes for their function of angiogenesis, but MSCs and pericytes trail distinct lineages from development hierarchy.

F1005

CONSTRUCTION OF A LIVER LOBULE-LIKE TISSUE

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Three-dimensional organoid technologies have revolutionized research in human organs and diseases. However, available liver organoid systems failed to construct a perfusable vasculature and in vivo liver lobule architecture. The lack of vascular networks restricts the size of organoid and limit the multicellular communications. Here we develop a millimeter-scale vascularized liver lobule-like tissue(vLB) with a perfusable capillary by taking advantage of microfluidic chip and cellular self-assembly. In vLB, hepatocytes interacted with non-parenchymal cells, forming structures analogous to the in vivo hepatic plate and space of Disse while maintaining the functionality of hepatocytes. Endothelial cells in vLB acquired certain molecular and structural characteristics of liver sinusoidal endothelium. Based on vLB, we also integrate innate immune cells into this tissue by perfusing monocyte-derived macrophages. Macrophages reside in the vessels, in prime position to respond to any pathogens entering the vLB via the vessels. The concept of extrinsically guiding the self-organization of multiple cell types into functional vascularized tissues is broadly applicable and holds promise for achieving more physiologically relevant tissue structures and functions.

F1007

FIBROBLAST-MEDIATED EPITHELIAL REGENERATION CAPTURED IN BIOENGINEERED HUMAN MINI-COLONS

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While organoid-based models offer valuable insights into gut biology, their lack of key microenvironmental components—such as vasculature, mesenchyme, and immune cells—limits their physiological relevance and utility. As such, intestinal fibroblasts play essential roles in gut epithelial homeostasis, matrix remodeling, and inflammation. Here, we leveraged bioengineered human mini-colons to establish a co-culture platform with intestine-derived fibroblasts. The biomimetic extracellular matrix (ECM) surrounding mini-colon tissue readily accommodated fibroblasts, enabling direct interactions between the two compartments.

Notably, fibroblast addition facilitated microchannel epithelialization and epithelium formation, mirroring colonic repair in vivo. Bulk RNAseq and immunofluorescence analysis revealed transient epithelial reprogramming into a regenerative state, with upregulation of regenerative and wound-associated epithelial (WAE) cell markers during early stages of the epithelium formation. Interestingly, this regenerative phenotype gradually diminished as cells established a tightly packed columnar epithelium and was entirely absent in mini-colons cultured without fibroblasts. Our findings demonstrate that this repair phenotype is driven by soluble factors secreted by fibroblasts, mediating epithelial changes via paracrine signaling. Proteomic profiling of fibroblast-conditioned media identified 467 unique proteins, categorized into ECM proteins, secreted growth factors, and other signaling molecules. Remarkably, the identified growth factors were conserved across the secretomes of two distinct fibroblast lines—both commercially available and in-house intestine-derived—cultured under the same conditions. Functional validation further revealed the key ligands playing a role in modulating epithelial restitution in mini-colons. Together, our results underscore the critical role of fibroblasts in epithelial repair and provide a dynamic, human-relevant co-culture system to investigate epithelial-stromal interactions observed in vivo.

Funding Source: This work was funded by support from the Swiss National Science Foundation (SNSF) research grant 310030_179447, the EU Horizon 2020 Project INTENS (#668294-2), Ecole Polytechnique Fédérale de Lausanne (EPFL), and Roche.

F1009

TRANSIENT PRMT-1 INHIBITION ENHANCES DEVELOPMENT OF HESCS INTO PANCREATIC ENDOCRINE LINEAGE IN VITRO

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Neurogenin 3 (NGN3) is a key transcription factor in the pancreatic endocrine cell development. Transient expression of NGN3 is essential for transition of endocrine progenitors (EPs) into endocrine cells (ECs). It is known that the transcriptional activity of NGN3 and its degradation are regulated by post-translational modifications such as phosphorylation. By using knockout system of protein arginine methyltransferase-1 (PRMT-1) in human embryonic stem cells (hESCs), recently, we found that methylation on 65 arginine of NGN3 is crucial for its transcriptional activity and rapid degradation. In this study, we hypothesized whether PRMT-1 inhibition supports the development of hESCs into pancreatic endocrine lineage. To address this question, hESC-derived EPs were transiently treated with a PRMT-1 inhibitor X and then cultured until the EC stage. As results, transient PRMT-1 inhibition increased expression levels of EP-specific markers (NGN3, NEUROD1 and PAX6) in the EPs, and enhanced the transcriptional expression of EC-associated genes such as INS and MAFA in the ECs. Proportion of ECs expressing c-peptide was significantly enhanced in the PRMT-1 inhibition group compared to the non-treated group. Furthermore, pancreatic islet-like organoids (PIOs) developed from the ECs secreted human insulin upon glucose stimulation. Collectively, our findings demonstrate that transient inhibition of PRMT-1 in EPs can induce efficient differentiation of ECs during pancreatic development of hESCs in vitro.

Funding Source: Korea Fund for Regenerative Medicine.

F1011

DYNAMIC ROLES OF RNA MODIFICATIONS IN HUMAN IPSC-DERIVED HEART ORGANOID

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As one of the earliest organs formed during human development, the heart plays an essential physiological role. However, related research relies on animal models due to the difficulty of obtaining human specimens. Differences between species result in specific physiological structures and molecular mechanisms that cannot fully reflect human cardiac development. The use of induced pluripotent stem cell (iPSC)-derived heart organoids allows for the self-assembly of three-dimensional tissues. Compared to animal models or monolayer two-dimensional cells, organoids can better simulate tissue structure and function in vitro. RNA modifications are critical regulatory during embryonic development by controlling RNA biogenesis and are essential in various cellular developmental processes, including embryonic, hematopoietic, reproductive, and neural development. However, research focusing on their role in cardiac development remains unclear. The heart organoids (HO) we established can differentiate into the heart's main cell types, including ventricular and atrial cardiomyocytes, endothelial cells, and fibroblasts. Additionally, HO exhibits contractile function and electrophysiological characteristics, closely resembling the human heart, making it an important platform for developmental research. Furthermore, we found that during different stages of HO development, RNA modifications and the expression of related regulatory proteins undergo dynamic changes. Therefore, this study will investigate the potential roles of RNA modifications in HO development and explore their possible regulatory mechanisms. These findings may provide valuable insights into the molecular mechanisms underlying human cardiac development and disease.

Funding Source: This project was partially supported by the National Science and Technology Council under the grant NSTC 112-2320-B-A49-049-MY2.

F1013

HYDROGEN GAS AND ITS ROLE IN MAINTAINING INTESTINAL STEM CELL INTEGRITY DURING MESENTERIC ISCHEMIA AND REPERFUSION

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Patients with mesenteric ischemia frequently suffer from bowel necrosis even after revascularization. Hydrogen gas has showed promising effects for ischemia-reperfusion injury by reducing reactive oxygen species in various animal and clinical studies. We examined intestinal tissue injury by ischemia and reperfusion under continuous initiation of 3% hydrogen gas. Aim: To clarify the treatment effects and target cells of hydrogen gas for mesenteric ischemia. Three rat groups underwent 60-min mesenteric artery occlusion (ischemia), 60-min

reperfusion following 60-min occlusion (reperfusion), or ischemia-reperfusion with the same duration under continuous 3% hydrogen gas inhalation (hydrogen). The distal ileum was harvested. Immunofluorescence staining with caspase-3 and leucine-rich repeat-containing G-protein-coupled 5 (LGR5), a specific marker of intestinal stem cell, was conducted to evaluate the injury location and cell types protected by hydrogen. mRNA expressions of LGR5, olfactomedin 4 (OLFM4), hairy and enhancer of split 1, Jagged 2, and Neurogenic locus notch homolog protein 1 were measured by quantitative polymerase chain reaction. Tissue oxidative stress was analyzed with immunostaining for 8-hydroxy-2'-deoxyguanosine (8-OHdG). Systemic oxidative stress was evaluated by plasma 8-OHdG. Ischemia damaged the epithelial layer at the tip of the villi, whereas reperfusion induced extensive apoptosis of the cells at the crypt base, which were identified as intestinal stem cells with double immunofluorescence stain. Hydrogen mitigated such apoptosis at the crypt base, and the LGR5 expression of the tissues was higher in the hydrogen group than in the reperfusion group. OLFM4 was also relatively higher in the hydrogen group, whereas other measured RNAs were comparable between the groups. 8-OHdG concentration was high in the reperfusion group, which was reduced by hydrogen, particularly at the crypt base. Serum 8-OHdG concentrations were relatively higher in both reperfusion and hydrogen groups without significance This study demonstrated that hydrogen gas inhalation preserves intestinal stem cells and mitigates oxidative stress caused by mesenteric ischemia and reperfusion.

F1015

MACROPHAGE-MEDIATED ACTIVATION OF HAIR FOLLICLE STEM CELLS IN ALLERGIC CONTACT DERMATITIS

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Allergic contact dermatitis (ACD) has been utilized as a therapeutic approach for alopecia areata, an autoimmune hair loss condition driven by T cell-mediated Th1 responses. Despite its clinical use, the underlying mechanisms by which ACD stimulates hair regrowth remain unclear. In this study, we demonstrate that ACD induces early anagen entry in wild-type mice, suggesting a direct role in promoting hair growth. Hair follicle stem cells (HFSCs), critical for hair regeneration, are regulated by dynamic signals from the local microenvironment. We hypothesize that macrophages recruited during the ACD response mediate HFSC activation through specific signaling pathways, thereby initiating a new hair cycle. To test this hypothesis, we employed diphenylcyclopropenone (DPCP) as an allergen to establish a robust ACD-induced hair growth model. Transcriptomic analysis using bulk RNA sequencing (RNA-seq) revealed significant changes in gene expression associated with immune cell recruitment and activation in the skin. Further, we utilized immune cell depletion strategies to dissect the role of macrophages in this process. Our findings highlight macrophages as key mediators in ACD-driven hair regeneration, shedding light on the crosstalk between immune responses and HFSC activation. This study not only advances our understanding of ACD-induced hair regrowth but also opens new avenues for developing macrophage-targeted therapies for

alopecia.

F1017

PROMOTING CARDIOMYOCYTE PROLIFERATION BY INDUCING CENTROSOME ASSEMBLY

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Adult human cardiomyocytes (CMs) have limited ability to regenerate. When they are lost after injury or disease, the heart often suffers permanent damage. Promoting cardiomyocyte proliferation is therefore an important avenue to repair the heart. Centrosomes are an established mediator of proliferation in many cell types but its role in CM proliferation is not well understood. We recently showed that verapamil, an L-type calcium channel blocker, could promote the proliferation of human pluripotent stem cell-(hPSC-) CMs. We aim to comprehensively evaluate its effect on CMs and dissect its mechanisms, particularly in relation to centrosomes. Our results showed that verapamil enhanced hPSC-CM proliferation, as measured by EdU incorporation and Ki67 staining, and increased nuclear and cell count. Both mononucleated and binucleated hPSC-CMs contributed to proliferation. Verapamil treated hPSC-CMs adopted a developmentally immature, embryonic-like phenotype, in terms of sarcomeric structure and mitochondrial function. The proliferative and immature phenotype induced by verapamil is reversible, and hPSC-CMs resumed maturation upon cessation of treatment. Although verapamil promoted an immaturity, treated cells retained cardiac identity, and did not express markers of pluripotency. Mechanistically, transcriptomic analysis of verapamil treated hPSC-CMs revealed a molecular profile consistent with increased cell cycle activity and reduced maturation. Bioinformatics analysis revealed upstream regulators governing these changes. Lastly, we demonstrated that hPSC-CMs underwent centrosome reassembly upon verapamil treatment, and this was positively associated with proliferation. Altered centrosome assembly could modulate hiPSC-CM proliferation. In conclusion, our data confirms that verapamil can induce cardiomyocyte proliferation, and that this is accompanied by the acquisition of an embryonic phenotype. The effect of verapamil is reversible, thus allowing controlled, regulatable proliferation. We further revealed centrosome assembly as a key contributor to hiPSC-CM proliferation induced by verapamil. Our study provides new understanding of about CM proliferation and centrosome assembly to aid in the development of new strategies to promote cardiac regeneration.

Funding Source: Health and Medical Research Fund.

F1019

RECONSTRUCTING THE COMPLEX HUMAN AIRWAY NICHE TO STUDY TISSUE REPAIR AND AGING

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Human organoids derived from reprogrammed and tissue stem cells offer a valuable in vitro platform for studying human development, regeneration, and disease modeling. Current platforms mainly focus on generating airway epithelial cells from human ESCs and iPSCs but do not effectively translate complex communications across various cellular and tissue niches of the human airway. Moreover, there is a lack of consensus benchmarking standards across different organoid platforms and human samples. Addressing this knowledge and technological gap, we developed an improved method to create human airway organoids containing both epithelial and stromal lineages. We introduced a co-culture approach incorporating blood-circulating monocytes, leading to enhanced mesenchymal-epithelial crosstalk, the emergence of fetal-specific pulmonary neuroendocrine cells, and the differentiation of monocytes into self-sustaining alveolar macrophages. Using these complex organoids, we demonstrate the repair trajectory of airway progenitors upon viral infection and chemical-induced epithelial loss. In addition, we characterize the effect of inflammaging on airway repair mediated through macrophages derived from young and aged donors. Our analyses underscore the importance of constructing a complex signaling niche for a better understanding of human tissue regeneration, highlighting the potential of this research in translating discoveries into clinical applications.

F1021

REFATE IDENTIFIES CHEMICAL COMPOUNDS TO TARGET TRANS-REGULATORY NETWORKS FOR CELLULAR CONVERSION

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The identification of chemical compounds to target trans-regulatory networks (TRNs) that cut across molecular and cellular programs for directed cellular conversion is a crucial step towards regenerative medicine. Recent advances in single-cell resolution multi-omics technologies provide a new opportunity for capturing the complexity and cell-type-specificity of TRNs for controlling cell identity and cell-fate decisions. Here, we introduce Refate, a computational framework that integrates large-scale multimodal single-cell atlas data from humans and mice and a repertoire of six drug databases for identifying genes with high cell propensity and subsequently predicting chemical compounds that target TRNs for converting cells from a starting type to a target type with minimum input from users. The multilayered prediction of TRNs, comprised of protein complexes and gene regulatory networks, and chemical compounds that drive the cellular conversion by Refate increases biological interpretability and enhances efficacy. We evaluated Refate on its ability to uncover previously known TFs and chemical compounds that drive experimentally validated cellular conversions of various cell types. This demonstrates the potential of Refate as a valuable tool for identifying

chemical compounds to target TRNs for cellular conversion.

Funding Source: National Health and Medical Research Council Investigator grant (1173469).

F1023

SENESCENCE OF EXPANDED CARDIOMYOCYTES AND ITS IMPACT ON MYOCARDIAL REGENERATIVE THERAPY

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Regenerative medicine using cardiomyocytes derived from human-induced pluripotent stem cells (hiPSCs) has already entered clinical trials. However, to establish this approach as a standard treatment, several challenges remain, including improving cell engraftment rates and reducing production costs. Cardiomyocytes were differentiated from hiPSCs through monolayer culture. On day 12 of culture, cells were passaged and treated with a GSK-3β inhibitor to generate expanded cardiomyocytes (E-CM). Non-expanded cardiomyocytes (N-CM) were prepared in parallel by simply exchanging the culture medium, without passage or GSK-3β inhibitor treatment. Both cell types were harvested on day 20. Myocardial infarction models were created using athymic rats, and 2×10^7 N-CM or E-CM were transplanted one week after infarction. Four weeks post-transplantation, the hearts were excised for histological analysis. Compared to N-CM, E-CM exhibited approximately a threefold increase in cell numbers at the time of harvest. However, E-CM showed a decreased Ki67-positive rate and significantly fewer viable cells 12 hours post-seeding. Moreover, E-CM exhibited increased expression of senescence markers, including SA-β-galactosidase, p16, and p21. The graft size in the E-CM transplantation group was significantly smaller, with a higher rate of apoptotic cells. This study demonstrated that cardiomyocytes proliferated using GSK-3β inhibitors underwent senescence, leading to reduced cell engraftment capacity post-transplantation. To advance myocardial regenerative therapy as a routine treatment, it is crucial to suppress cardiomyocyte senescence prior to transplantation.

Funding Source: This work was supported by JST SPRING, Grant Number JPMJSP2144 (Shinshu University).

F1025

SUSTAINED VIABILITY AND FUNCTIONAL MATURITY OF ISLET ORGANOIDS FROM INDUCED PLURIPOTENT STEM CELLS AMELIORATES DIABETES

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Islet organoids derived from pluripotent stem cells offer a promising solution for the shortage of cadaveric donors in diabetes treatment. However, great challenges in the improvement in differentiation, viability, functional maturity, and engraftment of iPSC-derived islet organoids remain prominent. Here, we reported the generation of improved islet organoids with high viability and functionality by employing decellularized extracellular matrix (ECM) hydrogel of amniotic membrane (dAM) as a mechanical and physiological support. dAM sheet is able to facilitate islet organoid engraftment and rapidly restore normoglycemia in diabetic mice, accompanied by increased body weight and augmented insulin release in response to glucose. Interestingly, Collagen VI (Col VI) was identified as a key component of islet niche, enhancing islet cell viability and biological function. Col VI-based biomimetic ECM scaffold recapitulates the native environment for islet organoids and exhibits better physiological properties both in vitro and in vivo. Importantly, the cellular composition and endocrine function of optimized hiPSC-derived islet organoids are comparable to those of human islets. Our findings thus offer a unique and valuable platform for future endeavors in organoid transplantation-based therapy of diabetes.

Funding Source: NSFC (81821003, 82273180, and 82472713), NSFG (2024A1515011365), High-level Hospital Construction Project (KJ012021074 and KJ012019517) and Guangdong Basic and Applied Basic Research Foundation (2021B1515130004).

F1027

SPATIAL TRANSCRIPTOMIC ANALYSIS OF GASTRIC ORGANOIDS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELL

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The gastric muscularis mucosa (MM) is a thin layer of smooth muscle within the gastrointestinal wall. However, the development of human gastric MM remains poorly understood. Previously, we investigated gastric MM development using gastric organoids (GOs) derived from human induced pluripotent stem cells (hiPSCs). We reported that long-term culture led to the formation of GOs accompanied by MM, and that epithelial-derived Sonic Hedgehog (SHH) and TGFβ signaling, as well as mechanical factors, were involved in MM development. To further explore this process, we conducted a time-course analysis of spatial transcriptomics using the Xenium in situ platform on hiPSC-derived GOs. Spatial transcriptomic analysis is particularly suitable for studying specimens containing diverse histological structures, such as tissues and organoids. UMAP analysis of this dataset revealed that epithelial and smooth muscle clusters were positioned on opposite sides relative to the hiPSC cluster. Additionally, RNA expression profiling of individual clusters has the potential to identify MM precursor cells. Based on this analysis, differentially expressed genes (DEGs) in the gastric epithelial cluster were significantly associated with gene ontologies related to gland development and epithelial morphogenesis. In contrast, DEGs in the mature smooth muscle cluster were significantly enriched in gene ontology terms related to the collagen-containing extracellular matrix. These findings suggest that the subepithelial mechanical environment contributes to MM formation. UMAP analysis, incorporating GOs treated with SHH and TGFβ inhibitors, revealed that while a candidate MM precursor cluster was maintained, mature smooth muscle cells were markedly reduced, indicating that these inhibitors prevent smooth muscle maturation. Furthermore, pseudotime analysis based on single-cell RNA expression demonstrated that the gastric

epithelial cluster preceded smooth muscle development. This suggests that epithelial maturation in GOs precedes MM formation, which is consistent with our previous report demonstrating that epithelial-derived factors contribute to MM development.

Funding Source: JSPS KAKENHI Grant Number 23K15041.

F1029

3D-BIOPRINTED FUNCTIONAL LIVER UNIT FOR TISSUE REPAIR AND LIVER FAILURE TREATMENT

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Acute liver failure is associated with a 93% one-month mortality rate after onset, along with a 12.2% mortality rate for patients awaiting liver transplantation. While bioartificial liver offers a potential alternative for improving liver function, it faces significant challenges, including limited cell sources, lack of internal vascularization, and the inability to restore immune homeostasis. Therefore, tissue engineering that aim to restore the liver microenvironment by integrating bioprinting and stem cell technologies hold great promise. In this study, direct ink writing-based multi-nozzle bioprinting was employed to construct an interlaced prototype using degradable microfibrous bioink and sacrificial bioink. Mesenchymal stem cells (MSCs), umbilical vein endothelial cells (HUVECs), and expanded potential stem cell (EPSC)-derived immune cells were embedded into the bioinks. The printed construct was evaluated through imaging. The bioengineered tissue was then transplanted into the liver subcapsular space to assess in vivo tissue compatibility and treatment efficacy. The results demonstrated that the tissue composition, comprising alginate, gelatin, and gelatin methacrylate, ensured the anisotropy, printability, and biocompatibility of the hydrogels. HUVEC-MSC co-culture in microfibrous hydrogels exhibited a more elongated and stretched morphology compared to HUVEC monoculture by day 14. HUVECs lined the heterogeneous interconnected lumens, facilitating macroscale vessel formation after a one-day incubation and the removal of HUVEC-laden sacrificial bioink. Subsequently, HUVECs autonomously formed capillary networks (microscale vessels) within the microfibrous bioinks. After seven days of co-culture, these cells sprouted and invaded the surrounding microfibrous hydrogels, promoting angiogenesis. In the mouse model, blood cells and CD31+ endothelial cells were observed infiltrating the bioengineered tissue. This study represents a novel application of 3D bioprinting and stem cell technologies to create a transplantable structure with well-formed capillary networks, offering a potential solution to the shortage of liver donors. This innovation also holds promise for developing vascularized artificial livers for liver failure treatment.

Funding Source: Theme-based Research Scheme (T12-703/19-R) General Research Fund (17113924, 17109422).

F1031

COMPARATIVE ANALYSIS OF TTNPB AND CONVENTIONAL GROWTH FACTORS IN CHONDROGENIC PELLET DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELLS

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Cartilage regeneration is crucial for treating joint disorders, yet conventional differentiation methods rely on TGF-β3 and BMP-2, which are costly and require complex protocols. Small-molecule alternatives have been explored to simplify the differentiation process while maintaining efficiency. Retinoic acid (RA) and its derivatives, including Retinoic acid analogs like TTNPB, play an essential role in limb bud formation and subsequent chondrogenesis during skeletal development. Previous studies have demonstrated that TTNPB can efficiently induce chondrogenesis in human pluripotent stem cells using a 2D differentiation approach (Kawata et al., 2019). However, to further evaluate its efficacy in a physiologically relevant environment, this study investigates whether TTNPB, a retinoic acid analog that activates retinoic acid receptors (RARs), can support chondrogenic differentiation of induced pluripotent stem cells (iPSCs) in a 3D pellet culture system, which more closely mimics native cartilage tissue formation. iPSCs were differentiated into embryoid bodies (EBs) and further into EB outgrowth cell cells (EBOGCs) following our established protocols. At the final stage, cells underwent chondrogenic pellet culture. As a control, differentiation was carried out using conventional BMP-2 and TGF-β3 supplementation, while the experimental group was treated with TTNPB. Chondrogenic differentiation was assessed by analyzing key cartilage-specific markers, including COL2A1, ACAN, and SOX9. Immunofluorescence (IFA) staining was performed to visualize the expression and localization of these markers at the protein level. Additionally, Alcian blue and Safranin O staining were used to evaluate glycosaminoglycan deposition, a critical indicator of cartilage formation. Based on previously confirmed results, we believe that TTNPB has the potential to simplify the chondrogenic differentiation process while maintaining comparable efficacy to conventional growth factors.

Funding Source: This work was supported by the National Research Foundation of Korea (NRF) and ministries including Science and ICT, Trade, Industry and Energy, Health and Welfare, and Food and Drug Safety (RS-2023-KH142779, RS-2019-NR040058).

F1033

DENTAL PULP STEM CELL-MEDIATED TOOTH REPAIR AND REGENRATION

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Dental caries is a common disease and represents a major public health problem. Untreated, it causes pain, leads to endodontic therapy, restoration or tooth loss. An alternative is therapeutic dentin regeneration. Yet, this remains clinically elusive. This study aims to enhance dentin regeneration in injured teeth by investigating the role of brain-derived neurotrophic factor

(BDNF) and its receptor TrkB in dental pulp stem cell (DPSC)-mediated dentinogenesis, particularly under inflammatory conditions. Additionally, we explore the potential of stem cell engineering to improve dentin regeneration. TrkB expression and activation in DPSCs were assessed during odontogenic differentiation, with and without inflammatory inducers such as TNFα, LPS, and LTA. A mouse pulp-capping/caries model was used for the in vivo evaluation of dentin formation, where CRISPR-engineered DPSCs overexpressing BDNF were transplanted into inflamed pulp tissue. Transcriptomic profiling was performed on TNFα-treated DPSCs, with and without TrkB antagonist CTX-B, to identify downstream pathways involved. TrkB expression and activation in DPSCs were significantly upregulated during odontogenic differentiation, especially under inflammatory stimulants by 301 ± 17, 320 ± 15.2, and 250 ± 19, respectively vs control 165 ± 12.4 ($p < 0.01$). In vivo, the transplantation of BDNF-overexpressing DPSCs led to enhanced dentin regeneration in the mouse model by increasing the dentin volume to 1241 ± 51 mg HA/ccm vs control 1169 ± 9 mg HA/ccm ($p < 0.05$). Transcriptomic analysis revealed that TrkB inhibition led to significant transcriptional alterations related to immune response, cytokine signaling, and extracellular matrix interactions. This study underscores the crucial role of BDNF and TrkB in DPSC-mediated dentin regeneration, particularly in the presence of inflammation. Through BDNF overexpression, stem cell engineering shows promise for enhancing dentin repair in injured teeth, providing potential therapeutic strategies for improved dental tissue regeneration.

Funding Source: NIH R01 DE029816.

F1035

EFFECTS OF GLYCOSAMINOGLYCAN-RICH SCAFFOLDS ON CHONDROGENIC MICROTISSUES

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A key feature of osteoarthritis is the depletion of glycosaminoglycans (GAGs), leading to impaired cartilage hydration, reduced compressive strength, and disrupted chondrocyte homeostasis. Stem cell-based tissue engineering holds promise for cartilage regeneration. To mimic the GAG-rich extracellular matrix (ECM) of native cartilage, we have developed a biomimetic scaffold, aminated collagen-GAG (aCol-GAG), allowing precise control over the GAG content (glycosaminoglycan-to-hydroxyproline ratio, GAG/HYP). Here, human mesenchymal stem cells(hMSCs) from commercial sources and human chondrocytes isolated from patients were expanded and encapsulated in scaffolds with different GAG incorporation, including Col, Col/aCol-GAG, and aCol-GAG with GAG/HYP ratios of 0, 2:1, and 5:1, respectively. Upon chondrogenic differentiation of hMSCs or encapsulation of human chondrocytes, histological and biochemical evaluations of the chondrogenic microtissues were performed to analyse cell viability, chondrogenic phenotype, and ECM deposition on days 7, 14 and 21. Immunohistochemical staining revealed that chondrocytes in GAG/HYP rich scaffolds (aCol-GAG, GAG:HYP = 5:1) exhibited greater type-II collagen expression at later time points as compared with Col (no GAG) and Col/aCol-GAG (low GAG) groups. Live-Dead staining

revealed high cell viability. This work demonstrated the importance of engineering the GAG content of scaffolds in engineered chondrogenic microtissues. Delineating the cell-matrix interactions that lead to such phenotype changes in future investigations will contribute to better understanding of matrix niche engineering in tissue engineering and regenerative medicine.

Funding Source: This work was supported by Research Impact Fund (R7036-20) from RGC and ITF (ITS/293/21) from ITC, of the Hong Kong SAR government.

F1037

ESTABLISHMENT OF VASCULATURE IN HYPER-CROSSLINKED CARBOHYDRATE POLYMER AS SCAFFOLDING FOR TISSUE ENGINEERING AND REGENERATION

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Vascularization is one of the key components of tissue engineering and must accompany the ingrowth of new tissue to establish an environment conducive to the repair and regeneration of damaged tissue. In reconstructive orthopedic procedures, bone graft substitutes (BGS) are implanted to facilitate the repair of osseous tissue, a process during which osteoprogenitor cell infiltration into the BGS scaffold is mediated by the vascular endothelium and dependent on angiogenesis at the defect site for cytokines, growth factors, and osteoblasts necessary for bone formation. Poor bone regeneration is primarily caused by deficient vascularization of the implanted scaffold, which leads to atrophic non-union, excessive fibrotic scar formation, and osteogenesis impairment. To address these issues, porous polymer-based BGS materials have been considered due to high biodegradability, antifibrotic, and pro-angiogenic properties, yet their ability to promote angiogenesis in the repair of critical-sized bone defects has not been well-studied. The overarching objective of this study was to investigate whether hyper-crosslinked carbohydrate polymer (HCCP) BGS could promote the establishment of new vasculature compared to hydroxyapatite/beta-tricalcium phosphate (HA/βTCP) scaffolds, which are widely used in orthopedic procedures. Sprague Dawley rats (n=12) were implanted subcutaneously with HCCP or HA/βTCP and evaluated histologically for the ingrowth of new vasculature at 3, 14, and 28-days post-implantation. Vascularization was assessed by microvessel density, qualitative histological evaluation, and blood perfusion. HCCP demonstrated 5.06X more blood perfusion at 28 days compared to HA/βTCP ($p < 0.05$), and substantially higher microvessel density (0.073) than HA/βTCP (0.009) at 28 days ($p < 0.05$). Additionally, HA/βTCP showed transient inflammation at 14-days post-implantation whereas minimal immune activities were noted in HCCP. These findings suggest that HCCP promotes the establishment of new vasculature without a significant immune response. Further studies are underway to assess osteogenic differentiation, matrix remodeling, scaffold degradation, and stem cell recruitment using techniques such as RNA-seq and convolutional neural network architecture.

F1039

HARNESSING BASE EDITING TO TARGET STAT3 FOR CARDIAC FIBROSIS TREATMENT

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Cardiac fibrosis is a major driver of heart failure progression, yet therapeutic options remain limited. Signal transducer and activator of transcription 3 (STAT3), a key transcription factor, plays a pivotal role in fibrosis by promoting fibroblast activation and extracellular matrix deposition. In this study, we explore the use of base editing, a precise genome-editing technology, to introduce targeted mutations in the STAT3 gene, aiming to disrupt its pro-fibrotic functions. We have established an in vitro model using fibroblasts cell lines treated with TGF-β1 to induce fibrotic differentiation. Using a lentiviral vector, we delivered a cytosine base editor targeting the STAT3 gene to these cells. Preliminary data show reduced expression of fibrosis markers (α-SMA and collagen I) in edited cells compared to controls. Building on these findings, future work will employ stem cell-derived cardiac organoids to model fibrosis in a 3D multicellular system that recapitulates human cardiac pathophysiology. Leveraging high-throughput base editing screening, we aimed to identify specific STAT3 mutations that effectively inhibit fibrotic pathways in cardiac fibroblasts and develop a precision therapy that not only halts fibrosis progression but also preserves cardiac function.

F1041

INFRAPATELLAR FAT-PAD DERIVED MSCS: AN ALTERNATIVE MSC SOURCE FOR CARTILAGE AND OSTEOCHONDRAL TISSUE ENGINEERING

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Osteoarthritis (OA) is one of the most prevalent joint diseases worldwide, causing progressive degeneration of articular cartilage and subchondral bone on weight-bearing joints such as the knees and hips. Available conservative and surgical treatments remain inadequate due to the lack of effective disease-modifying options. Tissue engineering enables innovative therapeutic approaches to restore, replace or regenerate the site of damage. In the context of OA, cartilage and osteochondral tissue engineering requires large quantities of cells to effectively fabricate cartilage grafts or osteochondral constructs of sufficient size. Infrapatellar fat-pad derived MSCs (IFP-MSCs) exhibit advantageous properties over bone marrow derived MSCs (BM-MSCs), including the absence of age-dependent decline in cell proliferation and differentiation potential. In this study, the ability of human IFP-MSCs (hIFP-MSCs) to differentiate towards chondrogenic and osteogenic lineages was assessed, hIFP-MSC derived cartilage and bone microtissues were also compared to those derived from human BM-MSCs (hBM-MSCs). Our results demonstrated that hIFP-MSCs developed superior cartilage and bone microtissues

compared to hBM-MSCs under identical differentiation conditions. Specifically, hIFP-MSC derived cartilage microtissue exhibited better cartilaginous matrix formation with significantly higher quantification of glycosaminoglycan and hydroxyproline, whereas hIFP-MSC derived bone microtissue demonstrated significantly higher calcium content. These findings suggest that IFP-MSCs hold potential as a viable alternative MSC source for cartilage and osteochondral tissue engineering, paving the way for future OA-related therapeutics.

Funding Source: This work was financially supported by Research Grants Council, Healthy Longevity Catalyst Awards (Hong Kong, China) 2022; Research Impact Fund 2025 (R-7036-20); ITC Tier 3 (ITS/293/21); ITC Raise+ (RAI/23/1/040A).

F1043

LABEL-FREE, MORPHOLOGY-BASED CLASSIFICATION AND ISOLATION OF IPSC DERIVED HEPATOCYTES AND THEIR PROGENITOR STATES ON GHOST CYTOMETRY

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Cell differentiation, a complex process transforming stem/progenitor cells into diverse mature cell types, is driven by intra- and extracellular stimuli and orchestrated by dynamic gene expression changes mediated by key transcription factors. Most biomarkers for identifying differentiation states are transcription factors, rather than surface markers. This limits the ability to sort cells using conventional fluorescence-activated cell sorting (FACS), especially in development and regenerative medicine. Even using intracellular proteins as biomarkers, it is often difficult to identify the cellular state while keeping them alive for further downstream analysis. Ghost cytometry (GC) is a novel flow cytometry technique that analyzes high-content optical signals that reflect cellular morphology using a static patterned light pattern and a single-pixel detector. By applying machine learning directly to these signals, GC enables label-free cell analysis and sorting based on intrinsic properties. In this study, we induced human iPSCs(201B7) differentiation towards hepatocytes using a monolayer differentiation protocol and harvested them at Day 3 (definitive endoderm), Day 7 (hepatoblast), and Day 15 (hepatocyte). All samples including undifferentiated iPSCs were mixed and measured on GC, where morphological features were analyzed and visualized via Uniform Manifold Approximation and Projection (UMAP) of the GC data. By applying the UMAP model to individual samples, we identified four cell subtypes that were distributed on different locations on UMAP. Based on their distribution, three subpopulations were defined and gated, presumed to represent the endoderm, hepatoblasts, and hepatocytes. Cells from each gate were sorted and enrichment of each cell type with the expression of marker genes, SOX17 and FOXA2 for endoderm, AFP, HNF4A, ALB and SERPINA1 for hepatoblast and hepatocyte was confirmed. These results demonstrate that GC effectively captures morphological transitions during hepatocyte differentiation from iPSCs, enabling assessment and sorting of transitional states without the need for external labels. This concept can be extended to various other differentiation processes to develop label-free workflows for cell monitoring and sorting in the

field of regenerative medicine.

F1045

MEGF10 SUPPORTS MUSCLE STEM CELL RETENTION AND REPOPULATION

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Early-onset myopathy, areflexia, respiratory distress and dysphagia (EMARDD) is a highly fatal skeletal muscle disorder that does not have available cures. EMARDD is a poorly understood disease that is the result of a loss of function mutation in the MEGF10 gene, a membrane-bound protein linked to muscle stem cell support. In EMARDD patients, muscle stem cells (SCs) have impaired myogenic capacities, and thus skeletal muscle myogenesis is stunted. To combat EMARDD, SC transplantation therapies offer an appealing avenue, and induced pluripotent stem cells can be differentiated to skeletal muscle before transplantation to ensure a patient-specific treatment. However, pluripotent derived skeletal muscle offers a relatively weak treatment since less than one percent of transplanted cells persist as PAX7+ stem cells after engraftment. Helping support the retention of transplanted SCs as a quiescent stem cell pool is paramount to addressing EMARDD and other skeletal muscle disorders. We set out to overexpress MEGF10 to improve the retention of quiescent transplanted SCs. We incorporated a Tet-On system of doxycycline-driven inducible overexpression into pluripotent stem cells using CRISPR gene editing. Tet-On pluripotent derived PAX7 cells were transplanted in vivo, and MEGF10 was overexpressed at different timepoints (Day0-10, Day10-20, Day20-30, or Day0-30), to determine the kinetics of MEGF10 expression for PAX7 cell support. We found that after MEGF10 induction for the first 10 days following transplantation resulted in more human myofibers and better SC retention and support. Our work indicates that support of transplanted stem cells after engraftment leads to their long-term stability. To showcase this improved PAX7 support in MEGF10 treated groups, we plan to conduct reinjury assays that demonstrate their predicted improved regenerative abilities. We have collected spatial transcriptomic data on engrafted muscle stem cells with and without MEGF10 treatment at late regenerative timepoints to compare the PAX7 and myofiber transcriptomic landscapes. With this spatial RNA-seq data, we hope to elucidate the dynamic mechanism of MEGF10-mediated muscle stem cell support in vivo.

Funding Source: UCI MAXIMUS T32 Pre-Doctoral Training Fellowship.

F1047

SINGLE-CELL TRANSCRIPTOMICS IDENTIFIES KEY REGULATORS OF MATURE CARDIOMYOCYTE TRANSITION

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Ischemic heart disease remains the leading cause of mortality worldwide, highlighting the urgent need for regenerative therapies. Human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) offer a promising approach. Here, we used a chemically defined, xeno-free laminin-221-based differentiation protocol to generate committed cardiac progenitors (CCPs) and investigated their maturation at later stages. Single-cell RNA sequencing of hPSC-CMs at days 21 and 42 revealed a progressive shift towards a cardiomyocyte-dominant population compared to fibroblast, myofibroblasts, epithelial, and endothelial cells. Gene expression analysis highlighted the upregulation of key cardiac maturation markers, including those involved in sarcomere organization, metabolism, and ion handling, consistent with known hallmarks of cardiac maturation. While day 21 cells exhibited features of immature cardiomyocytes. Day 42 cells showed enhanced RYR2, CACNB2, and MYL2 expression, indicating partial maturation. Functional analysis further highlighted a transition from RNA processing pathways at day 21 to cardiac-specific pathways at day 42. Furthermore, transcriptional regulatory analysis identified ESRR and TEAD isoforms as potential key regulators driving cardiomyocyte maturation. This study provides a single-cell transcriptomic profile of the critical transition phase during cardiomyocyte differentiation, offering insights into molecular mechanisms that can be leveraged to enhance cardiomyocyte maturation for regenerative applications.

Funding Source: This work has been supported by grants from the MOE, and NRF of Singapore (MOE-RS06/23, CRP24-2020-0083, RS06/23 and MOE start-up grant, 022976-00001).

F1049

SPATIAL REMODELING OF RNA SPLICING FACTORS ORCHESTRATES LIVER REGENERATION

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Tissue injury and regenerative impairment drive hepatic failure and progressive liver pathologies. However, the spatial and molecular dynamics governing early-stage liver regeneration, particularly the balance between hepatocyte proliferation and metabolic adaptation, remain elusive. By integrating spatiotemporal sequencing of regenerating liver tissues with single-cell RNA sequencing of liver organoids mimicking regeneration initiation, we uncovered that spatial reorganization of splicing factors initiates preferential periportal hepatocyte reactivation. A reprogrammed hepatocyte subpopulation with elevated splicing factor activity exhibited regenerative hepatoprogenitor characteristics, prioritizing proliferative capacity over metabolic functionality. Mechanistically, pharmacological inhibition of RNA splicing upregulated ribosomal proteins, attenuating proliferative signaling. Functional screening identified spliceosomes (e.g., heterogeneous nuclear ribonucleoprotein U, HNRNPU) as critical regulators of hepatic zonation and regeneration. Hnrnpu knockout disrupted liver repair architecture and exacerbated metabolic dysfunction-associated steatotic liver disease (MASLD) phenotypes in both clinical cohorts and murine models. Our findings delineate a splicing factor-mediated spatial hierarchy controlling hepatocyte fate specification during regeneration, proposing a paradigm where spliceosomal activation licenses hepatoprogenitor transition. Crucially, chronic splicing factor deficiency provoked spontaneous hepatic steatosis, implicating spliceosomes as molecular hubs coupling regeneration with lipid metabolism. This

work establishes a mechanistic framework for initiating hepatic repair and identifies spliceosomal targets such as HNRNPU for therapeutic interventions in chronic liver diseases, informing advanced treatment strategies including gene therapy and RNA-targeted modalities.

Funding Source: The National Natural Science Foundation of China (32122031 to Huili Hu and T2321004 to Huili Hu); the National Key Research and Development Program of China (2019YFA0111400 to Huili Hu).

F1051

TRANSCRIPTOMIC AND EPIGENOMIC CHANGES IN DUCTAL EPITHELIUM AFTER REPEATED LIVER DAMAGE

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Tissue repair relies on the ability of cells to respond to internal and external cues, proliferate, and differentiate into functional components. The liver stands out for its remarkable regenerative capacity, driven by hepatocytes or ductal cells following injury. However, the loss of this regenerative ability and its dysregulation can lead to disease. Using an in vivo mouse models of liver regeneration, in vitro cholangiocyte organoids we had previously developed and multiple sequencing techniques, we investigated the dynamic transcriptional and epigenetic changes to the ductal epithelium after repeated liver damage. Although the liver recovered histologically and functionally, the ability of ductal cells to generate organoids declined. We identified compositional shifts in the ductal cell population, alongside with transcriptomic and epigenomic changes. Cell population and gene regulatory network analyses revealed potential mechanisms underlying ductal-driven liver regeneration. These findings lay the foundation for understanding the diminished regenerative capacity observed in chronic liver injury.

Funding Source: This research is funded by the Deutsche Forschungsgemeinschaft– 514150034 and from the European Research Council under the European Union's Horizon Europe research and innovation programme (grant agreement No 101088869).

F1053

4D BIOPRINTING OF GRADED HYDROGELS FOR BONE TISSUE ENGINEERING

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3D bioprinting of cell-laden hydrogels enables the fabrication of complex structures and delivery of stem cells in situ for bone tissue engineering. However, certain bone defects such as those in cranial bone, possess inherent curvature, which makes it challenging for 3D bioprinted hydrogels to perfectly match the structure. Therefore, developing 4D bioprinted hydrogels with shape-morphing capacities to conform to the native structure of cranial bone defects is essential. In the current study, we prepared a photocrosslinkable bioink consisting of gelatin methacryloyl (GelMA), oxidized alginate (OAlg), magnesium-doped hydroxyapatite (MgHAp), and bone marrow-derived mesenchymal stem cells (BMSCs). Methacryloxyethyl thiocarbamoyl rhodamine (RhB), an effective UV absorber, was employed to create a graded crosslinking density in the hydrogel and thus realize its shape morphing behavior. The printing inks exhibited excellent printability, and that the printed hydrogels displayed high fidelity. Subsequently, a comprehensive study was conducted to evaluate the effects of various parameters, including UV crosslinking time, photoinitiator concentration, RhB concentration, the thickness and length-to-width ratio of printed hydrogels, and medium types, on the shape morphing behavior of hydrogels. Notably, our simulation results corroborated the significant influence of these parameters and mirrored the experimental observations. Furthermore, BMSCs showed a significant survival rate (> 90%) in 4D printed hydrogels and exhibited expanded morphology due to the dynamic network formed by GelMA and OAlg. The 4D printed hydrogels could sustainably release Ca2+ and Mg2+, which promoted the osteogenic differentiation of BMSCs by enhancing the expression of osteogenic-related genes and proteins. In vivo rabbit skull bone defect models indicated that 4D bioprinted hydrogels perfectly matched the native curvature of cranial bone defects and significantly facilitated the regeneration of the defects. As a result, the current study presents a novel and versatile approach to overcoming the hurdle of achieving shape changes in cell-laden hydrogels to match the native curvature of specific bone tissues, demonstrating significant promise in bone tissue engineering.

F1055

A CELL CULTURE PLATFORM FOR ELECTRICAL AND MECHANICAL STIMULATION OF HIPSC-DERIVED CELLS

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The potential of induced pluripotent cells (hiPSCs) has opened new opportunities in disease modeling, drug discovery, and cell-based therapies. Despite advances in protocol development, creating functional tissues has always been a challenge. Replicating their natural environment and experiences within the human body has proved difficult. The development of iPSC-based culture systems has brought us closer to biomimicking systems, but there are still gaps that need to be overcome. Since electrogenic cells function based on electrical conduction, understanding the parameters for differentiation and maturation of hiPSC-derived cells has always been a challenge. To overcome these huddles, we have developed a cell culture platform in which mechanical and electrical stimulations can be applied during or after differentiation of hiPSCs. For example, we demonstrated that hiPSC-derived cardiac cells subjected to electrical and mechanical co-stimulation were maturated more efficiently. As an extension, in this study, we aimed to understand the effect of electrical and mechanical stimulations on the differentiation of iPSC-derived Neural Stem Cells (NSCs). We first differentiated iPSCs into NSCs and then subjected these iPSC-derived NSCs to various

electrical and mechanical (stretching) stimulations. We then analyzed the effect of these stimulations using various post-simulation analyses to understand the resulting neuronal network's composition. We found that different stimulation conditions affected the composition of supporting and neuronal cells with different neurotransmitter receptors. We were able to identify astrocytes, acetylcholine, dopaminergic, and even GABAergic networks. Mechanical stretching further facilitated the arrangement and rearrangement of the structure, opening possibilities to explore interactions between other neurons. Further exploration in this approach will help us develop in vitro neural networks for drug studies, disease modeling, and cell-based therapies.

F1057

A NOVEL APPROACH TO HARNESS MESENCHYMAL STROMAL CELLS TO AUGMENT ARTICULAR CARTILAGE REGENERATION: AN IN VITRO AND IN VIVO ASSESSMENT

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Articular cartilage damage significantly contributes to joint pain and leads to osteoarthritis (OA) if untreated. The Articular Cartilage Paste Graft technique repairs cartilage damage through autologous grafting of healthy bone and cartilage harvested from the intercondylar notch of the knee. Morselization of the damaged cartilage allows for infiltration of endogenous bone marrow stem cells resulting in de novo cartilage formation. Regeneration of normal hyaline cartilage is observed in one-third of patients; however, this technique lacks consistent defect fill and attachment of naive cartilage to neighboring tissue. We hypothesized that adding allogeneic human mesenchymal stromal cells (hMSCs) would amplify the effects of endogenous bone marrow stem cells and the addition of synthetic PEG hydrogel to the paste graft would serve as a scaffold to improve integration of regenerated tissue. In vitro, addition of hMSCs to human cartilage-hydrogel constructs increased GAG content significantly. Our in vivo rabbit model bolstered these findings, demonstrating the addition of MSCs and hydrogel to the paste graft resulted in almost full defect fill and significantly increased attachment to surrounding native tissue. Both histological analysis and confirmatory microCT imaging showed this combination of hMSCs, hydrogel, and autogenous paste treatment to consistently produce a robust repair of tissue architecture that integrated with endogenous tissue. Our novel paste graft construct harnesses the capacity of hMSCs to extensively regenerate and repair cartilage with the potential to prevent the onset of post-traumatic and degenerative OA. We are now advancing this approach into large animal trials under FDA and CVM guidance, with the future aim of significantly reducing the burden of cartilage injury and OA.

Funding Source: California Institute of Regenerative Medicine (DISC2-13131).

F1059

A NOVEL NON-INVASIVE WORKFLOW FOR DIFFERENTIATION AND CHARACTERIZATION OF IPSC-DERIVED HEPATIC ORGANOIDS

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The liver is essential for critical physiological processes, such as detoxification, protein synthesis, metabolism, and hormone regulation. Despite its remarkable in vivo regenerative capacity, in vitro expansion of hepatocytes remains challenging. Induced pluripotent stem cells (iPSCs) offer a versatile source for generating hepatic cells and can be used to create hepatic organoids, which mimic liver structure and function. Effective differentiation of iPSCs into hepatic organoids requires monitoring both differentiation and organoid development. This study outlines a novel non-invasive workflow for analyzing iPSC-derived hepatic organoid development. iPSCs (StemCell Technologies) were differentiated into hepatic progenitor cells (HPCs) and hepatocyte-like cells (HLCs) using supplier protocols. HLCs represent a more advanced stage of hepatocyte lineage differentiation. Morphological changes during differentiation were monitored using the Omni live-cell imager. Subsequently, HPCs and HLCs were embedded in individual Matrigel domes to form organoids. Organoids were cultured for one week, passaged, and then imaged every 4 hours for 72 hours using the Omni to measure organoid area, diameter, and roundness. Clear morphological differences were observed across various differentiation stages. iPSCs had a highly compact, small-cell morphology, while definitive endoderm cells were even smaller and more compact. In contrast, HPCs were larger with a tightly packed cobblestone morphology. HLCs were the largest cells, maintaining the tightly packed cobblestone arrangement. Both HPC- and HLC-derived organoids were spherical with a prominent lumen. The average roundness across all time points was similar for both organoid types (0.85). Initially, HPC-derived organoids were smaller (diameter: 53 µm; area: 3027 µm²) than to HLC-derived organoids (diameter: 67 µm; area: 6885 µm²) but grew 3.6-fold over 72 hours as compared to 3.0-fold for HLC-derived organoids, indicating growth differences that may be related to cell maturity. This non-invasive workflow highlights the utility of live-cell imaging for real-time monitoring of iPSC differentiation and hepatic organoid formation, advancing liver research and applications in disease modelling and therapy.

F1061

A SPATIOTEMPORALLY DEFINED SUBSET OF EMVE CELLS IS ESSENTIAL FOR THE FORMATION OF THE GUT ENDODERM

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The gut endoderm forms the foundation of the respiratory and digestive systems, including the lung. Recent studies have shown that during mouse early development the gut endoderm has

a dual origin, deriving from both the definitive endoderm and the embryonic visceral endoderm (emVE) of mouse gastrula. However, the contribution of emVE cells and the molecular mechanisms underpinning their gut endoderm integration remain poorly understood. Here, we employed deep single-cell transcriptomic profiling and spatial transcriptomics to construct the spatiotemporal distributions and molecular transitions of emVE cells during gastrulation. Our findings reveal that a spatially defined subset of emVE cells undergoes molecular transition, significantly contributing to the formation of the gut endoderm. Notably, we identified several key transcription factors that govern this transition and play a critical role in determining emVE cell fate during gut endoderm specification. These insights deepen our understanding of the molecular events driving gut endodermal patterning and provide a framework for exploring stem cell-based regenerative strategies in gut tissue engineering.

F1063

ALIGNED 3D BIOPRINTING STIMULATES MATURATION OF HESC-DERIVED CARDIOMYOCYTES

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3D bioprinting has been widely used for cardiac tissue engineering, offering precise control over cellular organization. Anisotropic alignment of cardiomyocytes is critical for mimicking native myocardium, as it enhances cytoskeletal elongation, sarcomere organization, and intercellular connectivity. While methods such as micropatterning and electrospun nanofibers have promoted alignment, the role of Au nanowires in directing anisotropic organization and their molecular effects remain unclear. This study aims to develop a 3D bioprinting strategy using gold (Au) nanowires to induce anisotropic alignment of human embryonic stem cell-derived cardiomyocytes (hESC-CMs) and enhance their structural organization, maturation, and intercellular communication. Aligned 3D bioprinting with Au nanowires improved cytoskeletal organization, increasing F-actin structures and sarcomere length. Aligned hESC-CMs exhibited upregulated maturation markers, including cTnI, MYH7, and MYL2, compared to randomly aligned CMs. Additionally, the expression of N-cadherin and connexin 43 increased, facilitating intercellular communication. Ang-1 and Ang-2, key regulators of cardiac contractility, were also upregulated. We found that Wnt signaling, particularly the noncanonical pathway, was activated in aligned hESC-CMs, influencing cytoskeletal remodeling and cell adhesion. Given the role of Wnt in connexin regulation, we propose that anisotropic bioprinting enhances intracellular communication through cell junction-mediated crosstalk. This study demonstrates that 3D bioprinting with Au nanowires is an effective strategy for engineering anisotropic cardiac tissues, with potential applications in regenerative medicine.

Funding Source: Supported by the National Research Foundation of Korea (NRF, RS-2023-00208466) and the Korean Fund for Regenerative Medicine (KFRM, RS-2022-00070441) from the Korea government, MSIT, and MOHW.

F1065

APPLICATION OF TRANSGENE-CONTROLLABLE SENDAI VIRUS VECTOR FOR STEM CELL RESEARCH

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Viral vectors are attractive gene delivery vehicles for stem cell technology because of their broad tropism, high transduction efficiency, and durable expression. With no risk of integration into the host genome, the vectors developed from RNA viruses such as Sendai virus (SeV) are especially promising. However, RNA-based vectors have limited applicability because they lack a convenient method to control transgene expression by an external inducer. We engineered a Csy4 switch in SeV-based vectors by combining Csy4 endoribonuclease with mutant FKBP12 (DD: destabilizing domain) that becomes stabilized when a small chemical Shield1 is supplied. In this Shield1-responsive Csy4 (SrC) switch, Shield1 increases Csy4 fused with DD (DD-Csy4), which then cleaves and downregulates the transgene mRNA containing the Csy4 recognition sequence (Csy4RS). Moreover, when Csy4RS is inserted in the viral L gene, the SrC switch suppresses replication and transcription of the SeV vector in infected cells in a Shield1-dependent manner, thus enabling complete elimination of the vector from the cells. We also find an anti-virus drug can support such a removal of the vector. By temporal control of BRN4 expression, a BRN4-expressing SeV vector equipped with the SrC switch achieves efficient, stepwise differentiation of embryonic stem cells into neural stem cells, and then into astrocytes. SeV-based vectors with the SrC switch should find wide applications in stem cell research, regenerative medicine, and gene therapy, especially when precise control of reprogramming factor expression and final vector-free cells are desirable.

F1067

BICONVEX LENTOID BODIES WITH INTACT CAPSULES CONSTRUCTED ON ULTRA-SOFT HYDROGEL SUBSTRATES

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To develop three-dimensional lentoid bodies (3D-LBs) with double-sided convexity and complete capsule wrapping that highly mimics the morphology and structure of the natural human lens. Polyacrylamide hydrogels of 1, 5, 20, and 100 kPa stiffness were prepared, and urinary human induced pluripotent stem cells (UiPSCs) were implanted on the hydrogels and induced into 3D-LBs by the “Fried egg” differentiation method. The key mechanistic signaling pathways affecting the differentiation were explored by transcriptome sequencing, adjusting the extracellular matrix content, YAP inhibitor, and ROCK inhibitor. The morphology, composition and molecular expression characteristics of the organoids were depicted with the help of hyperfield microscopy, confocal 3D fluorescence imaging, transmission electron microscopy, and qPCR. 3D-LBs, which were as convex as the bottom and top surfaces and were encapsulated by the collagen membrane, could be obtained on 1 kPa hydrogels. And it could advance the maturation time from 25 days for hard substrates to 12 days for soft substrates. Lens fiber differentiation of 1kPa-LB was rapidly activated after day 9, and transcriptome sequencing revealed significant downregulation of the extracellular collagen-related pathway and the Hippo pathway prior to fiber differentiation. 1% relatively low extracellular collagen

concentration and transient inhibition of the ROCK/Hippo pathway were both beneficial for a more rapid differentiation of LBs. The biconvex 3D-LBs were successfully cultured on 1kPa hydrogels and greatly reduced the differentiation maturation time. The low stiffness mechanical environment may promote lens fiber differentiation by reducing extracellular collagen and inhibiting the ROCK/Hippo pathway early in development.

F1069

CELL-DERIVED SECRETOME-BASED BIOLOGICS TO REVERSE AXONAL DEGENERATION IN DIABETIC NEUROPATHY

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Diabetic neuropathy (DN), a complication of diabetes mellitus, causes axonal degeneration and loss of peripheral sensation, contributing to diabetic wounds and increasing the risk of limb amputation. Current therapeutic approaches for DN have limitations in addressing the underlying nerve damage, necessitating the exploration of novel approaches to reverse axonal degeneration. This project seeks to develop pro-reparative composite biologics based on the decellularized extracellular matrix (ECM) or conditioned media (CM) obtained from mesenchymal stromal cells (MSCs). By harnessing MSCs' trophic factor release capabilities, the goal is to explore the therapeutic efficacy of synthesized biologics in potentially reversing axonal degeneration in vitro. Several candidate secretome-based biologics were synthesized based on the previously established methods and solubilized by urea extraction to facilitate delivery into uninjured neuropathic skin. Schwann cell progenitors (SCPs) and Schwann cells (SCs) were differentiated from induced pluripotent stem cells (iPSCs) and neuron-like cells were derived from PC12 cells to evaluate the bioactivity of the biologics through functional assays in vitro. Preliminary findings indicate that soluble extracts of MSC-derived ECM enhanced SCP proliferation and neurite outgrowth in neuronal cultures, while SCP migration remained unaffected. The proposed approach for synthesising ECM-based biologics warrants further investigation as a potential alternative approach for DN.

Funding Source: Center for Neuromusculoskeletal Restorative Medicine, The Chinese University of Hong Kong, Hong Kong SAR.

F1071

CEREBRAL ORGANOIDS MODEL POSTNATAL INTERNEURON MIGRATION OF THE HUMAN CORTEX

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Much of what is known about human cortical development has been derived from rodent models, due to the highly conserved neural types and processes. However, a unique feature of human brain development is the protracted period of neurogenesis and interneuron migration. In humans, recent findings in postmortem tissue of the Arc migratory stream, the arc-ACC, and the EC stream into the postnatal cortex have changed the dogma that cortical interneuron migration is complete before birth. These studies shed light on a prolonged stage of human brain development and a longer plasticity window for fine-tuning of the developing circuit with local inhibitory inputs. This also leaves a wider window for potential insults, leading to neurological disorders such as autism and epilepsy. Here we have derived an iPSC dorsal::ventral assembloid model with fusion at day 120 and analysis after up to 390 days of culture, to model the migration dynamics of this postnatal process. Interestingly, we observed after over 200 days that newly born migratory interneurons arrange themselves into connected chains that are surrounded by astrocytes, unlike the dispersed migration seen at earlier time points. These interneurons express caudal ganglionic eminence (CGE) markers and are born late, shown with EdU birth dating. Analysis by electron microscopy reveals an architecture essentially indistinguishable from what has been seen in early postnatal human brains. Using a combination of time-lapse imaging, mathematical modelling, and single cell spatial transcriptomics, we uncovered that this unique mode of migration requires both intrinsic cues from the late-born interneurons, as well as specific interactions between the interneurons and surrounding astrocytes for chain formation and migration. For the first time, our work reconstitutes events of human brain development that occur after birth, allowing a genetic and cell biological analysis of this important phenomenon.

F1073

CHARTING POSTNATAL HEART DEVELOPMENT USING IN VIVO SINGLE-CELL FUNCTIONAL GENOMICS

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The transition at birth, marked by increased circulatory demands and rapid growth, necessitates extensive remodeling of the heart’s structure, function, and metabolism. This transformation requires precise spatial and temporal coordination among diverse cardiac cell types, yet the intrinsic and extrinsic regulatory mechanisms driving these changes remain incompletely understood. Here, we generated a single-cell-resolution temporal and spatial atlas of postnatal hearts by coupling time-course single-nucleus RNA sequencing with in situ single-

cell spatial imaging analysis. Our integrative approach not only provided a spatial map of the postnatal heart's transcriptome at the single-cell level but also unveiled the dynamic of localized intrinsic and extrinsic regulatory mechanisms driving postnatal heart development. To further interrogate intrinsic and extrinsic regulators' function within the native cardiac environment in a high-throughput manner, we developed an in vivo Probe-based Indel-detectable Perturb-seq (PIP-seq) platform. Applying PIP-seq to postnatal cardiomyocyte maturation uncovered key regulatory nodal points underlying postnatal cardiomyocyte maturation. Through these efforts, we have not only created a single-cell-resolution temporal and spatial atlas of postnatal hearts but also demonstrated the functional importance of cell-cell communication during postnatal heart development. Importantly, our PIP-seq opens up new possibilities for research, no longer limiting to a single gene but allowing for exploring a network of genes' function without sacrificing in vivo physiological context.

F1075

COCKTAIL CHEMISTRY MEETS BIOMATERIALS: ADVANCED FUNCTIONALIZATION FOR REGENERATIVE MEDICINE

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Engineering biomaterial surfaces with synergistic peptide combinations unlocks new possibilities for directing stem cell fate and advancing regenerative medicine. This study explores a novel multi-peptide functionalization strategy to enhance the osteoinductive potential of biomaterial surfaces. Accordingly, the existing synergy between RGD peptides and BMP-2 was leveraged by incorporating a third peptide designed to activate complementary signaling pathways, therefore enhancing the recruitment and differentiation of stem cells. This approach represents a paradigm shift in biomaterial design, taking advantage of the cooperative action of multiple ligands to optimize the cellular microenvironment. To achieve precise and reproducible surface modifications, an optimized spin-coating method was developed and employed for the first time to facilitate controlled grafting of biomolecules onto the surfaces. Unlike traditional immersion-based silanization techniques, this advanced approach enabled the achievement of a well-organized, self-assembled monolayer with superior bioactivity. Systematic optimization of the spin-coating parameters allowed for fine-tuning surface properties for subsequent peptide functionalization. Experimental results demonstrated that multi-peptide functionalized surfaces significantly outperformed single- or dual-peptide coatings in promoting osteogenic differentiation. Early upregulation of key osteogenic markers was observed through qPCR, Western blot, and immunofluorescence analyses. The introduction of additional peptides amplified stem cell signaling by targeting distinct molecular pathways, resulting in a highly osteoinductive environment. This study highlights the transformative potential of multi-peptide functionalization to orchestrate complex biological processes at the cell-material interface. This strategy paves the way for advancements in biomaterial design for regenerative medicine and tissue engineering applications by offering an efficient and adaptable approach for engineering bioactive surfaces.

F1077

COMPARISONS OF MIRNA PROFILES OF EXOSOMES DERIVED FROM HUMAN IPSCS, ADSCS AND BMSCS AND EFFECTS ON CHONDROCYTE FUNCTION

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This study aimed to identify and compare the miRNA profiles of exosomes derived from human iPSCs, BMSCs, and ADSCs (hiPSC-Exos, hBMSC-Exos, and hADSC-Exos) and their functional effects on human articular chondrocytes (hACs). hiPSC-Exos, hBMSC-Exos, and hADSC-Exos were collected from the appropriate cells cultured in 10% bovine exosome-depleted FBS (de-Exo-FBS) for 48 hours. NGS and bioinformatics were used to analyze the small RNA profiles of these exosomes. The biological functions of hACs were examined after a 12-day treatment with exosomes. hBMSC-Exos and hADSC-Exos had similar miRNA profiles but were largely different from hiPS-Exos. There were 17 highly expressed miRNAs in hiPSC-Exos, 13 miRNAs in hADSC-Exos, and 11 miRNAs in hBMSC-Exos. Among them, 7 miRNAs overlapped between the hBMSC-Exos and hADSC-Exos, and only 3 of them (hsa-miR-16-5p, hsa-miR-25-3p, and hsa-miR-93-5p) overlapped among all 3 exosomes. The putative target genes of the 3 overlapping exosomal miRNAs, and high-scoring target genes, including MAN2A1, ZNFX1, PHF19, GPR137C, ENPP5, B3GALT2, FNIP1, PKD2, and FBXW7, were identified. GO and KEGG enrichment analyses revealed that these genes are involved in cell growth, bone ossification, and cartilage development/differentiation, possibly via the MAPK signaling pathway. Accordingly, we confirmed the biological effect on cartilage differentiation and found that hiPSC-Exos, hBMSC-Exos and hADSC-Exos maintained hAC viability, prevented senescence, promoted the formation of a normal cartilage matrix (glycosaminoglycan and type II collagen), and downregulated fibrocartilage matrix (type I collagen) in normal hACs. Comparatively, hBMSC-Exos had the greatest effect on hAC function. Bioinformatics revealed differences and possible mechanisms of action of exosomes derived from pluripotent hiPSCs, multipotent hADSCs and multipotent hBMSCs and these exosomes effectively suppressed cell senescence and promoted normal functional extracellular matrix formation in hACs. Further investigations of the different functions of exosomes from pluripotent-hiPSCs other than those from multipotent-hMSCs are needed.

F1079

CONTROL OF MUSCLE STEM CELL BEHAVIOR BY THE CIRCADIAN CLOCK

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Adult muscle stem cells (MuSCs) allow the regeneration of skeletal muscle. They represent a potential therapeutic interest to treat trauma and muscular disorders. It is therefore essential to better understand the mechanisms that govern MuSCs engraftment, activation and self-renewal in order to design new strategies and enhance the potential of MuSCs. In this context, the role of the biological clock is poorly understood, whereas the regeneration of many tissues, such as the skin or the intestine, has been shown to be coordinated by the circadian clock. Our hypothesis is that the biological clock controls the regenerative capacity of skeletal muscle by gating MuSCs function to the right time window, leading an optimal response at the time of day when the risk of injury is greatest. Our results show a circadian remodeling of the MuSCs proteome associated with rhythmic changes in MuSCs metabolism and behavior during the circadian cycle. Through an innovative approach combining original mouse models, proteomics, and human MuSCs analysis, we identified the molecular targets of the biological clock in MuSCs. We showed that the MuSCs clock controls their behavior during activation by regulating their response to stress, to better respond to the activation at the right time of day, therefore optimizing skeletal muscle regeneration. In conclusion, our study paves the way for the chronobiology of MuSCs, which represents an important step towards the development of cellular chronotherapies in the context of trauma and myopathies.

Funding Source: AFM-Téléthon Agence Nationale de la Recherche (ANR) " MUSCLO" and “European Genomic Institute for Diabetes,” ANR-10-LABX-0046, INSERM CPER-CTRL, Région Hauts-de-France/Fonds Européen de Développement Régional.

F1081

DECODING THE ROLE OF ALTERNATIVE POLYADENYLATION IN DILATED CARDIOMYOPATHY

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Cardiovascular disease accounts for one-third of deaths worldwide, yet the molecular mechanisms underlying a healthy heart and its dysfunction in disease remain incompletely understood. While genetic factors play a significant role, the limited contribution of mutations in the coding genome has shifted the focus toward non-coding regulatory elements. Alternative polyadenylation (APA) is a co-transcriptional mechanism that modifies the 3' untranslated regions (UTR) of mRNA, thereby influencing mRNA stability, localisation, translation, and subsequent protein localisation and function. Emerging evidence highlights that APA significantly affects cardiac gene regulation and may present novel therapeutic opportunities for combating heart disease and dysfunction. To characterise normal APA signatures in cardiac genes, we analysed snRNA-seq data from the Human Heart Cell Atlas using PolyApiper, an innovative tool for detecting APA from scRNA-seq data. Our analysis identified 7,864 genes

expressing alternative 3'UTR isoforms and 550 genes undergoing differential APA between atrial and ventricular cardiomyocytes. Extending this investigation to hearts with dilated cardiomyopathy, which exhibit activation of the fetal gene program, we found 6,564 genes subject to APA in cardiomyocytes. These findings also enabled us to prioritise genes for in vitro studies based on 3'UTR isoform expression. For instance, we observed that longer 3'UTRs of the TBX5 gene were associated with reduced mRNA levels, resulting in decreased protein expression. These insights advance our understanding of how APA regulates gene expression and protein function in the healthy heart and reveal how its dysregulation contributes to cardiovascular disease.

F1083

DEVELOPMENT AND FUNCTIONAL ASSESSMENT OF IPSC-DERIVED ENDOTHELIAL CELLS USING A NOVEL NON-INVASIVE WORKFLOW

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Induced pluripotent stem cells (iPSCs) are widely used in studies of embryonic development, disease modelling, and tissue engineering. A promising application for iPSCs is the generation of endothelial cells, critical for forming blood vessels within larger tissue constructs to ensure oxygen and nutrient delivery. Effective differentiation of iPSCs into mature, functional endothelial cells presents several challenges, requiring monitoring of the differentiation process and recapitulating complex vascular networks. This study presents a novel non-invasive workflow for analysis and functional testing of iPSC-derived endothelial cells (id-ECs). First, iPSCs (StemCell Technologies) were differentiated into endothelial cells using the suppliers' 14-day protocol. Morphological changes (e.g. size and shape) were monitored using the Omni live-cell imager. Endothelial differentiation was verified via fluorescence staining of the endothelial marker CD31 and the absence of stem cell markers SSEA-4 and TRA-1-60. Functional assessment of id-ECs was performed to validate important endothelial behavior like migration (scratch assay), tubule formation and barrier integrity (transendothelial electrical resistance (TEER)). The actin polymerization inhibitor Cytochalasin D (CytoD) was added at various concentrations to validate functionality. Brightfield imaging of the scratch assay using the Omni showed 100% wound closure by id-ECs within 24 hours. Scratch closure was reduced 59% and 86% by 500 nM and 1000 nM CytoD, respectively. Interconnected tubular networks, characterized by long tubules and large mesh sizes were formed in the tubule formation assay. The addition of CytoD disrupted this in a dose-dependent manner, reducing tubule length and mesh integrity from CytoD concentrations as low as 50 nM. TEER measurements with the Maestro Z revealed that CytoD reduced barrier integrity, with barrier index values (TEER normalized to confluency) dropping by 34% (500 nM) and 69% (1000 nM), correlating with tight junction disruption. This non-invasive workflow combines live-cell imaging and real-time impedance measurements for efficient monitoring and functional validation of id-ECs, supporting advancements in vascularized tissue engineering and iPSC-based regenerative medicine.

F1085

DEVELOPMENT OF CHONDRO-PASTE USING HIPSC-DERIVED CHONDROCYTE PROGENITORS AND PHOTO-CROSSLINKABLE SCAFFOLDS

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Osteochondral defects are characterised by a limited capacity for self-repair, rendering treatment via regenerative medicine highly anticipated. However, challenges associated with regenerative medicine for osteochondral defects are primarily related to graft adhesion and the complexity of transplantation techniques. Human-induced pluripotent stem cell-derived expandable PRRX1+ limb bud mesenchymal cells, which function as human chondrocyte progenitor cells (hCPCs), were developed. The objective of this study is to establish a novel technique for cartilage regeneration by developing a paste-like, easily transplantable structure, designated as "Chondro-paste," which is composed of hCPCs and scaffolds. The differentiation of hCPCs into chondrocytes was induced through two-dimensional culture in 24-well culture plates or 10 cm Petri dishes. Subsequently, "Chondro-paste" was prepared by combining the cells with Mebiol gel(Ikeda), 2% methylcellulose(Wako), and a mixture of 5% gelatin methacrylate(CELLINK) and 1.2% alginate gel(Wako). Immunodeficient rats (F344-Il2rgem1Iexas) were used to create osteochondral defects on the femoral knee joint surface, and Chondro-paste was implanted using a 10 µL pipette. Four weeks after transplantation, Safranin O staining and immunostaining (human Vimentin, Aggrecan, and COL2) were performed on the grafts for histological evaluation. The growth of cartilage-like tissue resembling vitreous chondrocytes was observed within osteochondral defects in each application of Chondro-paste. The compatibility and continuity between the graft and the host osteochondral tissue were most effectively observed in the Chondro-paste containing 5% gelatin methacrylate and 1.2% alginate gel. hCPCs have the potential to differentiate into cartilage tissue when combined with a scaffold and transplanted into osteochondral defects. This study introduces a novel technique for cartilage regeneration using hCPCs and scaffolds, which can be transplanted in a straightforward and easily manageable manner. Further studies using larger animal models are necessary to validate the potential of Chondro-paste in treating osteochondral defects of different sizes and shapes.

Funding Source: This research was supported by the Grant-in-Aid for Scientific Research (C) from the Japan Society for the Promotional of Science (Grant Number 24K12308).

F1087

DEVELOPMENT OF HIPSC-DERIVED SCAFFOLD-FREE CARTILAGE TISSUES FOR PEDIATRIC TRACHEAL DISEASES

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Pediatric tracheal reconstruction presents significant challenges due to the need for growth-compatible solutions. Current approaches, including autologous tissues, tissue-engineered constructs, and allogeneic grafts, remain clinically underdeveloped. We developed human induced pluripotent stem cell-derived expandable PRRX1+ limb bud mesenchymal (ExpLBM) cells, functioning as human chondrocyte progenitor cells (hCPCs). These hCPCs can be mass-produced, cryopreserved, and used to generate stable scaffold-free cartilage tissues through a cell self-aggregation technique (CAT). This study evaluates the feasibility of applying hCPC-derived cartilage in pediatric tracheal reconstruction. Male immunodeficient rats (F344-Il2rgem1Iexas, 5 weeks old, ~150 g) were used to establish tracheal defect models. Two experimental groups were examined: hCPC-derived cartilage grafts and autologous rib cartilage grafts. hCPCs were processed into chondro-plates using the CAT method. After surgery, rats were monitored for three months to evaluate graft adaptation, tracheal integration, and epithelial remodeling. The hCPC-derived cartilage group exhibited significant tracheal adaptation, with no evidence of stenosis. The tracheal diameter expanded by approximately 1.5–2.0 times, and epithelial attachment was robust, facilitating successful graft integration. In contrast, rib cartilage grafts showed limited epithelial integration and tracheal stenosis. Notably, hCPC-derived chondro-plates retained their functionality after cryopreservation, highlighting their potential for on-demand clinical applications. This study demonstrates the feasibility of scaffold-free cartilage tissues derived from hCPCs for addressing critical challenges in pediatric tracheal reconstruction. The CAT method enhances graft biocompatibility and adaptability. Further investigations in large animal models and refinement of clinical application protocols are essential to advance their pediatric applications.

Funding Source: This research was supported by AMED (Grant Number: 18bm0704024h0001, 20bm0404064h0001) to Takeshi Takarada.

F1089

DEVELOPMENT OF SPINAL CORD-DERIVED EXTRACELLULAR MATRIX FOR SPINAL CORD ORGANOID TRANSPLANTATION IN SPINAL CORD INJURY MODEL

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Stem cell transplantation has been widely explored as a promising therapeutic approach for spinal cord injury (SCI). However, the lack of functional scaffolds in cell transplantation for SCI treatment leads to low therapeutic efficacy due to poor transplanted cell survival. To address these challenges, we aim to enhance regenerative potential of spinal cord organoids (SCOs) by employing an extracellular matrix (ECM) recapitulating spinal cord-specific microenvironment. Decellularized spinal cord-derived ECM (ScEM) matrix supports 3D culture for development, maturation, and functionality of human induced pluripotent stem cell-derived SCOs comparable to standard organoid culture matrix such as Matrigel. In a rodent SCI model, SCOs transplantation with ScEM hydrogel promotes axonal regeneration with neovascularization in lesions and induces functional restoration of locomotive activity. The combination of tissue-specific decellularized matrix and organoid technology presents a promising strategy for SCI treatment, offering a physiologically relevant three-dimensional environment that supports both organoid development and functional tissue regeneration. Our findings demonstrate the potential of engineered matrix-organoid constructs as an advanced therapeutic platform for spinal cord repair.

Funding Source: 1. The National Research Foundation of Korea (NRF) grant funded by the Ministry of Science and ICT (MSIT) (RS-2021-NR059722) 2. The Bio and Medical Technology Development Program of NRF funded by MSIT (No. RS-2024-00395393).

F1091

DIFFERENTIATION OF LUNG EXPANDABLE EPITHELIAL PROGENITORS IN AIR-LIQUID INTERFACE OR IN 3D CULTURE FOR FUNCTIONAL MODELING OF ALVEOLI STRUCTURE FORMATION

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The regeneration of human respiratory epithelium is a continuous and complex process. Cultivating cells from the distal regions of the lungs that form pulmonary alveoli has been particularly challenging due to their intricate, highly organized structure and the rapid onset of differentiation or replicative senescence in vitro. Recent advancements have enabled the differentiation of lung progenitors into a mosaic of surfactant-producing alveolar type 2 (AT2) cells and gas-exchanging alveolar type 1 (AT1) cells. This process relies on the activation of Wnt/Yap signaling and the inhibition of TGFβ pathways to achieve a more mature phenotype. However, the molecular and cellular mechanisms underlying alveolar development remain poorly understood, and reproducible in vitro models that accurately mimic the biology and pathology of the respiratory system are still lacking. Our lab established a protocol for generating human embryonic stem cell (hESC)-derived expandable lung epithelial progenitors (ELEPs) that express NK2 Homeobox 1 (NKX2.1) and prosurfactant proteins B and C, forming airway and alveolar structures in 3D cultures or in vivo. ELEPs can also be derived from human-induced pluripotent stem cells (iPSCs) from fibroblasts. We aimed to differentiate ELEPs into mature alveolar-type cells under air-liquid interface (ALI) or 3D culture conditions using various factors and inhibitors. Differentiation was evident morphologically and at the protein level, including SPC processing, caveolin-1 induction, and nascent extracellular matrix protein production (e.g., collagen I) in ALI cultures. Prolonged differentiation under these conditions activated ER stress pathways, leading to senescence and transitional AT2-AT1 phenotypes, which are typical in lung pathologies. In contrast, 3D cultures prolonged cell viability and supported protein production as well as the formation of structures resembling the alveolar cell network. These findings suggest that our progenitor cells can serve as a valuable in vitro model of human respiratory epithelium for studying development, disease mechanisms, and potential applications in preclinical drug screening.

Funding Source: This work was supported by the Czech Science Foundation (grant no. GA23-06675S), MZČR-RVO (FNBr, 65269705) and by Masaryk University (grant no. MUNI/A/1598/2023).

F1093

EARLY OMIC CHANGES IN THE BRAIN OF AXOLOTLS AFTER TISSUE REMOVAL REVEAL ACTIVATION OF NEUROREPAIR AND NEUROREGENERATION MECHANISMS

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The brain of axolotls regenerate in few weeks after tissue damage. In this study, we analyzed the early molecular changes by analyzing transcriptomic, miRNA and proteomic profiles within the first 6 hours, in the surrounding brain tissue, after partial removal of the dorsal pallium in wild-type animals from Mexico City. We identified a rapid activation of genes related to neural development, immune response, and tissue repair. Among the differentially expressed genes, key regeneration-associated genes and transcription factors were up-regulated, suggesting a swift molecular response conducive to neural regeneration. Proteomic analysis supported these findings, with early upregulation of proteins involved in cytoskeleton dynamics and synapse reorganization. miRNA analysis revealed downregulation of specific miRNAs, such as miR-199a-5p, correlating with the upregulation of regeneration-related genes. Cell-type enrichment bioinformatic analysis highlighted the involvement of ependymoglial cells in early post-injury events. The mechanical brain lesions made in this work are similar to the traumatic brain injury (TBI), which in mammals often causes tissue damage that leads to irreversible conditions of disability, due its limited regenerative capacity. A comparative analysis between axolotl and human TBI datasets underscored the unique regenerative pathways in axolotls, but also allowed the identification of 39 differentially expressed genes coincident in both species, offering insights that could aid to the development of future therapeutic strategies for enhancing neural repair in humans.

Funding Source: This work was supported by Conacyt-Tubitak 255793; additional support for I.V. laboratory includes PAPIIT-UNAM IN219122 and Pew Innovation Fund Biomed Innovation 2022-A-22568.

F1095

EFFICIENT HEPATIC DIFFERENTIATION OF HYDROGEL MICROSPHERE-ENCAPSULATED HUMAN PLURIPOTENT STEM CELLS FOR ENGINEERING PREVASCULARIZED LIVER TISSUE

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Liver tissue engineering is promising as an alternative strategy to treat liver failure. However, generating functional hepatocytes from stem cells is conventionally restricted by the immature status of differentiated cells. Besides, embedding hepatocytes in bulk scaffold is limited by a lack of vascularity and low cell-packing density. Here, we fabricate collagen type I (COL1) microspheres for efficient hepatic differentiation of pluripotent stem cells and subsequent assembly of prevascularized liver tissue (PLT). Using a microfluidic platform, we demonstrate that hydrogel COL1 microspheres (mCOL1) encapsulating human embryonic stem cells (hESCs) can be reproducibly generated and efficiently differentiated into hepatocyte-like cells (HLCs) microspheres for the first time. Compared with other culture configurations such as encapsulation of hESC in a bulk COL1 hydrogel and 2D monolayer culture, mCOL1 with high uniformity produce HLC microspheres of improved maturity based on comprehensive analyses of cell morphology, transcriptome profile, hepatic marker expression and hepatic functions. In addition, these HLC micro-spheres can be applied as building blocks to self-assemble with endothelial cells to construct a dense PLT. The PLT resembles native liver tissue with high cell-packing density, shows successful engraftment in mice liver following implantation, and exhibits improved hepatic function in vivo. Overall, it is believed that this multiscale technology will advance the fabrication of stem cell-based liver tissue for regenerative medicine, drug screening, and in vitro liver modeling.

Funding Source: National Key Research and Development Program of China (2019YFA0111300; 2018YFA0903400); Research Grants Council of Hong Kong (ECS (24204819); NSFC and RGC (N_CUHK432/20).

F1097

ELONGATING HEART ORGANOIDS RECAPITULATE EARLY HUMAN HEART TUBE MORPHOGENESIS AND DEVELOPMENTAL PROCESSES

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The early development of the heart involves the formation of a heart tube that undergoes elongation and looping, processes critical for shaping the mature heart. Disruptions in these morphogenetic events lead to congenital heart diseases, which affect 1% of newborns. Despite significant progress, our understanding of human heart development and the etiology of congenital malformations remains limited, largely due to the reliance on animal models and the absence of human in vitro systems capable of replicating these dynamic processes. Existing human heart organoids fail to capture the elongation and looping of the heart tube. Through

enhancing self-organization with minimal external cardiogenic signaling cues, we generated elongating heart organoids (eHOs) that successfully undergo heart tube formation, elongation, and looping, mimicking key aspects of natural heart morphogenesis. These eHOs displayed distinct regions corresponding to the atrium, ventricle, and sinus venosus along the longitudinal axis of the heart tube. Additionally, the eHOs exhibited directional propagation of contractile waves initiated from the sinus venosus region, mirroring the functional integration of the cardiac domains. Remarkably, the eHOs also generated proepicardial cells, which protruded from the sinus venosus and migrated away to form an epicardial layer. This novel platform offers an unprecedented human in vitro model of early heart development, enabling mechanistic studies of heart tube morphogenesis and providing new opportunities for investigating the origins of congenital heart defects. Furthermore, these eHOs hold promise for advancing preventive and therapeutic strategies targeting congenital heart diseases.

F1099

ENGINEERING A UTERUS-MIMETIC EXTRACELLULAR MICROENVIRONMENT FOR ENDOMETRIAL ORGANOID DEVELOPMENT AND REGENERATION

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The endometrium plays a vital role in blastocyst implantation during the implantation window, a period of peak receptivity regulated by estrogen, progesterone, and growth factors. However, existing in vitro models fail to accurately replicate the native tissue environment and its functions. This study presents a novel in vitro implantation model incorporating endometrial organoids (EOs) and stromal cell cultures within a decellularized uterus extracellular matrix (UEM) to better simulate physiological conditions. Mouse-derived EOs were successfully cultured and encapsulated in UEM, exhibiting formation efficiencies comparable to Matrigel. Hormonal treatment with estradiol and progesterone enhanced mucin secretion and proliferation, demonstrating the EOs' functional mimicry of endometrial tissue. In vivo, the combination of EOs and UEM effectively restored thin endometrium, significantly increasing epithelial thickness and reducing fibrosis. All treated mice achieved successful pregnancies. Co-culturing stromal cells with EOs in UEM facilitated epithelial monolayer formation, while GFP-labeled E3.5 blastocysts migrated toward the basal layer. Additionally, EOs cultured in UEM exhibited higher expression of endometrial genes and greater hormone responsiveness compared to Matrigel. These findings underscore UEM's potential in promoting endometrial regeneration and function, leading to improved pregnancy outcomes and reduced fibrosis.

Funding Source: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (NRF-2021R1C1C2009131) and the Brain Korea 21 Project for Medical Science, Yonsei University.

F1101

ENGINEERING MESENCHYMAL STEM CELL - DERIVED EXOSOME MIMETICS FOR BONE REGENERATION IN OSTEOPOROSIS

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Osteoporosis, a prevalent disorder among aging populations, is characterized by impaired bone remodeling largely due to increased marrow fat and reduced bone density. Bone marrow mesenchymal stem cells (MSCs) in osteoporosis favor adipogenesis over osteogenesis, exacerbating bone loss. Modulating aberrant MSC differentiation presents a promising therapeutic strategy for bone regeneration. Tribble 3 (Trb3), a critical regulator of MSC lineage commitment, has emerged as a potential therapeutic target for restoring osteogenic balance. This study aims to develop Trb3-enriched exosome mimetics (EM-B3) with enhanced bone-targeting property to promote osteoporotic bone regeneration. EM-B3 were generated from hMSCs treated with small molecules using an extrusion method. Comprehensive characterization of EM-B3, including structural and functional analyses, was conducted. To augment their bone-targeting efficiency, EM-Trb3 were metabolically engineered with alendronate (bEM-B3). Bone-binding capabilities of bEM-Trb3 were evaluated ex vivo using labeled particles on mouse bone chips. Their in vivo therapeutic efficacy was subsequently assessed in an ovariectomized (OVX) mouse model of osteoporosis through μCT imaging and quantitative analyses. TEM revealed the intact and spherical EM-B3 with an average size of ~100 nm, as confirmed by nanoparticle tracking analysis. Confocal microscopy demonstrated efficient cellular uptake of EM-B3. ELISA assay showed a significant upregulation of endogenous Trb3 within EM-B3. Functional assays highlighted that EM-TB3 enhanced osteogenic differentiation, evidenced by increased osteogenic gene expression, ALP activities, and mineral deposition. Conversely, EM-TB3 suppressed adipogenic differentiation, as indicated by reduced expression of adipogenic markers. Ex vivo studies confirmed the high bone-binding affinity of bEM-B3. In vivo μCT analysis revealed that bEM-B3 treatment significantly improved bone regeneration in OVX mice, as demonstrated by increased bone mineral density and bone volume fraction. Overall, bEM-B3 represent a novel and effective therapeutic strategy for addressing osteoporotic bone loss. This exosome-mimetic platform holds promise for clinical translation and offers a potential breakthrough in stem cell treatment.

Funding Source: NIH/NIDCR DE030539; Osteo Science Foundation; NSF 2401861; Princeton PACRI award.

F1103

ENHANCED IN VIVO SURVIVAL AND YIELD OF SUBTYPE-SPECIFIC DOPAMINE NEURONS FROM HUMAN PLURIPOTENT STEM CELLS BY MODULATION OF TNF-NFKB-P53 AXIS AND WNT-FGF18 SIGNALING

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While clinical trials are ongoing using human pluripotent stem cell (hPSC)-derived midbrain dopamine (mDA) neuron precursor grafts in Parkinson's disease (PD), unresolved challenges remain, including extensive cell death following transplantation and suboptimal mDA differentiation protocol from hPSC leading to low yield of mDA neuron proportion from the grafted cells. In particular, the yield of TH+ mDA neurons after in vivo grafting and subtype-specific mDA neuron markers can be further improved. For example, characterization of mDA grafts by single cell transcriptomics has yielded a small mDA neuron proportion and a considerable contaminating cell fraction. Through a pooled CRISPR/Cas9 screen, we identified p53-mediated apoptotic and TNFα-NFκB signaling as key contributors to postmitotic dopamine neuron loss. Coupled with novel cell surface marker-based mDA neuron purification strategies, a clinically approved TNFα inhibitor, adalimumab, significantly improved mDA neuron survival and functional recovery in a preclinical PD mouse model, offering a clinically translatable approach to optimize mDA neuron-based therapies in PD. To further improve mDA neuron differentiation, we present an optimized mDA neuron differentiation strategy that builds on our clinical grade ("Boost") protocol but includes the addition of FGF18 and IWP2 treatment ("Boost+") at the mDA neurogenesis stage. Boost+ mDA neurons show higher expression of EN1, PITX3, and ALDH1A1. Improvements in both mDA neuron yield and transcriptional similarity to primary mDA neurons are observed both in vitro and in grafts. Furthermore, grafts are enriched in authentic A9 mDA neurons by single-nucleus sequencing. Functional studies in vitro demonstrate increased dopamine production and release and improved electrophysiological properties. In vivo, analyses show increased percentages of TH+ mDA neurons resulting in the efficient rescue of amphetamine-induced rotation behavior in the 6-OHDA rat model and rescue of some motor deficits in non-drug induced assays, including the ladder rung assay that is not improved by Boost mDA neurons. The Boost+ conditions present an optimized protocol with advantages for disease modeling and mDA neuron grafting paradigms.

Funding Source: This work was supported by DGIST start-up funds from the Ministry of Science and ICT (2024010330) to T.W.K, and the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. RS-2024-00351442) to T.W.K.

F1105

ENHANCING CARDIOMYOCYTE PURIFICATION VIA LACTATE-BASED METABOLIC SELECTION

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Direct reprogramming, which converts one cell type directly into another without passing through a pluripotent state, provides a unique and promising source for therapeutic applications. Reprogramming fibroblasts into chemically induced cardiomyocyte-like cells (CiCMs) using small molecules has emerged as a valuable approach for cardiac regeneration and therapeutic development. However, the presence of contaminating non-cardiomyocytes, primarily untransformed fibroblasts, undermines the functionality of CiCMs in various applications. Although several strategies exist for enriching cardiomyocytes such as transgenic selection methods via drug-selectable elements or fluorescence-activated cell sorting (FACS), these approaches often involve significant technical challenges. To address these limitations, our group investigated a metabolic selection approach that leverages the ability of cardiomyocytes to utilize lactate as an energy source in addition to glucose. Primary mouse embryonic fibroblasts (pMEFs) were reprogrammed into CiCMs and treated with a glucose-depleted medium supplemented with lactate. Under lactate-enriched conditions, the proportion of non-cardiomyocytes was significantly reduced while CiCMs (CiCM-LAC) remained largely unaffected. The CiCM-LAC group exhibited higher expression levels of cardiac-related genes and showed more pronounced and intense expression of cardiac troponin T (cTnT) compared to the untreated group (CiCM-NT). Moreover, metabolically purified CiCMs exhibited enhanced contractile force, higher contraction frequency, and elevated drug responsiveness compared to non-purified CiCMs. Taken together, our study suggests that lactate-based metabolic selection not only provides a highly purified cardiomyocyte population but also preserves their functional integrity, offering a promising platform for potential clinical applications.

Funding Source: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (NRF-2021R1C1C2009131) and the Brain Korea 21 Project for Medical Science, Yonsei University.

F1109

ESOPHAGUS EXTRACELLULAR MATRIX FOR CULTURE AND TRANSPLANTATION OF ESOPHAGEAL ORGANOIDS

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Esophageal organoids hold significant potential as therapeutics for esophageal mucosal damage, although their clinical application remains largely unexplored. To investigate esophageal organoids as regenerative therapeutics, there is an urgent need to develop alternatives to tumor-derived extracellular matrix (ECM), the standard culture matrix for organoids, due to its safety concerns. In this study, we leverage decellularized esophagus-derived ECM (EEM) for the culture and transplantation of esophageal organoids. Comprehensive proteomic analysis revealed that EEM provides a microenvironment with a complexity suitable for esophageal organoids. Furthermore, the reproducibility of EEM was confirmed by comparing proteomic profiles of EEM samples derived from different batches or donors. Esophageal organoids cultured in EEM hydrogel contained proliferative basal cells and differentiated suprabasal cells, similar to the structure of esophagus tissue. Additionally, the EEM hydrogel supported the long-term maintenance of esophageal organoids in vitro. The transplantation of esophageal organoids with EEM promoted epithelial regeneration and reduced fibrosis at the wound site in a mouse model of esophageal ulcer. Collectively, our

EEM-based platform offers a stable and refined matrix, thereby advancing the clinical applications of esophageal organoids.

Funding Source: 1. The National Research Foundation of Korea (NRF) grant funded by the Ministry of Science and ICT (MSIT) (RS-2021-NR059722) 2. The Nano and Material Technology Development Program through the NRF funded by the MSIT (RS-2024-00449435)

F1111

EVALUATION OF INJECTABLE ADIPOSE STEM CELL-DERIVED MICRO-CARTILAGE HYDROGEL FOR REGENERATIVE TREATMENT OF LUMBAR DISC HERNIATION USING A NOVEL PRECLINICAL MODEL

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Lumbar Disc Herniation (LDH), primarily caused by intervertebral disc degeneration (IDD), affects 10-20% of the population globally, leading to pain and disability. Available treatments mainly focus on symptom management. Stem cell therapies hold promise for treating LDH but often show low recovery rates and poor in vivo remodeling. Current implantation techniques for tissue-engineered lumbar discs or nucleus pulposus face challenges due to the spine's complex, load-bearing structure, particularly in LDH patients. The lack of effective in vitro and in vivo models for LDH limits progress in regenerative therapy research. To address these challenges, we developed an injectable platelet-rich plasma (PRP) micro-cartilage hydrogel (PAM) containing adipose stem cell-derived chondrogenic cell masses (ASCC) under 1 mm in diameter and in situ cross-linkable PRP. Additionally, we developed a novel rat spine motion segment LDH load-bearing model (rSMS-LDH), which was combined with an in vitro 'sandwich' model and an in vivo rabbit LDH model to validate PAM’s integrability and remodeling ability in the intervertebral disc. Histological and immunofluorescent staining results showed that PAM integrates well with the nucleus pulposus and fibrosis, with less cell leakage and better cartilage-like extracellular matrix (ECM) formation compared to the PRP stem cell hydrogel (PSH) in vitro. Although both PAM and PSH showed therapeutic benefits in four-week animal studies, the PAM group demonstrated enhanced anti-ferroptosis effects and more consistent ECM production, which are crucial for cell survival in the harsh disc microenvironment and long-term intervertebral disc repair. This study revealed that PRP micro-cartilage hydrogel provides better niche-rebuilding conditions in the harsh intervertebral environment. This minimally invasive injectable hydrogel could transform the management of LDH. The rat SMS-LDH ‘spine organoid’ avoids inhumane LDH generation methods and offers a valuable preclinical model for LDH regenerative treatments.

Funding Source: Research and Innovation Team Project for Scientific Breakthroughs at Shanxi Bethune Hospital.

F1113

EXPLORING THE HEPATIC STELLATE CELL DYNAMICS IN 3D ACELLULAR LIVER SCAFFOLDS: THE ROLE OF THE EXTRACELLULAR MATRIX

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Acellular liver scaffolds (ALS) are powerful tools to overcome organ shortage problems. To date, it is unknown whether transplanted ALS are affected by cirrhotic livers, either becoming cirrhotic themselves or instead remaining as a robust template for healthy cell growth after transplantation (tx). Since Hepatic Stellate Cells (HSCs) play a key role in fibrogenic processes, the aim of this study was to analyse whether the change in the ECM impacts HSC behaviour (pro-fibrotic reverses into a more quiescent state?). To address this, in vivo and in vitro experiments using human 3D ALS were performed. For in vitro experiments, primary human HSC (250k) were cultured in cirrhotic ALS for 14 days. HSC were then extracted from cirrhotic ALS and reseeded in healthy ALS for 14 days. Histological analysis (H&E), cell viability, RT-PCR (Col1alpha1, PDGFR, ACTA2, and CYTGB), and western blot analysis were performed (n=4). For in vivo analysis, decellularized livers obtained from Wistar rats (Animal Care approval-UFRJ 097/20) were transplanted into cirrhotic recipient rats. Cirrhotic recipient rats (n=5) received 5% ethanol in drinking water and i.p injections of carbon tetrachloride (1 ml/Kg) for 8 weeks, underwent hepatectomy (10%) and partial ALS orthotopic tx. H&E staining, immunohistochemistry (alpha-SMA), and microcirculation analysis were performed. RT-PCR and western blot analysis from in vitro experiments revealed that the fibrotic HSC reverses to a more quiescent state when HSC are extracted from cirrhotic and subsequently seeded and cultured in healthy ALS. Results from in vivo analysis showed that HSC migrated from the recipient cirrhotic liver to healthy ALS after tx. Histological and microcirculation analyses revealed that HSC underwent remodeling, transitioning into a more quiescent state 30 days after tx. Our results showed that the ECM affected HSC behaviour, reversing from a profibrotic to a quiescent state both in vivo and in vitro. Currently, hepatocytes derived from induced pluripotent stem cells are cultured with HSC to investigate whether cell-cell and cell-ECM could affect HSC behaviour. Thus, 3D ALS represents a robust template for healthy cell growth stimulation that can impact on liver regeneration after hepatectomy.

Funding Source: CNPq, Capes, FAPERJ, INCT-REGENERA, Ministério da Saúde.

F1115

EX-VIVO GENERATION OF IMMORTALIZED ERYTHROID PROGENITOR CELL LINES FROM HEMATOPOIETIC STEM CELLS FOR RED BLOOD CELL PRODUCTION

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Blood transfusion is a critical component of modern medicine, yet the global demand for red blood cells (RBCs) continues to outpace supply, particularly in low- and middle-income countries. Developing reliable methods for the sustainable in vitro production of red blood cells is crucial to offering an alternative source of clinical-grade blood, especially for individuals with rare blood group phenotypes. Immortalized erythroid progenitor cell lines represent the most promising emerging technology to accomplish this objective. Current methods for in vitro generation of red cells from adult and bone marrow progenitors fail to provide a sustainable supply, and existing systems relying on pluripotent stem cells as progenitors cannot produce viable red cells. To address this challenge, we have adopted an alternative strategy by immortalizing early adult erythroblasts from cord blood hematopoietic stem cells to generate a stable cell line that provides a continuous supply of red cells. We have successfully generated immortalized erythroid progenitor cell lines derived from CD34+ hematopoietic stem cells (HSCs) derived from cord blood using lentiviral vectors to introduce HPV16 E6/E7 oncoproteins. By employing a three-stage erythroid culture system, these immortalized cells efficiently differentiate into mature, functional reticulocytes that can be isolated through filtration. Comprehensive characterization has shown no functional or molecular differences between these reticulocytes and in vitro-cultured adult reticulocytes, with no evidence of aberrant protein expression. This work establishes a viable method for producing red cells in vitro for clinical applications. This work represents a significant advancement in addressing blood shortages, offering a sustainable and scalable solution for transfusion-grade RBC production. The potential implications of this achievement extend beyond addressing transfusion demands, providing a platform for future research into erythroid biology and the development of personalized cellular therapies. Our findings demonstrate a promising pathway to revolutionize blood supply systems globally.

Funding Source: This work was funded by the Aga Khan University (AKU) University Research Council (URC) grant No. 191018CRM.

F1117

FUNCTIONAL CHARACTERIZATION OF HUMAN TYMPANIC MEMBRANE-DERIVED PROGENITORS AND THEIR EXTRACELLULAR VESICLES UNDER SERUM-FREE CULTURE CONDITIONS

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The transplantation of stem or progenitor cells derived from human tympanic membrane and their extracellular vesicles (EVs) into damaged sites can stimulate numerous regenerative functions through the secretion of various bioactive molecules. However, currently there is limited data on the cellular and phenotypic properties of progenitors and EVs responsible for regeneration of tympanic membrane. Therefore, the present study investigated the potency features along with markers expression in human tympanic membrane progenitors and their EVs for clinical applications using serum-free media. Tympanic membrane samples were used for establishing the cells in vitro. A plastic adherent progenitor cells displayed a small spindle-shape with enhanced cell number due to increased proliferation, and the growth was higher during culture expansion. The FACS assay determined the varied proportion of cells expressing integrin beta 1, cytokeratin 19 (epidermal/epithelium), nestin, beta III tubulin (neural), and vimentin (fibrous/dermal) and the positively stained cells were thus considered to be potential progenitors in the tympanic membrane. Further, an enriched round and discoid vesicles from the progenitors of tympanic membrane were successfully separated and the results of transmission electron microscopy (TEM) have shown the circular or elliptical vesicles with disc and cup-shaped structures, possessing a diameter about 50-150 nm. Nanoparticle tracking analysis (NTA) suggested the main peaks of EVs around 100 nm. The results of Western blotting showed that three positive protein markers (CD9, CD63, and CD81) were highly expressed and negative protein (Albumin) was very low or absent in EVs. Moreover, progenitors isolated EVs displayed anti-inflammatory and regenerative ability, and promoted angiogenesis and extracellular matrix remodeling, in vitro. In conclusion, tympanic membrane derived clinical-grade progenitors showed their potency features and the growth was relatively higher. Further, the expression of phenotypic markers indicated their plasticity and stemness properties. Morphology, particle size, and labeled protein marker detection confirmed EVs separation from progenitors of tympanic membrane, and provided a basis for investigating their regenerative functions in vivo.

Funding Source: Funded by Nitte (Deemed to be University).

F1119

GENERATION OF CORNEAL ENDOTHELIAL-LIKE CELLS FROM ADULT NEURAL CREST-DERIVED CELLS IN SERUM-FREE CONDITIONS

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Corneal endothelial dysfunction is one of the leading causes of vision loss, and endothelial transplantation is the only available curative option. However, due to the global shortage of donors and the low in vivo proliferative capacity of corneal endothelial cells (CECs), there is increasing focus on efficient culture methods to differentiate CECs from pluripotent stem cells (PSCs) through a neural crest (NC) lineage. In this study, we generated induced neural crest-like cells (iNCLCs) from adult mouse palate-derived fibroblasts (mPF) via a non-genetic approach. These iNCLCs could be easily acquired and expanded for as long as 9 passages in serum-free NC induction medium (NCIM). We demonstrate that NCIM is suitable for selectively supporting the growth of NC-derived cells with distinct morphological features. The iNCLCs exhibited a dramatic increase in neural crest stem cell markers, including AP2, Nestin, and Sox2. Notably, these iNCLCs possessed a high proliferative potential and were capable of differentiating into CEC-like cells with regular hexagonal morphology. Consequently, our

protocol enables the efficient generation of CEC-like cells from NC-derived cells. We propose that palate-derived iNCLCs may serve as a promising cell source for personalized regenerative therapies to repair damage in NC-derived tissues such as corneal endothelium.

F1121

GENERATION OF HUMAN SKELETAL ASSEMBLOIDS FROM IPSC-DERIVED GDF5-POSITIVE INTERZONE-LIKE ORGANOIDS

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Human pluripotent stem cells (hPSCs) have shown great potential in recapitulating developmental processes for tissue engineering and disease modeling. However, generating functional skeletal tissues, especially those involved in limb development, remains a major challenge. In this study, we describe a novel methodology for differentiating human induced pluripotent stem cells (iPSCs) into region-specific skeletal progenitor cells, including those giving rise to articular cartilage and growth plate cartilage. We successfully induced limb bud mesenchymal cells (LBM) from iPSCs, derived from the lateral plate mesoderm, and used these cells as a foundation for generating articular cartilage and growth plate cartilage, two key structures in limb development. Through a combination of molecular signaling manipulations, 3D chondrogenic induction, and in vivo transplantation, we identified conditions that promote GDF5-positive (GDF5+) and GDF5-negative chondrogenic fates in a controlled manner. Moreover, we constructed human skeletal assembloids, combining chondrocyte progenitors from different regions of the limb, which mimicked endochondral ossification and longitudinal growth when transplanted into animal models. This study provides a significant advancement in stem cell-based limb regeneration and offers a powerful platform for investigating developmental biology and disease.

Funding Source: The JSPS (T.T and T.Takao) AMED (T.Takao) the JST FOREST Program (T.T.) the Astellas Foundation for Research on Metabolic Disorders the Cooperative Research Program of the LiMe Kyoto University.

F1123

GENERATION OF INDUCED-T-TO-NATURAL KILLER CELLS FROM HUMAN EXPANDED POTENTIAL STEM CELLS

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Human pluripotent stem cells (hPSCs) have been considered a promising source for the generation of immune cells for immunotherapy and transplantation. However, lympho-myeloid hematopoiesis has been masked by the dominant primitive and myeloid-restricted waves of hematopoiesis in hPSC differentiation. Generating functional immune cells remains a great challenge. Among them, T cells and natural killer (NK) cells are of special interest due to their substantial potential in immunotherapy. T cells play a critical role in adaptive immunity while natural killer (NK) cells are important for the innate immune system. By knocking out a key T cell regulator gene Bcl11b, T cells can acquire NK features, termed induced-T-to-NK (ITNK) cells, which are highly potent in tumor killing. We establish an efficient, highly reproducible and cell line-independent system for human embryonic hematopoiesis and develop a method to generate ITNK cells from human expanded potential stem cells, which can be further engineered for antitumor functions. Our human EPSC-based hematopoiesis system can in a week produce a large number of CD34+ hematopoietic stem/progenitor cells, which process multilineage potentials. The HSPCs can differentiate to T/ITNK cells robustly, serving as a novel type of cell source for cell-based cancer therapy. Human EPSCs are genetically and epigenetically stable and are amenable to efficient precision genome editing. Our human EPSC-based hematopoiesis system can thus offer a useful tool to genetically dissect human hematopoiesis.

Funding Source: This project is supported by Health@InnoHK, Innovation Technology Commission, HKSAR.

F1125

GENERATION OF RAT LUNG TISSUES IN FGF10-DEFICIENT MICE VIA BLASTOCYST COMPLEMENTATION

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The generation of pluripotent stem cells (PSCs)-derived lung tissues is one of the ultimate goals in the field of regenerative medicine. Fibroblast growth factor 10 (Fgf10) is a gene involved in the development of limbs and lungs in mice. Fgf10-/- mice present phenotypes

indicating limb and lung deficiencies. We have earlier generated functional lung organs from mouse embryonic stem cells (ESCs) via blastocyst complementation in Fgf10 compound heterozygous mutant (Fgf10 Ex1mut/Ex3mut) mice. Here, we report the generation of rat lung tissues in interspecies rat-mouse chimeras using Fgf10 Ex1mut/Ex3mut model mice. A total of 1136 mouse blastocysts were microinjected with rat ESCs and 1107 of these blastocysts were transferred into pseudopregnant mouse uteri, and 242 neonates (21.9%) were obtained. Genotyping revealed that a total of 53 of 242 neonates (%) was derived from Fgf10 Ex1mut/Ex3mut blastocysts. A total of 13 of 242 neonates (5.3%) was identified as rat-mouse chimeras by confirming GFP expression and 5 of 53 (9.4%) Fgf10 Ex1mut/Ex3mut mice were confirmed as rat-mouse chimeras. 3 of 5 Fgf10 Ex1mut/Ex3mut rat-mouse chimeras exhibited limb deficiencies. Lung morphogenesis was confirmed in all of the Fgf10 Ex1mut/Ex3mut rat-mouse chimeras including those with limb deficiencies. However, lobes of lung even in Fgf10 Ex1mut/Ex3mut rat-mouse chimeras without limb deficiencies were fewer compared with those in wild type mice. Histological analysis exposed that rat lung tissues were generated from rat ESCs in Fgf10 Ex1mut/Ex3mut mice via blastocyst complementation. However, generated lung tissues were mosaic of ESCs and recipient cells in the parenchymal regions as well as in the interstitial regions. In spite of the low efficiency of generating rat-mouse chimeras and incomplete lung morphogenesis in Fgf10 Ex1mut/Ex3mut rat-mouse chimeras, these findings provide useful insights to generate lung tissues in an interspecies setting.

Funding Source: This work was supported by JSPS KAKENHI Grant Number 20H03741 and 24K11760.

F1127

GONADOTROPHS HAVE A DUAL ORIGIN WITH MOST DERIVED FROM EARLY POSTNATAL PITUITARY STEM CELLS

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Reproductive health is crucial for fertility, and important for overall well-being. While reproductive capacity begins at puberty, reproductive development starts much earlier, shortly after birth, during a preparatory phase called minipuberty. This period is important for future fertility in males, and brain development in both sexes. The same hormones, control minipuberty, puberty, and reproduction. These hormones, namely LH (luteinizing hormone) and FSH (follicle-stimulating hormone) are produced by the pituitary, a crucial gland located underneath the brain. They act on the gonads where they induce steroidogenesis and the production of germ cells, as well as sexual development, bone strength and physical and psychological health. In turn, LH and FSH synthesis and secretion are controlled by the

hypothalamic gonadotrophin-releasing hormone (GnRH), and gonadal steroid feedback. In the pituitary, LH and FSH are produced by gonadotrophs. These first appear in the embryonic pituitary, along other endocrine types, and all expand after birth. While gonadotrophs may display heterogeneity in their response to GnRH, they appear, at least transcriptionally, as a homogenous population. The pituitary also contains a population of stem cells (SCs), whose contribution to postnatal growth is unclear, in part because endocrine cells maintain the ability to proliferate. Here we show an unsuspected dual origin of the murine adult gonadotroph population, with most gonadotrophs originating from postnatal pituitary stem cells, starting early postnatally and up to puberty, while embryonic gonadotrophs are maintained, as a locally distinct population. We further demonstrate that postnatal gonadotroph differentiation happens independently of GnRH or gonadal signals. The division of gonadotrophs based on separate origins has implications for our understanding of the establishment and regulation of reproductive functions, both in health and in disease.

Funding Source: Medical Research Council, UK (U117512772, U117562207, and U117570590) and the Francis Crick Institute (FC001107), which receives its core funding from Cancer Research UK, the UK Medical Research Council, and the Wellcome Trust.

F1129

HUMAN CORD LINING IPS CELL-DERIVED CARDIOVASCULAR PROGENITORS POTENTIALLY EVADE HOST IMMUNE REJECTION IN A MYOCARDIAL INFARCTION MOUSE MODEL

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In the field of regenerative cardiology, stem cell therapy holds the potential for replacing damaged heart muscle. Through a patented differentiation method based on human recombinant laminins, we have previously demonstrated the reproducible generation of cardiovascular progenitors (CVPs) from human pluripotent stem cells. These CVPs showed strong engraftment, significant improvements in cardiac ejection fraction, and a 50% reduction in ventricular arrhythmias when transplanted intramyocardially into myocardial infarction (MI) models in mice and pigs. Immunosuppressive drugs were typically used during these studies to prevent immune rejection of the human grafts. However, the animals experienced side effects such as anemia and stomach ulcers. Therefore, to develop a safer cellular transplantation therapy, these challenges must be addressed. This project investigates the hypothesis that differentiating induced pluripotent stem cells (CLiPS) derived from cord lining epithelial cells into CVPs will enable the progenitors to maintain their hypo-immune state and persist in an immune-competent animal model without the need for systemic immunosuppression. Initially, we adapted pluripotent CLiPS cells and fibroblast-derived iPS cells from the Matrigel™ culture system to a xeno-free system using laminin-521 and chemically defined Nutristem cell culture media. We then characterized the cells by flow cytometry to assess pluripotent markers (Tra1-60 and Oct3/4) and karyotyping. Following this, the cells were differentiated into cardiac

mesodermal lineage, and cardiac markers (Troponin-T (TNNT2) and a-actinin (ACTN2)) were quantified by flow cytometry and qPCR. Results showed that the newly adapted iPSCs maintained pluripotency and differentiation efficiency into cardiac lineage. Subsequently, luciferase-labeled CLiPS-CVP and control cells were transplanted into an immune-competent myocardial infarction mouse model for 4 weeks. Histological analysis and in vivo imaging were employed to monitor the cells' engraftment, survival, and biodistribution of the cells within the host. These results could pave the way for hypo-immune cellular therapies for regenerative medicine, potentially overcoming the need for immunosuppression.

Funding Source: This work has been supported by grants from the National Research Foundation (CRP24-2020-0083), Singapore, and the Ministry of Education, Singapore (MOE-RS06/23, MOE-RG38/24, and MOE start-up grant, 022976-00001).

F1131

HUMAN UMBILICAL CORD LINING EPITHELIAL CELLS AS A NON-NATIVE SOURCE FOR EPIDERMAL RECONSTRUCTION

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The concept of using "non-native" sources of cultured epithelial cells as a form of regenerative medicine is not new. Corneal reconstruction with cultured autologous oral mucosal epithelium remains in practice today. However, the question of whether cultured human umbilical cord lining-derived epithelial cells (hCLECs) can engraft as a non-native source for skin epidermal reconstruction of severe wound defects is still not fully answered. We first characterised and compared human umbilical cord and skin tissues through histological analysis and identified common proteins in both tissues. These include keratin combinations 5/14 and 1/10, epithelial stem cell markers Tp63 and ΔNp63, integrins α6 and β1, as well as the differentiation marker involucrin. Single-cell RNA sequencing of hCLECs isolated directly from the lining of umbilical cords revealed that epithelial cells constitute the majority of the four main cell populations detected, and which share many common genes associated with human epidermal keratinocytes (hEKs). The transcriptomic signature of one epithelial cell population cluster showed a 44.9% overlap with the holoclone of hEKs, and GO analysis suggested that this cluster possesses stem-cell-like characteristics. We evaluated the culture of hCLECs in a classical mouse feeder system using 3T3-J2, based on Green and Rheinwald's method. Colony forming efficiencies (CFE) of hCLECs were comparable to hEKs, averaging 20-40% for the first three passages. Subsequently, the majority of hCLEC colonies became aborted, with a significant reduction in size. Similarly, cumulative population doublings of hCLECs were considerably lower at approximately 20, reaching a plateau at passages 4-5, compared to hEKs with 60 cumulative doublings. Addition of ROCK inhibitor (Y-27632) into the culture system rescued the low growth potential and CFE of hCLECs. Functional assays on early-passage hCLECs demonstrated that these cells could attach and stratify on 3D skin organotypic cultures in vitro, and similarly engraft and stratify on a nude mouse flap in vivo. These findings suggest that hCLECs expanded ex vivo at early generations could serve as an alternative to hEKs, providing a non-native and autologous cell source for treating major burns, extensive congenital aplasia cutis, and epidermolysis bullosa.

Funding Source: National Medical Research Council, Ministry of Health, Singapore (OFIRG20nov-0024).

F1133

IGFBP7-MEDIATED PARACRINE EFFECTS OF MSCS' INSOLUBLE SECRETOME: A KEY DRIVER OF ANGIOGENESIS AND WOUND HEALING

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Mesenchymal stromal cells (MSCs) promote tissue repair via paracrine mechanisms. While past studies mainly identified responsible components within their soluble secretome, MSCs are also excellent producers of extracellular matrix (ECM), their insoluble secretome. ECM assembly allows spatially and stoichiometrically precise organization of signaling factors, thereby harnessing their full bioactivity. We thus hypothesized that MSCs' therapeutic effects might be mediated through insoluble factors in their ECM. To test this, bone marrow-derived MSCs were stimulated to deposit ECM in the presence of a heparan sulfate mimetic (HSM), facilitating ECM assembly. The resultant decellularized biologic was processed into Microparticles of Solidified Secretome (MIPSOS). Treatment of full-thickness skin wounds with MIPSOS enhanced revascularization and healing, surpassing naïve MSC-derived ECM (cECM), deposited in the absence of HSM. Proteomic analysis showed MIPSOS was enriched in pro-angiogenic factors compared to cECM, with strong protein-protein interactions identified in an IGF signaling cluster, where IGF binding protein 7 (IGFBP7) was most enriched. Prior research showed highly conflicting results on IGFBP7's pro-angiogenic properties, suggesting its activity may depend on its ECM microenvironment composition. Next, IGFBP7 was knocked down in MSCs synthesizing MIPSOS, creating MIPSOS (IGFBP7-KD). Endothelial cells showed slower adherence, reduced migratory and sprouting ability on MIPSOS (IGFBP7-KD), while their proliferation remained unaffected. MIPSOS enhanced skin wound revascularization and closure, while MIPSOS (IGFBP7-KD) exhibited less therapeutic effects. Application of soluble IGFBP7 or its supplementation to MIPSOS (IGFBP7-KD) had no therapeutic benefit. Our data suggest that MSCs promote tissue repair via their ECM, with IGFBP7 being a key driver of revascularization and wound healing. Proper incorporation of IGFBP7 into MSC-derived ECM appears crucial for this effect, although the exact mechanism by which ECM-incorporated IGFBP7 promotes wound repair remains to be determined. The insoluble format of MSC-derived ECM ensures long-term stability and sustained release of bioactive factors, rendering MIPSOS a suitable off-the-shelf biologic for wound repair.

Funding Source: This work was supported by the general research fund (GRF, 14115723, UGC) and by the research fund to the Center for Neuromusculoskeletal Restorative Medicine from Health@InnoHK program (ITC), Hong Kong SAR.

F1135

IN SITU 3D CELL SPHEROID FORMABLE AND TRANSPLANTABLE HYDROGEL CONCAVE FOR MYOCARDIAC INFARCTION MODEL

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Traditional cell spheroid formation culture platforms with micro-concave structures have been proposed as an effective system for cell spheroid formation and culture. However, this system presents challenges for transplanting cell spheroids and often results in a low cell survival rate in ischemic regions. To overcome these limitations, a dual-functional open and close (O/C) type micro-concave hydrogel patch has been developed for 3D cell spheroid formation and transplantation. The open-type patches were fabricated using rigid hydrogel, while the close-type patches were fabricated by combining highly swellable soft hydrogel with rigid hydrogel. A hypothesis has been set that the transplantation of open- type cell spheroids, along with the release of paracrine factors from close-type cell spheroids, would synergistically enhance therapeutic effects in ischemic lesions. The O/C-type patches were prepared with various concentrations in a ratio of swellable polyacrylamide (PAM) hydrogel, using a 3D-printed micropillar-like mold. Additionally, the characterization of PAM has been done to improve the compactness of 3D cell spheroids in the close-type patches. Transplantation of O/C-type patches containing spheroids significantly enhanced therapeutic outcomes in a rat cardiac infarction model compared to open- type patches alone. These results showed that defining a functionally advanced 3D micro-concave as in situ stem cell spheroid formation and transplantation could be a practical candidate for tissue regeneration.

F1137

IN VIVO INDUCTION OF RETINAL GANGLION CELLS WITH DEVELOPMENTAL TRANSCRIPTION FACTORS

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Glaucoma is the most prevalent retinal degeneration disease that is caused by the lesion and loss of retinal ganglion cells (RGCs). Finding a method to regenerate RGCs represents a critical direction toward curing glaucoma, but there is still no effective one to regenerate RGCs. In our previous study, we identified a combination of three transcription factors (TFs)—Ascl1, Brn3b, and Isl1(abbreviated as ABI)—that efficiently reprogrammed fibroblasts into RGC-like neurons (iRGCs) in vitro. In this study, we used mouse late-stage retinal progenitor cells (late-RPCs) which lack the competence to differentiate into RGCs under the established developmental process, as a model to examine the ability of ABI to induce the RGC fate through direct somatic cell reprogramming in situ. Our results showed that overexpressing ABI reprogrammed the developmental competent state of the late RPCs, enabling them efficiently to generate RGCs. The ABI-induced RGCs exhibited RGC-like electrophysiological activities, projected axons to and formed synaptic connections with the lateral geniculate body (LGN) and superior colliculus (SC). Single cell RNA sequencing confirmed the RGC nature of the induced cells and revealed the molecular route underlying the reprogramming process. Furthermore, CUT&RUN and ATAC-Seq analyses revealed roles of each TF in the ABI combination during the reprogramming process. Taken together, our study demonstrated the potent capability of Ascl1, Brn3b, and Isl1 in in vivo RGC fate induction. These findings may pave the way for translational applications in glaucoma treatment in the future.

Funding Source: The National Natural Science Foundation of China (32470848).

F1139

INVESTIGATING THE ROLE OF BIP IN UPR REGULATION AND APOPTOSIS IN HUMANEMBRYONIC STEM CELLS

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Human embryonic stem cells (hESCs) are susceptible to dissociation-induced stress, activating the unfolded protein response (UPR) and triggering apoptosis. BiP (GRP78), a key ER chaperone, regulates UPR signaling and maintains ER homeostasis. However, the specific role of BiP in dissociation-induced stress in hESCs is unclear. This study investigates how BiP knockout (BiP-KO) alters UPR pathways and apoptosis, and evaluates TUDCA's potential to improve cell survival. BiP was knocked out in hESC line (H9) using CRISPR/Cas9 technology. BiP-KO and wild-type (WT) hESCs were dissociated and analyzed over a 24-hour time course. UPR markers (EIF2α, ATF4, ATF6, ERN1, spliced XBP1, total XBP1) were quantified using qRT-PCR. Apoptosis markers (CHOP, cleaved caspase-3) were assessed by Western blotting and immunofluorescence. TUDCA treatment was used to assess protective effects on ER stress and apoptosis. BiP-KO cells exhibited significant alterations in UPR signaling. EIF2α levels increased over time and were significantly higher in BiP-KO cells, indicating enhanced UPR activation. In contrast, ATF4 and ATF6 levels decreased over time and were significantly lower in BiP-KO cells, while WT cells showed an increase, reflecting adaptive UPR activation. Spliced XBP1 levels increased over time and were significantly higher in BiP-KO cells, while total XBP1 levels decreased and were lower in BiP-KO cells, indicating altered XBP1 dynamics. No significant difference in ERN1 expression was observed between BiP-KO and WT cells. BiP-KO cells demonstrated increased apoptosis, with elevated cleaved caspase-3 and CHOP levels. TUDCA treatment partially alleviated ER stress and reduced apoptosis markers in both BiP-KO and WT cells. BiP is crucial for maintaining ER homeostasis and UPR signaling in hESCs under dissociation-induced stress. Its absence causes dysregulated UPR pathways, increased EIF2α, spliced XBP1 activation, and heightened apoptosis. While ERN1 expression remains unchanged, elevated spliced XBP1 suggests BiP influences ERN1 activity indirectly. TUDCA shows promise in reducing ER stress and apoptosis, supporting its potential as a therapeutic approach to enhance hESC viability and address ER stress-related diseases.

Funding Source: Shahnaz Babaei was supported by a Cumming School of Medicine Postdoctoral Fellowship.

F1141

INVESTIGATING THE ROLE OF NERVE GROWTH FACTOR IN NEURO-CARDIO-VASCULAR CO-DEVELOPMENT IN HUMAN 3D INNERVATED CARDIAC MUSCLE MODEL

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Nerve growth factor (NGF) is a neurotrophin crucial for the differentiation and survival of sympathetic neurons (SNs). NGF released by perivascular cells in the developing heart, controls cardiac sympathetic innervation. We developed a 3D innervated cardiac muscle model from hiPSC (iEHM) by fusing a sympathetic neuronal organoid and engineered heart muscle. Using optogenetics, we demonstrated functional connectivity between SN and cardiac cells (n=41 iEHM, 3 independent differentiations). Single nuclei RNA sequencing revealed among others the co-development of vascular and perivascular cells which were further validated by immunofluorescence. The extensive vascular network (PECAM1pos), surrounded by pericytes (PDGFR-betapos) was interlaced by neurons and sympathetic varicosities (SYN1/THpos) suggesting an interaction between SN and vascular cells. Orientation and proximity analysis, via machine learning-based system, showed a significant correlation in the directionality of SN and cardiac vessels in iEHM. snRNAseq showed that perivascular cells in the tissue were the main source of NGF, which is in line with in vivo data. To study the role of NGF in neuro-cardio-vascular development, we developed two hiPSC lines via CRISPR/Cas9 engineering: an NGF knock-out (NGF-KO) and an inducible NGF (iNGF). For iNGF, a TET-ON system linked to an NGF coding sequence and green fluorescence protein was integrated into the AAVS1 locus. Correct clones from both lines were validated by Sanger sequencing and characterized for stemness, karyotyping, germ layer differentiation, and function. NGF-KO was confirmed by qPCR in bioengineered neuronal organoids (BENO) that typically express NGF. For iNGF, green fluorescence following doxycycline treatment was detected in hiPSC and cardiomyocytes, indicating NGF induction. NGF quantification by ELISA validated NGF secretion with supernatant levels at 2944 pg/mL (n=15) vs. control at 10 pg/mL (n=6). Preliminary iEHM experiments showed that NGF induction led to an increase in cardiac muscle innervation compared to control while NGFKO to a reduction, supporting the role of NGF in human cardiac innervation. In the future, we will employ these tools to investigate neuro-cardio-vascular development and cell-to-cell interactions in iEHM.

F1143

IPSC-DERIVED V2A NEURON TRANSPLANTS RETURN LOST FUNCTION AFTER SEVERE SPINAL CORD INJURY IN MICE

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Traumatic spinal cord injury (SCI) is a devastating condition which causes irreversible damage to neural circuits and pathways. Therapeutic strategies involving neural stem cell transplants hold significant promise to replace lost tissue, restore continuity across the lesion and, in doing so, promote functional recovery from SCI. Propriospinal interneurons may be ideal for transplantation purposes as these cells naturally connect distant segments of the spinal cord through both ascending and descending projections, and they are also thought to have crucial roles in motor control. This study explored the feasibility of generating V2a propriospinal interneurons from mouse-induced pluripotent stem cells (iPSCs) and subsequently transplanting these cells syngeneically into SCI mice. To guide iPSCs towards a Chx10+ V2a interneuron fate, a 6-day differentiation protocol was employed, that included the precise

temporal addition of specific morphogens: retinoic acid, sonic hedgehog, and a notch signalling inhibitor. A constitutive tdTomato expressing Chx10- Blasticidin S deaminase (BSD) selectable cell line was then generated for purification and identification of Chx10+ transplants. Following differentiation, a significant downregulation of the pluripotency gene Oct4 was observed by day 4, along with the induction of the neural stem cell markers Nestin and NCAM. Expression analysis of the transcription factor Chx10, and also Tuj1 as a more general neuronal lineage marker, confirmed that V2a interneurons can indeed be generated from these mouse iPSCs (~17% efficiency). The transplantation of purified V2a interneurons into SCI mice resulted in their survival, extensive neurite outgrowth, and integration within the injured spinal cord. Importantly, these cellular changes corresponded with significant functional recovery at 1 month post-transplantation. Future experiments will focus on assessing the longer-term therapeutic potential of these cells as neuronal relays, with the ultimate goal of restoring function across the site of SCI.

Funding Source: Perry Cross Spinal Research Foundation.

F1145

KIDNEY MIMICKING HYDROGEL FOR TUBULOID CULTURE

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Adult stem cell-derived renal tubuloids are three-dimensional (3D) structures that replicate the epithelial cellular composition and spatial organization characteristic of kidney tubules. Likewise other tissue-derived organoids, tubuloids have generally been cultured using commercially available extracellular matrix (ECM) derived from mouse sarcomas. However, despite its widespread use, this tumor-derived ECM comes with critical limitations, including high costs, insufficient tissue specificity and safety concerns, which hinder its broader application in kidney research. To address these limitations, we developed biocompatible kidney mimicking hydrogel enriched with various components beneficial for tubuloid development. Our hydrogel provided an optimal microstructure and mechanical properties to support tubuloid culture. Moreover, tubuloids cultured in our hydrogel exhibited comparable or higher expression levels of kidney-specific genes and proteins compared to those grown in conventional matrix. We then demonstrated that our hydrogel enables the long-term cultivation of tubuloids and confirmed that these tubuloids showed robust trans-epithelial transport functions of kidney proximal tubule cells. In conclusion, our tubuloid culture platform holds great potential for broad applications including the evaluating drug toxicity, modeling kidney-related diseases, and understanding critical mechanisms of proximal tubule differentiation and function.

Funding Source: This work was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government, the Ministry of Science and ICT(MSIT) (RS-2021-NR059722).

F1147

LATROPHILIN-2, A SINGLE GENE, REPROGRAMS ADULT FIBROBLASTS INTO FUNCTIONAL CARDIOMYOCYTES VIA A DISTINCT PATHWAY FROM GATA4/MEF2C/TBX5 COMBINATION

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The heart lacks the ability to self-renew after ischemic injury, primarily due to the limited regenerative potential of mature cardiomyocytes. Latrophilin-2 (Lphn2), a G-protein-coupled receptor, has been identified as a critical player in cardiac development. This study aimed to compare the effectiveness of Lphn2 with that of Gata4, Mef2c, and Tbx5 (GMT)—well-known factors in direct cardiac reprogramming—in repairing the heart after ischemic disease by transdifferentiating cardiac fibroblasts into functional cardiomyocytes. Lphn2-OE and GMT-OE lentiviruses were injected into various transgenic mice, including Lphn2-hetero-KO and FSP1-Cre/Rosa-mTmG mice, following permanent ligation of the left anterior descending (LAD) artery. Cardiac function and heart regeneration efficiency were compared using echocardiography and immunofluorescence. Additionally, distinct induction pathways of Lphn2 and GMT were investigated in vitro using single-cell RNA sequencing. In vitro, the Lphn2-OE lentivirus successfully converted adult mouse fibroblasts into functional cardiomyocytes to a degree comparable to the GMT-OE lentivirus. The Lphn2-OE lentivirus induced direct conversion at an earlier time point and generated a more mature population of cardiomyocytes than the GMT-OE lentivirus. In a myocardial infarction model, Lphn2-het-KO mice exhibited severe fibrosis and reduced cardiac function, which were rescued by Lphn2 overexpression. Furthermore, the Lphn2-OE lentivirus alone significantly improved cardiac function and repaired damaged hearts after myocardial infarction, outperforming the GMT-OE lentivirus. Overexpression of Lphn2 directly converted adult mouse fibroblasts into mature and functional cardiomyocytes, resulting in reduced fibrosis and improved cardiac function following myocardial infarction. From these results, we can conclude that Lphn2 overexpression can be utilized as a gene therapy approach to reduce fibrosis and promote myocardial regeneration.

Funding Source: Bio and Medical Technology Development Program of the NRF, funded by Korean government (MSIT) (No.RS-2022-NR067329), “Korea Research-Driven Hospital” (HI14C1277) through the KHIDI, funded by the MOHW.

F1149

LYSOSOMAL CONTROL OF NEUROGENIN 3: A CRITICAL MECHANISM REGULATING IPSC TO PANCREATIC BETA CELL DIFFERENTIATION

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The differentiation of insulin-producing beta cells relies on precise regulatory mechanisms that govern cell fate decisions. Neurogenin 3 (Neurog3), a transcription factor essential for endocrine lineage specification, plays a critical role in this process. However, the mechanisms that regulate Neurog3 activity and stability remain incompletely understood. Our study identifies the lysosome as a key regulator of Neurog3 protein stability, providing a new layer of control over pancreatic endocrine differentiation. Using iPSC-derived pancreatic organoids, we have characterised a regulatory network through which lysosomal pathways influence the temporal dynamics of Neurog3 activity, directing progenitors towards beta cell fate. These findings provide insight into how lysosomal function contributes to pancreatic development and how disruptions in this pathway may contribute to disease. This work highlights the lysosome as a central player in transcription factor regulation and its potential as a target for improving beta cell differentiation. By linking lysosomal regulation to endocrine lineage decisions, this research advances our understanding of pancreatic biology and its implications for developing therapies for diabetes.

Funding Source: This work was supported by Wellcome Trust (207529/Z/17/Z), MRC (MR/S000011/1) and MRC/JDRF (MR/T015470/1) to R.S. A.G was funded by the Wellcome Trust (207529/Z/17/Z). T.M was supported by a Wellcome Trust (215138/Z/18/Z).

F1151

MECHANISMS OF MESENCHYMAL STEM CELLS MIGRATION AND THERAPEUTIC EFFECTS IN RENAL INJURY MODELS

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Mesenchymal stem cells (MSCs) have demonstrated significant therapeutic potential in renal injury models. However, the exact mechanism of their transport to the kidneys—whether as intact cells or extracellular vesicles (EVs)—remains debated. Additionally, immune cells that phagocytose MSCs may contribute to renal repair through anti-inflammatory effects. This study investigates how feline amniotic membrane-derived mesenchymal stem cells (fAM-MSCs) are delivered to the kidneys in a chemically induced renal injury mouse model, focusing on the timing and cellular forms of viable and apoptotic MSCs in the kidneys and bloodstream following intravenous (IV) and intraperitoneal (IP) administration. Renal injury was induced in male C57BL/6 or NSG mice via intraperitoneal injection of cisplatin (10 mg/kg) and lipopolysaccharide (LPS, 5 mg/kg). fAM-MSCs labeled with NIR815 or PKH26 were administered by IV or IP routes. Fluorescence imaging tracked MSC distribution over time, while PCR was employed to detect feline mitochondrial and nuclear DNA in the kidneys and bloodstream. Flow cytometry analyzed interactions between immune cells and transplanted MSCs. Fluorescence imaging revealed rapid migration of fAM-MSCs to the kidneys within 1 hour of injection. PCR detected feline mitochondrial DNA in the kidneys, but no nuclear DNA,

suggesting the absence of intact MSCs. Mitochondrial DNA persisted in the bloodstream for up to 3 hours post-injection, whereas nuclear DNA was undetectable. Flow cytometric analysis showed that macrophages and other immune cells phagocytosed more than 42% of transplanted MSCs. IV-administered MSCs were more frequently observed in the bloodstream at 3 hours post-injection compared to IP-administered MSCs. These results indicate that the therapeutic effects of fAM-MSCs are mediated by their components, such as EVs, and by macrophage-mediated anti-inflammatory processes, rather than intact viable cells. IP administration appears to bypass the bloodstream, delivering MSCs directly to the kidneys. This study provides critical insights into the mechanisms of MSC-based renal therapies and highlights the importance of EVs and immune cell interactions in achieving therapeutic outcomes.

Funding Source: This research was supported by a grant of Korean Cell-Based Artificial Blood Project funded by the Korean government (grant number: RS-2023-KH140925).

F1153

MICROWELL ARRAY-BASED 3D CULTURE SYSTEM FOR GENERATING "SEED ORGANOIDS" IN REGENERATIVE MEDICINE

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The development of organoid research provides new possibilities for regenerative medicine and translational applications. Organ and tissue reconstruction based on organoid research, as well as drug screening chips leveraging organoid technology, hold significant clinical and scientific value. However, achieving precise regulation of organoid proliferation and differentiation remains a critical bottleneck for large-scale clinical translation. Here, we present a novel microwell array-based three-dimensional culture system, which enables uniform production of salivary gland organoids in size and differentiation status through microenvironment regulation. In this system, AggreWell™800 was used to create a PDMS microwell array to mold an agarose culture dish. The agarose surface was coated with Matrigel, resulting in a 3D culture system featuring uniform trapezoidal microwells. Salivary gland organoids were self-assembled by co-culturing human minor salivary gland stem cells (hMSG-SCs) and human minor salivary gland mesenchymal stem cells (hMSG-MSCs). In the microwell array culture system, organoids with a uniform diameter of 223.74 ± 18.36 μm were obtained on day 3. Molecular biology analyses, including qPCR and Western blot, revealed that compared to organoids generated in traditional Matrigel-based 3D culture system, organoids derived from this system exhibited enhanced expression of stem cell markers (LGR5, SOX2) and proliferation marker KI67, accompanied by a significant reduction in differentiation-related acinar markers (AMY1B, AQP5) and ductal markers (KRT7, KRT19), suggesting better preservation of stem cell characteristics. This innovative approach offers a promising strategy for generating "seed organoids" with enhanced stemness and controllable differentiation capacity. This culture system enables large-scale production of early-stage organoids and

allows precise regulation of proliferation and differentiation by adjusting microwell size and biological culture conditions, supporting personalized configurations for organ reconstruction or chip fabrication.

Funding Source: This work was funded by the National Nature Science Youth Foundation of China (Grant Number 81801946).

F1155

MITOCHONDRIAL DYSFUNCTIONS IN A HUMAN IN VITRO BRAIN AGEING MODEL

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Aging is a complex process characterized by the progressive accumulation of molecular and cellular damage, driving functional decline and neurodegenerative disorders. Investigating human brain aging is challenging due to limited access to biomaterials and the inability of induced pluripotent stem cells (iPSCs) to retain age-related signatures post-reprogramming. To address this, we developed GFP-T2A-PROG, an iPSC line enabling inducible overexpression of Progerin, a mutant Lamin A protein linked to premature aging. This model allows controlled induction of aging phenotypes in neural lineages, particularly cortical organoids. Progerin overexpression in neurons and organoids recapitulated key aging hallmarks, including nuclear lamina abnormalities, a 40% reduction in H3K9me3 and HP1γ-marked heterochromatin, DNA damage accumulation indicated by γH2AX and p53BP1, elevated senescence-associated β-galactosidase activity, and increased p21. Furthermore, artificially aged models showed a 2-fold increase in mitochondrial reactive oxygen species (ROS) and glycolysis, suggesting metabolic dysfunctions previously implicated in aging. Transcriptomic analysis validated the age-associated phenotype observed at the cellular level, identifying 1,366 differentially expressed genes. We found, among others, upregulation of STAT3 and the metabolic gene PYCR1 and downregulation of mitochondrial genes OPA1 and TOMM20. We corroborated observations from post-mortem aged human brain tissue and Alzheimer's disease (AD) pathology, such as reduced expression of synaptic genes (SYN1, SNAP25, and CAMK4). Initial single-cell transcriptomic data suggest an imbalance in synaptic gene expression, which will be further explored through functional network analyses. Finally, we collected evidence highlighting the potential of GFP-T2A-PROG organoids for studying rejuvenation, in particular through reversal of DNA damage upon application of rapamycin. In summary, this model provides a robust platform for investigating cellular aging processes underlying neural dysfunction, including disrupted mitochondrial homeostasis and synaptic aberrations, and offers significant potential for developing age-reversal strategies and modeling

neurodegenerative diseases.

F1157

MUCOSAL MACROPHAGES GOVERN INTESTINAL REGENERATION IN RESPONSE TO INJURY

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Cancer patients treated with radiotherapy in the abdomen suffer from bleeding and malabsorption that impairs their quality of life. Radiation injury depletes the proliferative intestinal stem cells and progenitors, in response to this the intestine is reprogrammed into a fetal-like primitive state where committed cells de-differentiate giving rise de novo to the intestinal stem cells. Macrophages have garnered significant attention due to their multifaceted functions. While their role in inflammation and injury is well-established the putative contribution of macrophages to the intricate process of intestinal regeneration remains elusive. Using a plethora of approaches such as in vivo ablation of macrophages, bulk and single-cell RNA-seq, lineage tracing, 3D imaging, mouse and human organoid co-cultures we demonstrate that macrophages crosstalk with the intestinal epithelium to lead the process of regeneration. Our findings show that upon injury, macrophages are massively recruited near the intestinal stem cell compartment, acting as a temporary niche for de-differentiating epithelial cells by secreting two critical factors. NRG1 activates the regenerative genetic program, while SPP1 promotes the acquisition of ISC transcriptional traits. In the absence of macrophages, intestinal proliferation and de-differentiation are disrupted after injury. Moreover, co-culture experiments with human intestinal organoids and polarized macrophages show that macrophages influence cell differentiation trajectories and enhance organoids recovery after radiation injury. Consequently, we have identified a novel role of macrophages beyond their traditional innate immune functions that can be exploited to boost the process of intestinal regeneration to avoid anti-cancer treatments side effects.

F1159

MUTUAL REPROGRAMMING BETWEEN CARDIOMYOCYTES AND MACROPHAGES ENHANCES THE FUNCTION OF MULTICELLULAR CARDIAC ORGANOIDS

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Cardiac resident macrophages are pivotal in heart development, homeostasis, and disease response. Recent studies have shown that macrophages enhance cardiomyocyte (CM) function in cardiac organoids through various mechanisms. In this study, we generated cardiac

organoids using human pluripotent stem cell (hPSC)-derived cardiomyocytes and macrophages. Integrative transcriptomic and epigenomic analyses revealed that the cardiac tissue environment reprograms macrophages to express muscle-specific genes, while macrophages, in turn, promote CM maturation, contractile strength, and connectivity. Notably, macrophage-derived HBEGF activates ERBB4 signaling in CMs, further enhancing their functional properties. These findings highlight the bidirectional reprogramming between CMs and macrophages, leading to the generation of cardiac organoids that more closely resemble in vivo tissue, offering improved models for studying cardiac physiology and disease.

Funding Source: This work was supported by the National Key Research and Development Program of China Grants 2022YFA1103103 and 2023YFA1800302 and the National Natural Science Foundation of China (NSFC) Grants 32270784 and 31970819 to Jie Na.

F1161

THE TOPOLOGICAL CONTROL OF MESENCHYMAL STEM CELL BEHAVIOR ON POLY-EPSILON-CAPROLACTONE SCAFFOLDS FOR TISSUE ENGINEERING

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Control of cell behavior is essential for regulating the process of tissue regeneration. We aim to direct cells to adhere, migrate and proliferate predictably ultimately leading to the formation of new tissue and topology appears to be a very impactful signal for this process. Yet it is still unclear how the microtopology of such a widely used polymer as poly-ε-caprolactone (PCL) affects the cell behavior. PCL is a semicrystalline polymer and upon its surface distinct crystals known as spherulites form, giving rise to a topology with an as-yet unidentified influence on cell activity. So, we have developed two different types of PCL scaffolds with elaborate topology. The first one exhibits a variable area and height of PCL spherulites varying in the surface roughness from 50 to 220 μm and the median area from 100 to 700 μm2, depending on the production conditions. The second one features pore-like structures with diameter less than 0.5 μm, which arise in blend scaffolds from the dissolution of added polyethylene glycols (PEG) with molecular mass ranging from 1 to 15 kDa. It has been shown that fetal mesenchymal stem cells (MSCs) tend to adhere more actively to higher spherulites while proliferating extensively on the flatter ones. Moreover, migration patterns are more pronounced and rapid on the latter as well. Due to the surface tension, MSCs are more spread out on the higher roughness scaffolds, but there is no significant difference in average circularity. On the pore-like surfaces, the presence of leftover hydroxyl groups from PEG favorably affects cell behavior due to an increase in the hydrophilicity of the surface, but the high surface roughness offsets it. PEG 6kDa shows the lowest contact angle so MSCs prefer it more for both adhesion and proliferation. However, pores contribute to these processes and boosts them as well. So, this study underscores the significance and the effect of PCL surface topology for MSCs behavior and in the tissue engineering constructions fabrication.

F1163

OVERCOMING SENESCENCE IN OSTEOARTHRITIS AS A BARRIER TO STEM CELL THERAPIES

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Osteoarthritis (OA) is a degenerative joint disease characterised by progressive cartilage degradation, inflammation, and pain that diminishes quality of life. Although mesenchymal stem cell (MSC) therapies have shown promise, patient outcomes are heterogeneous; some patients report marked improvement, while others experience minimal relief. Emerging evidence indicates that this variability may be linked to senescence within the joint environment. Specifically, the senescence-associated secretory phenotype (SASP) may create a hostile joint milieu, affecting MSC efficacy. Despite the recognised impact of senescence in OA, there is no established strategy to address it; thus, we investigated two novel interventions using nicotinamide mononucleotide (NMN) and doxorubicin (DOX) in an in vitro OA model. Specifically, NMN was used to treat pelleted MSCs in chondrogenic medium supplemented with TNFα (mimicking in-situ treatment of cells within a hostile environment). In contrast, DOX was used as a pre-conditioning regimen for MSCs prior to chondrogenic induction (mimicking a pre-treatment strategy). In our in vitro models, we first confirmed that exposure to OA-associated SASP factors (TNFα) impairs MSC chondrogenic potential and upregulates senescence- and SASP-associated factors. We then assessed a DOX pre-treatment of MSCs, finding that this pre-conditioning enhanced chondrogenesis- (Agg, COMP) and suppressed senescence- (p16, p53) and SASP-associated (IL-1α) markers. Finally, when continuously treated with NMN, MSCs exposed to a hostile senescent OA-like environment exhibited significant reductions in senescence- (p16, p21, p53) and SASP-related (TNFα, IL6) genes. These findings demonstrate the potential of targeted interventions to recapitulate the regenerative efficacy of MSCs in senescence-driven OA, providing the framework for optimising MSC-based therapies by addressing senescence. These results demonstrate that targeting senescence in OA can restore MSCs' regenerative potential in hostile environments, and stratifying patients based on the senescent OA endotype – rather than grouping all cases – can improve therapeutic consistency. This approach offers a model for refining regenerative therapies in OA and addressing other pathologies driven by cellular senescence.

F1165

PROX1 IS REQUIRED FOR BONE MARROW MICROENVIRONMENT MAINTENANCE

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Bone marrow (BM) microenvironment comprises various types of cell populations, including endosteal and sinusoidal endothelial cells, mesenchymal stromal cells (MSCs), and osteoblast lineage cells, which support hematopoietic stem cell (HSC) maintenance through direct interactions or paracrine factors secreted by the niche cells. Transcription factor PROX1 (Prospero-related homeodomain transcription factor 1), is known to play critical functions in a variety of tissues including the lens, heart, liver, pancreas and central nervous system (CNS). We previously identified a new paracrine Wnt5a/Prox1 axis as a regulator of HSC regeneration

under conditions of injury and aging. Here we investigate role of PROX1 in BM microenvironment using mesenchymal specific Prox1 conditional knockout mouse model (Prox1f/fPrx1Cre), and found that mice deficient for Prox1 in the BM niche are hypersensitive to 5-FU challenge. Loss of Prox1 in the BM affects MSC differentiation under stressed conditions, and consequently compromises their hematopoiesis supportive function both in vitro and in vivo. Untargeted cytokine profiling reveals a significantly reduced levels of activin receptor-like kinase 1 (ALK1) in the BM of Prox1f/fPrx1Cre mice compared to that in the control Prox1f/f mice. Recombinant ALK1 treatment mimics Prox1f/fPrx1Cre phenotypes in the wild-type (WT) mice. Conversely, activation of ALK1 signaling pathway rescues MSC defects in Prox1-knockout mice. Taken together, our results uncovered a previously unknown function of transcription factor PROX1 in the BM microenvironment and provide novel insights on targeting PROX1 and its downstream signaling pathways to improve BM function in mice.

Funding Source: This work is supported by R01HL151390, R56HL169348, R01CA285400 to W.D., and a Children's Leukemia Research Association Research Grant (to W.D). This project used the Hillman Animal Facility, supported in part, by NIH P30CA047904.

F1167

PULMONARY ORGAN ENGINEERING USING HUMAN-DERIVED PARTIALLY REPROGRAMMED PROGENITOR-LIKE CELLS

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The bioengineered artificial organ can solve the problems around the current transplantation medicine, such as donor shortage and heterogeneous organ quality. If patient-specific cells are available for organ engineering, we could even overcome graft rejection after organ transplantation, which is particularly challenging in lung transplantation. This ambitious goal is only achievable after isolating and propagating the individual components of the target organ cells. However, we still lack the method to expand the highly diverse lung cell population at scale. Recently, researchers have investigated the potential role of Yamanaka reprograming factors for "rejuvenation." The idea is to ectopically express reprogramming factors in adult cells to reverse the biological clock. This can be translated into creating a fetal-type progenitor cells using patient-specific cells. The aim of the current study is to transform the terminally differentiated adult cells into highly proliferative but lineage-restricted progenitor-type cells by transfecting Yamanaka reprogramming factors yet only transiently enough to avoid pluripotency. Human umbilical code vein endothelial cells (HUVECs) and small airway epithelial cells (SAEC) were purchased from Lonza. mRNA cocktail coding human Oct4, Sox2, Klf4, c-Myc, Nanog, and Lin28 (OSKMNL, Stemgent) were transfected to these cells with Lipofectamine MessengerMAX (ThermoFisher) for 2 to 4 days. Cells were either directly passaged to the next dishes or colony-picked. After passage, the cells were subjected to the organ culture using decellularized mouse lung scaffold and perfusion-based bioreactor This "partial" reprogramming using an mRNA cocktail converted human primary cells into a highly proliferating state with loss of mature endothelial/epithelial markers such as CD31 or Surfactant protein B. These reprogrammed cells formed "shiny" colonies similar to induced pluripotent stem cells; however, the cells spontaneously recovered some of the original phenotypes after just changing the media to the original cell-specific media such as EBM2 or SAGM. Finally,

these reprogrammed “progenitor-like cells” seemed to recapitulate cellular heterogeneity in the mouse lung scaffold.

Funding Source: Grant-in-Aid for Scientific Research / KAKENHI (C) #20K09174, #23K08308, the Fund for the Promotion of Joint International Research (Fostering Joint International Research (B)) #22KK0132 for TS.

F1169

RECAPITULATING HUMAN CARDIOVASCULAR DEVELOPMENT AND YOLK SAC HAEMATOPOIESIS

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Organoids can recapitulate many aspects of the complex structures and functions corresponding to in vivo tissue. This makes them powerful models for studying development, physiology, and disease. Current cardiac organoid models lack the paracrine signalling from surrounding tissues as well as vascular networks with a perfusable tube formation. In this current study, we generate human multilineage cardiac organoids by first forming gastruloids which mimic key features of early embryonic development and then inducing cardiovascular differentiation. Single-cell RNA-seq analysis and immunostaining revealed that the cardiac organoids generate multiple cell types alongside cells from cardiovascular lineages such as haematopoietic progenitors and endodermal cells. Immunofluorescence staining revealed the cardiac organoids contained a network of vascular endothelial cells with cardiomyocytes found encapsulated in a layer of epicardial cells. Transcriptomic analysis of cardiac organoids taken at different timepoints during differentiation reveal how these organoids closely mimic early in vivo cardiac development with early upregulation of genes associated with the primitive streak, and then development into cardiac progenitors. Moreover, upregulation of genes associated with the yolk sac and primitive haematopoiesis after cardiac induction were observed and found that potential haematopoietic progenitors and erythroid-like cells are enclosed in a vascular structure of endothelial cells within a yolk sac-like cyst. These results suggest that our organoid model could be an excellent model to recapitulate early cardiac and haematopoietic development.

Funding Source: British Heart Foundation.

F1171

RECONSTRUCTION OF CORTICOSPINAL TRACTS BY TRANSPLANTATION OF HIPS CELL- DERIVED CORTICAL NEURONS

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Ischemic stroke is a disease characterized by cerebral blood vessel occlusion, resulting in neuronal death. The fact that many patients continue to suffer from long-term neurological deficits indicates that current therapies are insufficient. In this context, transplantation of human induced pluripotent stem cell (hiPSC)-derived cortical neurons has emerged as a promising approach for ischemic stroke. The corticospinal tract, extending from cerebral cortex to spinal cord, is a critical neural circuit damaged by stroke and crucial for sophisticated motor functions, including fine finger movements essential for daily activities. Previously, we demonstrated that hiPSC-derived cortical neuron transplantation in stroke model mice leads to axonal extension to the spinal cord and improved motor function. However, the mechanism underlying this recovery remained unclear. We hypothesized that functional recovery is mediated by the reconstruction of the corticospinal tract through cell transplantation, and tried to verify this hypothesis. Using rabies virus vectors (RABV), which propagate retrogradely across synapses between neurons, we investigated neural connections between graft and host. In experiments examining neural inputs to grafted cells, RABV selectively infected transplanted cells and spread to host neurons providing direct neural input. When investigating outputs, RABV infected host spinal neurons and propagated to transplanted cells, indicating functional integration. This RABV-based synaptic tracing revealed connections between transplanted and host neurons, providing evidence for structural reconstruction of the corticospinal tract within damaged neural circuits. These findings suggest that post-transplantation motor recovery occurs through direct neural circuit reconstruction rather than indirect mechanisms, representing a significant advancement in understanding cell-based therapy for ischemic stroke.

Funding Source: This research is supported by AMED under Grant Number JP23bm1223005h0001. This research is supported by Grant-in-Aid for JSPS Fellows Grant Number 24KJ1351.

F1173

REGENERATION OF LARGE BONE DEFECTS USING MOUSE-DERIVED CALVARIAL OSTEOBLAST-LIKE CELLS AND THREE-DIMENSIONAL COTTON-LIKE POLYLACTIC ACID SCAFFOLD

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In dentistry, bone substitutes have often been used to regenerate alveolar bone. In recent years, new bone substitutes with three-dimensional structures have been developed, and only small bone defects can be completely healed. However, the healing of large bone defects where there is no surrounding bone remains difficult and bone tissue engineering approach

combining scaffold materials and cells is thought to be necessary. Mesenchymal stem cells (MSCs) have been the main cell source for tissue engineering. Although MSCs have the ability to promote healing through their anti-inflammatory effects, there are few clinical reports that the cells themselves have a clear bone regenerative ability. Therefore, it is necessary to develop a new bone regeneration technique that combines cells with high bone-forming capacity with a scaffold that enables vertical bone formation. In this study, we developed a composite of mouse-derived calvarial osteoblast-like cells (MCOB) with bone regeneration ability and a three-dimensional cotton-like polylactic acid (3DPLA) scaffold. The MCOB-3DPLA composite demonstrated bone regeneration ability in mouse alveolar bone defect models, and nanoindentation analysis showed that the regenerated bone exhibited micromechanical strength similar to that of healthy bone. When titanium implants were placed in the regenerated bone, osseointegration with the implant surface was observed. Based on these results, it is believed that a new bone regeneration technology could be developed by isolating human cells using a method similar to MCOB.

F1175

RESPIRATORY MEDIATED MECHANICAL FORCE REGULATES LUNG REPAIR AFTER INJURY

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During respiration, the lungs experience mechanical stress, and these physiological forces impact cellular destiny and tissue equilibrium. Pulmonary neuroendocrine cells (PNECs) serve as sensory epithelial cells, communicating the status of the airways to the brain through sensory neurons and locally through the release of calcitonin gene-related peptide (CGRP) and γ-aminobutyric acid (GABA). Upon lung injury, these neuroendocrine cells multiply and differentiate into various cell types to facilitate the repair of the epithelium. A minority of these neuroendocrine cells act as stem cells, exhibiting characteristics of traditional stem cells. The majority of them engage in self-renewal through proliferation following injury, while others migrate to the site of damage. Approximately one week post-injury, specific cells, often singular within each cluster, shed their neuroendocrine identity (dedifferentiate), undergo a period of transit amplification, and then redifferentiate into alternative cell fates, resulting in the formation of extensive clonal patches for tissue repair. Nonetheless, how PNECs integrate mechanical signals from respiratory forces in vivo remain elusive. After unilateral lung airway ligation, we lineage-labeled PENCs using CGRPCreERT2; R26LSL-tdtomato mice and examined reprogram following the Naphthalene injury and we found clonal repair patches reduce after Naphthalene induced injury within two weeks. Furthermore our scRNA-seq data showed that mechanical force sensing ion channels-Piezo2 was upregulated in PNECs after blockage of mechanical force and inhibition of Piezo2 promoted the growth of clonal repair patches. We conclude that respiratory forces play crucial role for PENC stem cell differentiation into the repair lineage. The mechanism how PNECs integrate mechanical signals in their microenvironment remains a mystery and requires further research.

Funding Source: This work was supported in part by National Key Research and

Development Program of China (2024YFC3405600,SQ2024YFA1100190)，National Natural Science Foundation of China(32270854,32161160322,92469301).

F1177

REVEALING THE DEVELOPMENTAL ORIGIN OF NEURAL PROGENITORS WITHIN HUMAN BONE MARROW VIA SINGLE CELL ANALYSIS AND MIGRATIONAL ASSAYS

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Human bone marrow stromal cells (BMSCs) are an easily accessible and expandable progenitor population with the capacity to generate neural cell types in addition to mesoderm. Lineage tracing studies in transgenic animals have indicated Nestin + BMSCs to be descended from the truncal neural crest. Single-cell analysis provides a means to identify the developmental origin and identity of human BMSC-derived neural progenitors when lineage tracing remains infeasible. This is a prerequisite towards translational application. We attained transcriptomic profiles of embryonic long bone, adult human bone marrow, cultured BMSCs and BMSC-derived neurospheres. Integrated scRNAseq analysis was supplemented by characterization of cells during culture expansion and following provision of growth factors and signalling agonists to bias lineage. To demonstrate stereotypic neural crest migration and differentiation, BMSC subpopulations isolated via magnetic bead sorting were transplanted into the embryonic chick neural tube. Reconstructed pseudotime upon the integrated dataset indicated distinct neural and osteogenic differentiation trajectories. The starting state towards the neural differentiation trajectory consisted of Nestin + /MKI67 + BMSCs, which possessed a neural crest phenotype but could also be diverted towards the osteogenic trajectory via a branch point. Nestin + /PDGFRA + BMSCs responded to neurosphere culture conditions to generate a subpopulation of cells with a neuronal phenotype according to marker expression and gene ontogeny analysis that occupied the end state along the neural differentiation trajectory. Based on pseudotime findings, provision of exogenous BMP signalling agonist SB4 could direct BMSC-neurospheres to upregulate Pax6. NGFR+ BMSCs transplanted to the truncal embryonic chick neural tube resulted in cell migration to craniofacial structures as well as the heart. This study suggested BMSCs originating from truncal neural crest to be the source of cells within long bone marrow possessing neural differentiation potential. Unravelling the transcriptomic dynamics of BMSC-derived neural progenitors promises to enhance differentiation efficiency and safety towards clinical application in cell therapy and disease modelling.

Funding Source: This project was funded by GRF 17106722 and HMRF 06172326.

F1179

RUNX2 AND NOTCH: COORDINATORS OF EARLY OSTEOGENIC DIFFERENTIATION

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Runx2 is a transcription factor and a key regulator of osteogenic differentiation, playing an essential role in skeletal development. It not only controls the expression of genes involved in bone formation but also interacts with several signaling pathways, such as Wnt, Bmp, and Notch. Despite significant progress in understanding the role of Runx2 in bone formation, the relationship between Runx2 and the Notch signaling pathway, and the specific role of Runx2 in this process, remains poorly understood. The aim of this study was to investigate the dynamics of Runx2 activation at the RNA and protein levels during the early stages of osteogenic differentiation and to analyse the relationship between Runx2 and the Notch signaling pathway. Primary human osteoblasts derived from femoral trabecular bone served as the experimental model. Findings revealed that Runx2 expression begins to rise 12 hours after osteogenic induction, highlighting finely tuned regulation through transcriptional control and protein degradation. A critical threshold of Runx2 protein appears necessary to trigger differentiation. During the early stages of Runx2 activation in osteoblasts, we observed a rise in the expression of SPP1 and BGLAP. This suggests that the accumulation of RUNX2 transcripts during the induction of osteogenic differentiation affects the expression of various osteogenic markers. Proteasomal inhibition studies further confirmed that transient reductions in Runx2 are pivotal for initiating differentiation, while prolonged stabilization inhibits the process. Furthermore, we observed an increase in the expression of the NOTCH1 at 6 hours after the initiation of osteogenic differentiation. The accumulation pattern of transcripts, the Runx2 protein, and the components of the Notch signaling pathway suggest that all these elements are involved in the induction and progression of osteogenic differentiation.

Funding Source: The work was carried out with the financial support of the Russian Science Foundation (project no. 23-15-00320).

F1181

SCALABLE IPSC-DERIVED ITENOCYTES FOR TENDON/LIGAMENT REGENERATION

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Tendinopathy is the most prevalent among the musculoskeletal injuries. Due to hypocellularity and hypovascularity, adult tendon healing is compromised. This leads to incomplete tissue remodeling and as a result, scar tissue forms and a vicious cycle of chronic inflammation ensues. On the contrary, young tendon heals faster and deposit tendon tissue rapidly. In this context, we studied young and adult rat Achilles tendon healing. Young tendon demonstrated faster improvement in gait and tendon biomechanics, while single cell data revealed a significant increase of tenogenic precursors as early as 1 week compared to adult rat tendon. Informed by tenogenic cell clusters in young rat tendon, we differentiated the iPSCs towards triple positive Scleraxis+ (Scx+), Tenomodulin+ (Tnmd+) and early growth response-1+ (Egr1+) iTenocytes using developmental pathways in a high purity, xeno-free and stepwise manner. First, iPSCs were induced to form primitive streak through activation of TGF β , WNT and FGF signaling. Then, WNT/FGF were activated and TGF β /BMP were suppressed to complete

paraxial mesodermal differentiation. Next, somitogenesis was induced by inhibition of WNT, FGF, TGF β , BMP at the same time. Upon formation of somites, we established of PAX1>>PAX3 sclerotome (SCL) cells by activation of Hedgehog while inhibiting WNT and BMP. Subsequently, we achieved >90% Scx+ tenogenic precursor-like syndetome (SYN) cells by context dependent activation of TGF β 3/BMP14/FGF8. The SYN cells clearly illustrated the tendon trajectory at single cell level. Finally, we obtained readily scalable Tnmd+/Mkx+/Egr1+ iTenocytes within 12 days. We showed that two different iPSC lines can be used for the iTenocyte production with abolished side products. In addition to the remarkable efficiency of iTenocyte induction, the cells can be frozen stored and scaled up without loss of phenotype. Currently, we are investigating the translation potential of iTenocytes in full thickness adult rat tendon defect model. Collectively, the iTenocytes are highly translatable and scalable for future clinical applications in scarless tendon/ligament regeneration and functional restoration.

Funding Source: The study is supported by CIRM-DISC0-14350 and CIRM Scholarship CIRM EDUC4-12751.

F1183

SERGLYCIN PROMOTES SKELETAL MUSCLE REGENERATION THROUGH EZH2 DEGRADATION IN MYOBLASTS: A NOVEL PATHWAY FOR THERAPEUTIC MUSCLE REPAIR

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Elucidating the mechanisms of skeletal myogenesis is crucial for clarifying the pathophysiology of muscular disorders and facilitating muscle regeneration after injury. Enhancer of Zeste Homologue 2 (EZH2) is a catalytic subunit of Polycomb Repressive Complex 2 that regulates stem cell differentiation via transcriptional repression. In myoblasts, EZH2 contributes to maintaining the chromatin of muscle genes in a repressed state, whereas its downregulation leads to the transcriptional activation of the myogenic program. However, the precise mechanism of triggering this EZH2 downregulation after muscle injury remains unclear. Here, we show that serglycin promotes skeletal muscle regeneration through EZH2 degradation in myoblasts. We found CD90-positive human urine-derived cells (UDCs), which we previously reported as a cell population with a high potential for myogenic differentiation, excreted more serglycin than CD90-negative UDCs. Adding recombinant human serglycin to the culture medium significantly enhanced the fusion index of MYOD1-converted UDCs compared to untreated ones. Moreover, our immunoblotting data showed that serglycin activated the CD44-p38-EZH2 pathway, resulting in EZH2 degradation by phosphorylation of threonine 372. As a next step, to confirm whether serglycin could activate the same pathway in mouse myoblasts and promote muscle differentiation, we intramuscularly injected serglycin into the injured muscles of wild-type mice. As a result, in serglycin-injected mice, the percentage of embryonic myosin heavy chain positive fibers evaluated by immunohistochemistry showed its peak on day 5 which was earlier than day 8 in the control group. Simultaneously, serglycin-injected mice indicated significantly high muscle torque both on days 5 and 8 compared to the control group. Taken together, serglycin can enhance regeneration in injured muscles by degrading EZH2 in

myoblasts. Our findings should pave the way for developing regenerative medicine approaches using serglycin to mitigate skeletal muscle disorders.

F1185

SKELETAL MUSCLE HOMEOSTASIS – LEADS FROM CROSS-SPECIES ANALYSIS

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The commonality between various muscle diseases is the loss of muscle mass, function, and regeneration, which severely restricts mobility and impairs the quality of life. With muscle stem cells (MuSCs) playing a key role in facilitating muscle repair, targeting regulators of muscle regeneration has been shown to be a promising therapeutic approach to repair muscles. However, the underlying molecular mechanisms driving muscle regeneration are complex and poorly understood. Through a genetic screen in Drosophila, we have identified a transcriptional factor, Deformed epidermal autoregulatory factor 1 (Deaf1), as a new regulator of muscle regeneration. We showed that Deaf1 targets to PI3KC3 and Atg16l1 promoter regions and suppresses their expressions, thus inhibiting autophagy. Deaf1 depletion therefore induces autophagy which blocks MuSC survival and differentiation. In contrast, Deaf1 overexpression inactivates autophagy in MuSCs, leading to increases in protein aggregates and cell death. The fact that Deaf1 depletion and overexpression both lead to defects in muscle regeneration highlights the importance of fine tuning of Deaf1-regulated autophagy during myogenesis. Significantly, we further showed that Deaf1 expression is altered in sarcopenic and cachectic MuSCs. Manipulation of Deaf1 expression can attenuate muscle atrophy and restore muscle regeneration in the mouse models of sarcopenia and cancer cachexia. Our findings together unveil a critical role for Deaf1 in muscle regeneration, providing insights into the development of uncovering new therapies against muscle atrophy.

F1187

SOFT EXTRACELLULAR MATRIX REGULATING GLUCOSE METABOLISM AND MICROTUBULE ACETYLATION TO PROMOTE OSTEOGENIC DIFFERENTIATION IN 3D CULTURED MESENCHYMAL STEM CELLS

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The early stage of bone reforming contains low stiffness ECM. Matrix stiffness is crucial to regulating stem cell behavior. However, the mechanism of stem cells respond to matrix stiffness at the early stage of bone remodeling was still unrevealed. Our study aims to explore the effect of ECM stiffness on the differentiation of MSCs in 3D cultures and the potential mechanism. Human mesenchymal stem cells (hMSCs) and GelMA were used to construct a 3D culture system with various matrix stiffness. The osteogenic differentiation level was evaluated both in vivo and vitro. Meanwhile the energy metabolism level was detected to further investigate the mechanism of 3D matrix stiffness effects on MSCs differentiation. In our studies, it was found that the soft (5 kPa) matrix promoted osteogenic differentiation both in vitro [mRNA (~1.4-fold change), ALP staining (~2-fold change)] and in vivo, which was different from 2D culture. Compared with the stiff matrix (~ 20 kPa), the cells in the soft matrix spread faster and showed larger volume (~3000 μm3), had more filamentous-structure-like mitochondria (~80 %) with more active metabolism [ATP content (~1.8-fold change), the oxygen consumption (~2.5-fold change) and acid production rate (~2.7-fold change)]. We focused on the microtubules (MTs) cytoskeleton differences and its effects on mitochondria. Both mRNA level and protein level showed that the acetylation level of α-tubulin (~1.5-fold change) and the expression of microtubule acetylase αTAT1 were significantly up-regulated (~2.5-fold change) in the soft matrix group. The mitochondrial networking, and ALP activity was inhibited in the soft matrix group by si-αTAT1 or eliminating microtubule homeostasis with Taxol. This study revealed that the soft 3D matrix promoted MSCs spreading, cytoskeletal aggregation, mitochondrial networking, and metabolism function, thereby promoting osteogenic differentiation. The cytoskeleton, especially MTs, mediates the network of mitochondria and energy metabolism to regulate osteogenic differentiation. This study demonstrated the important role of soft ECM in the design of tissue engineering materials for bone repair and revealed the necessary effects of soft tissues such as collagen in promoting the osteogenic differentiation of cells at the early stage of bone reformation.

Funding Source: This work was supported by Beijing Natural Science Foundation [L242125, L234072, L242042]; NSFC [11972067, 32171310, 12472315, U20A20390, 12332019]; SKLOD2024OF01; Fundamental Research Funds for the Central Universities.

F1189

SPATIALLY RESOLVED LASER-ACTIVATED CELL SORTING (SLACS) FOR INTEGRATING SPATIAL AND MOLECULAR BIOLOGY

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Spatially Resolved Laser-Activated Cell Sorting (SLACS) was used to investigate the impact of Ahcyl knockout (Ahcyl-KO) on intestinal cellular dynamics in mouse models. By targeting Lgr5-positive intestinal stem cells, Alpi-positive absorptive enterocytes, and Muc2-positive goblet cells, SLACS enabled the precise isolation of these cell populations from both Ahcyl-KO and control tissues while preserving spatial and molecular integrity. Transcriptomic analysis revealed significant alterations in Lgr5 stem cell gene expression in Ahcyl-KO tissues, including

downregulation of regeneration-associated pathways, suggesting impaired epithelial turnover. Alpi enterocytes in the Ahcyl-KO group exhibited reduced expression of nutrient absorption genes, implicating compromised digestive function. Additionally, Muc2 goblet cells showed dysregulated mucus-related gene expression, indicating potential disruption of the intestinal barrier. Spatial mapping highlighted altered cellular organization and niche interactions in Ahcyl-KO tissues, particularly a diminished presence of Lgr5 stem cells in crypt regions. These findings demonstrate the utility of SLACS in dissecting the spatial and molecular effects of genetic perturbations, providing novel insights into the role of Ahcyl in intestinal homeostasis and its implications for gastrointestinal diseases.

F1191

STEM-LIKE CELL EXHAUSTION IN NON-HUMAN PRIMATE RENAL AGING

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Stem cell exhaustion is one of the hallmarks of biological aging. It has been shown that stem cell exhaustion is heterogeneous among aged organs and tissues. The kidney is one of the most vulnerable organs to aging, and aging plays a leading role in various renal diseases. However, the existence of stem cells in adult primate kidney and their alterations during aging remain unclear. Here, we made use of single-nuclei RNA sequencing (snRNA-seq) to uncover the stem cells in the adult kidney of the cynomolgus monkey (Macaca fascicularis). Notably, a subpopulation of principle cells, namely stem-like cells, was found in the root of pseudotime trajectory with distinctive expression of known stem cell markers, including prominin-1 (Prom1), compared to the other cell populations. In addition, the level of stemness and stemness-related transcription factors of these stem-like cells were significantly higher than the remaining cell populations as estimated using CytoTrace2 and Pyscenic. Further, we compared the stem-like cells from young, middle-aged, and old monkey kidney to characterize their aging-related alterations. Interestingly, the population of stem-like cells in the kidney was expanded during aging, which was thought to be reduced with age. We then examined the expression level of proliferation markers, the differentiation potential, and the stemness levels of these stem-like cells during aging. All of them were declined along with age. It suggested that the aberrant accumulation of stem-like cells in primate kidney is due to stem cell exhaustion characterized by impaired proliferation and differentiation capacity. Collectively, we identified a stem-like cell population in non-human primate kidney and found that exhausted stem-like cells accumulated in the kidney during aging, which could serve as a promising therapeutic target of kidney rejuvenation.

Funding Source: RGC Theme-based Research Scheme (T13-602/21N).

F1193

STUDYING CELLULAR MEMORY IN RESPONSE TO INTESTINAL INJURY

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Radiotherapy plays a major role in the treatment of abdominal malignancies; however, it leads to the development of unintended side effects in the intestine like radiation-induced enteritis. Radiation injury inflames the intestinal mucosa and depletes the proliferative intestinal stem cells (ISCs). Following injury, intestinal regeneration relies on the proliferation of surviving ISCs and the dedifferentiation of progenitors and differentiated cells to replenish the stem cell pool. However, the consequences of this process remain poorly understood. Here, we demonstrate that intestinal cells recovering from radiation injury develop an epigenetic inflammatory memory and retain transcriptional features of their previous identity. Intestinal cells retain higher chromatin accessibility and elevated expression of inflammation-associated genes post-recovery, enabling faster bodyweight and cell proliferation recovery upon subsequent radiation exposure. However, the dedifferentiation process causes a broad loss of cellular identity across all intestinal cell types, driving them into a stem-like, highly plastic state, which compromises their functional specialization. Consequently, the progeny of de novo formed ISCs exhibit enhanced dedifferentiation capacity after secondary injury, at the expense of their ability to perform differentiated cell functions. This adaptive state supports rapid regeneration following recurrent injuries, but we hypothesize that it may predispose to long-term complications, such as chronic radiation-induced enteritis or cancer. These findings highlight the dual role of epigenetic memory and cellular plasticity in intestinal regeneration, offering new insights into the biology of ISCs and potential therapeutic targets to mitigate intestinal damage.

F1195

TARGETING CARDIAC FIBROSIS WITH CHIMERIC ANTIGEN RECEPTOR NEUTROPHILS FROM HUMAN PLURIPOTENT STEM CELLS

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Cardiac fibrosis is a pathological hallmark of various forms of heart disease, characterized by excessive deposition of extracellular matrix (ECM) proteins by activated fibroblasts, leading to cardiac hypertrophy, arrhythmias, and heart failure. Current treatments, predominantly pharmacological, target signaling pathways involved in fibroblast activation but often come with side effects such as cardiac toxicities. There is a critical need for therapies that specifically target activated cardiac fibroblasts to mitigate these adverse effects. Recent advances have shown that chimeric antigen receptor (CAR)-T cells targeting fibroblast activation protein (FAP), expressed by activated fibroblasts, can significantly reduce fibrosis and improve cardiac function in mouse models. However, CAR-T cell therapies face challenges such as the requirement for large quantities of healthy primary immune cells, lengthy process, and the high cost of personalized treatments. To address these issues, we propose an innovative strategy using off-the-shelf CAR-neutrophils derived from human pluripotent stem cells (hPSCs). We hypothesize that FAP-targeting CAR-neutrophils will effectively reduce cardiac fibrosis and improve cardiac function post-injury due to their potent cytotoxic effects and ability to infiltrate infarct regions. To test this hypothesis, anti-FAP CAR hPSCs were generated by CRISPR/Cas9 genome editing and differentiated into neutrophils. The differentiated anti-FAP CAR hPSC-neutrophils exhibited molecular characteristics comparable to unmodified hPSC-neutrophils. We also established an in vitro cardiac fibrosis model utilizing a previously reported

protocol for the generation of hPSC-derived epicardial fibroblasts. Importantly, our anti-FAP hPSC-neutrophils exhibited significant cytotoxicity against activated epicardial fibroblasts, while unmodified hPSC-neutrophils showed minimal killing efficiency. This study suggests a proof-of-concept therapeutic approach against cardiac fibrosis utilizing FAP-targeting CAR-neutrophils. This strategy can potentially be adapted to treat fibrosis in other organs, thereby having a broad and significant impact on the treatment of various fibrotic diseases, ultimately contributing to longer, healthier human lives.

Funding Source: American Heart Association Post-doc Fellowship (23POST1025924) NIH NCI R37CA265926.

F1197

THE EFFECT OF MITOCHONDRIAL METABOLISM ON THE FUNCTION OF STEM CELL DERIVED PANCREATIC ISLETS

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Generating stem cell derived pancreatic islets (SC-islets) with functional beta cells has become a reality in recent years. SC-islets show great promise as a renewable cell source for the treatment of insulin-deficient diabetes. However, SC-islets differ metabolically from primary human islets, especially in having low mitochondrial coupling to glucose stimulated insulin secretion (GSIS). The aim of our study was to determine whether there is functional heterogeneity between beta cells within the SC-islets, and if so, what characterizes the more functional beta cells. Mitochondrial function was used to identify populations of interest. As we have previously shown in other systems that mitochondrial age can be used as a proxy for function, we created a reporter hESC line, H1 SNAP-OMP25, which enables temporally controlled labelling of mitochondria. We sorted out four cell populations differing in their mitochondrial age profiles as well as levels of ENTPD3, an ectonucleoside highly expressed in beta cells. Three of the four populations contained over 95% beta cells. We found that beta cells with older mitochondria had higher glucose-stimulated insulin secretion. Additionally, contrary to previous reports on sorting based on ENTPD3 levels, we found that moderate rather than high levels of ENTPD3 were associated with better functionality. The discrepancy is likely due to the old mitochondrial axis allowing us to sort out a pure beta cell population with moderate levels of ENTPD3. We conclude that there is functional heterogeneity within the beta cells of SC-islets identifiable by mitochondrial age as well as ENTPD3 levels. We are currently deciphering the transcriptomic and metabolic basis underlying the identified functional differences. This knowledge could be used to modify the SC-islet differentiation protocol further, allowing the generation of better islets for therapeutic and disease modelling applications.

F1199

THE ROLE OF INTERVERTEBRAL DISC DEGENERATION IN RESIDENT PROGENITOR CELL DIFFERENTIATION

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Lower back pain is one of the most common medical complaints in the US with up to 40% of the cases attributed to intervertebral disc (IVD) degeneration. Notochordal cells (NCs) are considered progenitor cells within the IVD and are thought to be lost in humans around age 10. The loss of NCs coinciding with degenerative changes suggests that NCs may play a role in IVD degeneration, however, the mechanism has not been identified. Due to disc size, the mechanism of degeneration in rodents differ substantially from human disease. Here, we developed a comprehensive porcine model for which we can quantitative measure discogenic pain via biobehavioral testing (BBT) and novel MRI. In addition, we applied a multi-omics approach to elucidate the cellular identities and mechanisms driving IVD degeneration and pain development at the molecular and cellular levels. Traditional MRI and histology of the injured discs confirmed development of IVD degeneration. Novel qCEST sequences, BBT, and bulk transcriptomics of DRGs showed the development of pain responses. Proteomic analysis of plasma identified an upregulation of inflammatory and differentiation marker with degeneration. Single cell transcriptomics determined that post injury, IVD makeup shifts from NC progenitors to terminally differentiated Nucleus Pulposus Cell (NPC) dominated. Trajectory analysis predicts cells from injured discs are located later in pseudotime and express markers associated with differentiation. Cell-cell communication analysis predicts terminally differentiated cell types are involved in multiple differentiation pathways and have high intercellular communication, while NCs are mostly silent. Pathway analysis of high communication clusters predicts increased cellular stress, neural outgrowth, inflammation, and differentiation pathways. When NCs were isolated from healthy IVD and exposed to degenerative culture conditions, they were found to differentiate toward fibroblast-like NPCs. Understanding the role of NCs in IVD degeneration will help to, not only to understand their role in degeneration process and pain induction, but also inform the development of cell therapies for low back pain. Additionally, development of a pain assessment assay in large animals can accelerate translation of promising treatments towards the clinic.

Funding Source: This study was supported by NIH (K01AR071512, R01AR066517) and CIRM (DISC2-14049, EDUC4-12751).

F1201

THE ROLE OF TGFBR3-DEPENDENT TGFB ANGIOCRINE SIGNALING IN GLOMERULAR MATURATION OF HIPSC-DERIVED KIDNEY ORGANOIDS

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Human induced pluripotent stem cell (iPSC)-derived kidney organoids (KORs) show great promise as viable alternatives for transplantation in regenerative medicine. We previously demonstrated that the transplantation of these KORs into the coelom of chicken embryos triggers both glomerular vascularization and podocyte maturation, suggesting a role for endothelial cell (EC)-derived factors (angiocrine factor) in glomerulogenesis. In this study, we leveraged scRNA-seq data from untransplanted and transplanted KORs, as well as human fetal kidneys (hFKs), to pinpoint angiocrine factors and receptors involved in this process. We identified specific ligand-receptor pairs between developing (glomerular) ECs and podocytes from transplanted KORs and hFKs using scRNA-seq data. Ligands whose signaling was active during podocyte maturation in transplanted KOR or hFKs were further analyzed, yielding TGFB1/TGFBR3 as the top ligand/receptor pair, along with possible downstream target genes in podocytes. Furthermore, pseudotime reconstruction of podocyte differentiation in KORs and hFKs revealed TGFBR3 upregulation at the latest stage of podocyte development, further supporting its potential role as the key angiocrine factor receptor required for advanced glomerular maturation. To explore this further, we generated an iPSC line with a podocyte-specific knockout of TGFBR3 when differentiated to KORs. We then developed an in vitro co-culture system combining KORs with blood vessel organoids. By comparing podocyte-specific TGFBR3 knockout KORs with unmodified controls, we aim to investigate the impact of TGFBR3 loss on vascularization and glomerular maturation. This research holds the potential to significantly advance our understanding of glomerular vascularization and podocyte maturation in KORs in vivo and translate these findings to in vitro applications.

Funding Source: The Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW, supported by Novo Nordisk Foundation grants (NNF21CC0073729)).

F1203

THERAPEUTIC EFFICACY OF INDUCED PLURIPOTENT STEM CELL-DERIVED CORNEAL ENDOTHELIAL CELLS MITOCHONDRIA IN RESTORING CELLULAR FUNCTIONS AND REDUCING INFLAMMATION

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Mitochondrial stress and dysfunction significantly impact cellular functions and contribute to the pathology of a variety of diseases, including Fuchs' syndrome. Recent studies have explored the regulatory role of mitochondria derived from stem cells as a novel therapeutic approach for various diseases. We aim to investigate whether mitochondria isolated from induced pluripotent stem cell-derived corneal endothelial cells (iPSC-CEC) can restore the functionality of damaged CEC. Inflammatory conditions were induced in cells by treatment with lipopolysaccharide (LPS) and interferon-gamma (IFN-γ) to iPSC-CECs. We assessed the effects of iPSC-CEC mitochondria on mitochondrial morphology under inflammatory conditions, using confocal microscopy to observe mitochondrial rescue. We also evaluate the influence of mitochondrial delivery on cellular function and proliferation using an in vitro scratch assay and CCK8 assay. Finally, Western blot analysis was conducted to evaluate cell survival and the changes of inflammatory responses. Treatment with iPSC-CEC mitochondria successfully rescued mitochondrial fusion morphology under inflammatory conditions induced by LPS and IFN-γ, as shown by confocal microscopy. Delivery of mitochondria significantly reduced the expression of pro-inflammatory cytokines IL-6, IL-1β, and TNF-α at both mRNA and protein levels. Scratch assays demonstrated increased cell migration and wound closure following mitochondrial treatment. Western blot analysis revealed increased Mfn-2 and p-AKT expression, indicating improved mitochondrial function and survival pathways. These results highlight the therapeutic efficacy of iPSC-CEC mitochondria in restoring cellular functions and reducing inflammation under pathological conditions of CECs, suggesting the potential of iPSC-CEC-derived mitochondria as a therapeutic agent in regenerative medicine of cornea.

Funding Source: This work was supported by a grant of the Korea Health Technology Research and Development Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health and Welfare, Republic of Korea (RS-2024-00438366).

F1205

TRANSFORMING FAT GRAFTING WITH 3D-CULTURED NON-AUTOLOGOUS ADSCS: A PARADIGM SHIFT IN SOFT TISSUE REGENERATION

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Fat grafting is a simple and minimally invasive approach for soft tissue reconstruction; however, poor graft engraftment due to insufficient vascularization remains a major challenge. Previous studies have shown that the addition of adipose-derived stem cells (ADSCs) to fat grafts enhances angiogenesis and improves engraftment. However, extracting autologous ADSCs requires invasive procedures and specialized facilities, limiting their widespread adoption in clinical practice. Despite ADSCs having both angiogenic and immune-tolerant properties, most studies have focused on autologous transplantation in immunodeficient models, and the efficacy and mechanisms of non-autologous transplantation under immune response remain unclear. To address this issue and aim for the realization of off-the-shelf fat grafting using pre-formulated, non-autologous ADSCs for immediate transplantation, we aimed to evaluate the potential of non-autologous ADSCs by using human ADSCs and immunocompetent mice. To better mimic the in vivo environment, human ADSCs were 3D-cultured, resulting in significant upregulation of angiogenic markers compared to 2D cultures. Fat grafts containing only human adipocytes (adipocyte-only group) and fat grafts supplemented with ADSCs (ADSC-supplemented group) were transplanted subcutaneously into immunocompetent mice and analyzed using immunohistochemical staining for fat engraftment and vascularization. In the adipocyte-only group, human fat was completely resorbed within 4 weeks, with no evidence of angiogenesis. In contrast, the ADSC-supplemented group demonstrated sustained graft engraftment even at 3 months, along with significant neovascularization. Furthermore, both the ADSCs and the transplanted adipocytes were non-autologous in this experiment. These findings suggest that non-autologous ADSCs not only promote angiogenesis under immune response conditions but also facilitate the engraftment of non-autologous fat grafts otherwise expected to face immune rejection, indicating the potential to achieve fat grafting solely with non-autologous cells. Our findings pave the way for an off-the-shelf, novel approach to regenerative medicine in soft tissue reconstruction.

Funding Source: This study was funded by Rohto Pharmaceutical Co., Ltd.

F1207

TRI-LINEAGE SPECIFICATION FROM HPSCS ENHANCES SKELETAL MUSCLE MATURITY AND REGENERATION

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Volumetric muscle loss (VML) injuries occur due to orthopedic trauma or the surgical removal of skeletal muscle (SkM) and results in irreversible damage in muscle structure and function due to the loss of a significant portion of SkM. Current clinical options to treat VML are inefficient, as recent advances in tissue engineered SkM offer a potential solution to address lost or damaged muscle tissue but are limited by donor availability and issues associated with immune incompatibility. The use of human induced pluripotent stem cells (hiPSCs) derived SkM is extremely promising for the treatment of VML due to their ability to generate personalized muscle cells in a dish. However, the Pyle lab has shown that currently available protocols for generating hiPSC muscle progenitors (SMPCs) are limited to modeling the embryonic stage of development. We hypothesized that introducing endothelial cells in our established protocol, allowing the co-differentiation of hPSCs into innervated muscle fibers, would enhance the proliferation and the maturity of the SMPCs. To optimize our model, we induced the co-differentiation of the lateral mesoderm along the paraxial mesoderm and the

neuroectoderm and then induced the differentiation and proliferation of endothelial cells. After 60 days of differentiation, as shown by immunofluorescence, we obtained vascularized millimeter long fetal muscle bundles innervated by motor neurons capable of repeated contractions and surrounded by a high number of SMPCs. Our snRNAseq analysis, as well as the fusion index of the sorted SMPCs, suggests that the presence of endothelial cells increases the proliferation and enhance the maturity of our SMPCs aligning with our data of fetal in vivo SMPCs. Finally, the engraftment of sorted CD82+/NCAM SMPCs into NSG-mdx mice resulted in the formation of hundreds of dystrophin+ muscle fibers demonstrating the high proliferation and fusion capacity of our SMPCs in vivo. The Pyle lab has shown that fetal SMPCs can better regenerate skeletal muscle compared to embryonic SMPCs and our ongoing studies are evaluating SMPCs and endothelial cells in mouse models of VML for future use in a cell-based therapy for treatment of VML injuries.

Funding Source: Kilian Mazaleyrat acknowledges the support of the UCLA Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research Training Program for resources, mentorship, and funding.

F1209

UTILIZATION OF LGR5+ INTERZONE CELLS IN KNEE JOINT DEVELOPMENT FOR ACHILLES TENDON REPAIR

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Tendinopathy has a high prevalence in the elderly and athletes, with adverse effects due to slow and poor repair, affecting quality of life. Lgr5 and Lgr6 are markers for stem cells, and we have recently identified Lgr5+ cells in the developing knee joint and Achilles tendon. Expression of Lgr5 and Lgr6 in Achilles tendon development is dynamic. There are both Lgr5+ and Lgr6+ cells in early postnatal tendon. However, Lgr5+ cells are no longer detectable beyond 4 weeks of age, while Lgr6+ cells exist in adulthood, suggesting Lgr5+ cells are more relevant in development and growth. Tendon healing following injury or rupture decreases significantly with age that may correlate with low or absence of Lgr5+ cells. Thus, we propose that Lgr5+ progenitors are related to repair potential of tendons in young mice. Interestingly, we found that during healing of tendon injuries in adult mice, there is a sporadic reactivation of Lgr5-GFP+ cells, noted at 7 days post-surgery, underscoring a role of Lgr5+ cells in Achilles tendon repair. This led us to investigate the possible application of exogenous Lgr5+ cell as therapy in Achilles tendon repair. To assess the contribution of transplanted Lgr5+ cells to Achilles tendon repair following injury, we encapsulated Lgr5-GFP+ tdTomato+ cells isolated from E13.5 knee joint interzone in hydrogel, before implantation into the injury site. One week after surgery, exogenous Lgr5-GFP+ cells were found within the existing tendon, aligned parallel to the orientation of the collagen fibers, indicating engraftment of these cells that will contribute to the repair process. Additional works are needed to investigate the positive outcomes of such a therapeutic approach for tendinopathy.

TRACK: ORGAN GENERATION AND REGENERATION (OGR)

Poster Session 3: (EVEN)
5:00 PM – 6:00 PM

F1002

A NOVEL CGMP-GRADE MEDIUM FOR LARGE-SCALE MSC EXPANSION

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Mesenchymal stem cells (MSCs) have emerged as a promising tool in regenerative medicine and cell therapy due to their ability to differentiate into various cell types and their immunomodulatory properties. However, the traditional use of serum-containing media in MSC culture has raised concerns regarding variability, contamination, and ethical issues associated with animal-derived components. Serum-free culture medium (SFM) offers a viable alternative that addresses these challenges. In this study, we developed a novel cGMP-grade medium for large-scale MSC expansion. DOE (Design of Experiments) methods were adapted, and multiple rounds of optimization were performed. All the components were cGMP grade and commercially available. Cell growth was investigated by calculating doubling time. MSC surface markers were measured using flowcytometry. The capability of MSC differentiation was assessed using standard protocol. The medium developed in this study incorporates chemicals and human recombinant proteins, such as bFGF and other growth factors, and is free of undefined animal origin components. MSCs can be expanded up to 20 passages. The cell doubling time ranges from 15.5 hours in passage 5 to 24.4 hours in passage 16 and the cells stopped growing after passage 20. High purity of MSCs was achieved and evidenced by cell surface marker staining. The percentage of cells positive for CD73, CD90, and CD105 but negative for CD31, CD34, CD45, and HLA-DR was 99%. The medium was used to culture MSCs in multi-layer cell factory and generated consistency production with very little batch-to-batch variability. No significant difference was observed among MSCs from different donors in terms of identity, purity and the capability to be differentiated into adipocyte, chondrocyte, and osteocyte lineages. We have systematically developed a serum-free culture medium for MSC expansion. The MSC manufacturing processes are more consistent and scalable by using this medium. This serum-free culture medium is a significant advancement in MSC research and application, offering enhanced safety, consistency, and efficiency. Its use is expected to facilitate the translation of MSC-based therapies into clinical practice, providing new treatment options for a wide range of medical conditions.

F1004

SCLEROSTIN IN REGULATING ENDOSTEAL SKELETAL STEM CELLS IN MICE: IMPLICATION FOR CELL THERAPY

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The adult human skeleton is constantly renewed in a process called remodeling, which is essential for maintaining healthy bones. However, up to one in three people, both young and old, suffer from issues related to abnormal bone mass. Osteogenesis Imperfecta (OI) is a rare group of diseases that result in fragile bones that break easily. Traditionally, OI is treated with drugs and hormones, but these treatments can lead to problems like unusual fractures or bone death over time, and although they may increase bone density, the bone quality often remains poor. Researchers have looked into using mesenchymal stem cell (MSC) transplants as a long-term treatment for OI, but the benefits observed in clinical trials were only short-term. Recently, unique skeletal stem cells (SSCs) were identified that may be more preferable for bone regeneration. These SSCs are found in certain parts of long bones in both mice and humans. SSCs are very rare among the broader family of MSCs, which may explain why MSC transplants haven't been very effective in the long run. Our lab has created a mouse model, (13del-tg) where a mutant form of Collagen X causes ER-stress in osteocytes, impairing maturation and the expression of Sclerostin (SOST), resulting in excessive bone growth, of both the periosteal and endosteal origin. To understand how SSCs from the bone marrow contribute to endosteal bone growth, we assessed SSCs in various bone regions using flow cytometry and single cell transcriptomics. Further, we showed that these SSCs are in close association with blood vessels in the bone marrow. We propose that in 13del mice, there may be a mechanism of enhanced recruitment and/or differentiation of SSCs on the inner bone surface that promoting bone growth. This population of SSCs could be candidate for cell therapy in patients with OI, and we plan to test this in an OI mouse model.

Funding Source: Hong Kong Health Bureau - Health and Medical Research Fund.

F1006

DEVELOPMENT OF 3D PACKAGING TECHNOLOGY WITH BRAIN ORGANOIDS FOR HIGH-PERFORMANCE BIOPROCESSORS

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The human brain exhibits exceptional computational efficiency, integrating sensory information and solving complex problems with minimal energy consumption. This efficiency arises from neuronal parallel processing and synaptic plasticity, presenting a promising alternative to conventional silicon-based computing, which has limitations related to power consumption and scalability. However, the performance improvement of three-dimensional (3D) organoid-based bioprocessor is constrained by the structural limitations inherent to 3D organoids. Increasing

the number of cells and synapses to enhance bioprocessor performance often leads to inadequate oxygen and nutrient delivery to the inner regions of the three-dimensional organoid structure. This insufficient supply causes cell death, ultimately resulting in a decline in processor performance. To address this challenge, we propose a novel 3D packaging strategy for bioprocessor, wherein uniformly cultured brain organoids are systematically stacked within a structured matrix to enhance network scalability while mitigating necrotic core cellular apoptosis. Layered architecture significantly increases the number of neurons and synapses while improving inter-organoid connectivity, enabling richer neural signal processing. These findings suggest that 3D organoid integration approach can surpass the performance of conventional two-dimensional (2D) neuron-based bioprocessor, providing a foundation for the development of next-generation energy-efficient and high-performance neuron cell-based computing systems.

Funding Source: This work was supported by NRF and MFDS grants funded by the Korean government (RS-2024-00441103 and RS-2024-00331678).

F1008

HUMAN IPSCS AND HUMAN IPSC-ENDOTHELIAL CELLS DERIVED FROM PAD PATIENTS AND HEALTHY DONORS HAVE SIMILAR CHARACTERISTICS AND POTENCY: IMPLICATIONS FOR AUTOLOGOUS CELL THERAPY IN PERIPHERAL ARTERY DISEASE

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Peripheral artery disease (PAD) can lead to amputation in advanced cases, making cell therapy using human induced pluripotent stem cells (hiPSCs) a promising therapeutic option. hiPSC-derived endothelial cells (hiPSC-ECs) have shown favorable effects in treating experimental ischemic cardiovascular disease. An autologous approach for PAD patients is preferable to avoid immunological reactions. However, it is yet unknown whether hiPSCs and hiPSC-ECs derived from PAD patients have similar characteristics and potency compared to those derived from healthy volunteers. Therefore, we explored whether there are significant differences in the characteristics and potency of hiPSCs and hiPSC-ECs between non-PAD donors and PAD patients. We successfully generated hiPSCs from seven non-PAD donors and

eight PAD patients. Both non-PAD and PAD-derived hiPSCs exhibited similar expression levels of pluripotency markers, as determined by qRT-PCR, flow cytometry, and immunostaining. All hiPSCs, regardless of the group, formed teratomas and showed normal karyotypes. RNA-seq analyses revealed similar gene expression profiles between the groups. We then differentiated hiPSCs into endothelial cells (ECs) in a clinically compatible manner. hiPSC-ECs derived from both groups exhibited similar expression levels of EC markers at both the gene (qRT-PCR) and protein levels (immunostaining and flow cytometry). RNA-seq analyses showed no significant overall differences in gene expression profiles between the groups. In vitro nitric oxide assays and tubular structure formation assays demonstrated similar endothelial characteristics and function in hiPSC-ECs from both groups. When injected into the hindlimb muscle following induction of hindlimb ischemia, both groups showed similar perfusion recovery, limb salvage, and vessel-forming capacity. Engrafted hiPSC-ECs from both groups also exhibited similar angiogenic and vessel-forming capabilities. Our study demonstrated no significant differences in hiPSCs and hiPSC-ECs derived from non-PAD donors and PAD patients in terms of molecular and cell biological characteristics, therapeutic effects, and vessel-forming capability. Our study indicates that hiPSCs and hiPSC-ECs derived from PAD patients can serve as a novel platform for autologous cell therapy.

Funding Source: This work was supported by grants from the NRF of Korea funded by the Korean government (MSIT; RS-2025-00554363) and Korean Fund for Regenerative Medicine funded by MSIT and Ministry of Health and Welfare (RS-2024-00333839).

F1010

YAP ACTIVATION REVERSES AGING-RELATED VISUAL DYSFUNCTION CAUSED BY IMPAIRED CELL-MATRIX ADHESION

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Cellular senescence of retinal pigment epithelium (RPE) cells plays a pivotal role in age-related visual decline, particularly in the progression of age-related macular degeneration (AMD). Yet, the molecular mechanisms underlying RPE senescence remain largely elusive. In this study, we utilized single-cell RNA sequencing (scRNA-seq) to analyze RPE cells from young and aged mice, uncovering a strong association between dysregulated cell-matrix adhesion and RPE senescence. Using hydrogel-based models, we demonstrated that impaired integrin-mediated adhesion triggers a novel form of senescence, termed softness-induced senescence, in RPE cells. Mechanistically, a mechanically compliant microenvironment attenuates integrin signaling, leading to reduced activation of Yes-associated protein 1 (YAP), a pivotal mechanotransducer that maintains cellular homeostasis. Loss of YAP activity precipitates hallmark senescence phenotypes, including irreversible cell cycle arrest, upregulation of

senescence-associated beta-galactosidase (SA-β-GAL), and increased secretion of senescence-associated secretory phenotype (SASP) factors. Notably, reactivation of YAP in senescent RPE cells reinstated stem cell-like transcriptional programs and reversed senescence-associated phenotypes. Furthermore, pharmacological activation of YAP using TRULI, a small-molecule YAP agonist, significantly improved visual function in both an AMD mouse model and physiologically aged mice. These findings establish the integrin-YAP mechanotransduction axis as a fundamental regulator of RPE senescence and highlight YAP activation as a promising therapeutic strategy for mitigating RPE aging and preserving visual function.

Funding Source: This work was supported by the National Research Foundation of Korea grant funded by the Korea government.

F1012

HIGH RESOLUTION SPATIAL TRANSCRIPTOMIC PROFILING OF HUMAN UPPER AIRWAY

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The mucociliary apparatus of the airway epithelium serves as a critical defense system, orchestrating the secretion of mucins and other proteins to entrap and neutralize pathogens. Secretory cells within this barrier exhibit a high degree of molecular and functional heterogeneity, facilitating dynamic responses to environmental challenges. Recent scRNA-seq studies have elucidated the transcriptional heterogeneity of secretory cells, yet their lack of spatial context limits understanding of localized functionality. Here, we leveraged Stereo-seq to generate a high-resolution map of human tracheal biopsies, capturing the spatial transcriptomic landscape of the surface epithelium and submucosal glands. Our analysis revealed discrete regional expression patterns of secretory protein families, including MUC5AC and MUC5B mucins, SCGB3A1 secretoglobins, and serous proteins such as PRH and PRB family. These spatially resolved profiles suggest functional compartmentalization critical for mucosal homeostasis and pathogen clearance. The spatial data also revealed a coordinated immune network, with IgG- and IgA-producing plasma cells preferentially localized near submucosal glands. Furthermore, ex vivo infection experiments using influenza virus revealed region-specific transcriptional responses, providing mechanistic insights into spatially resolved host-pathogen dynamics.

F1014

INCORPORATION OF MESENCHYMAL STEM CELL-DERIVED EXTRACELLULAR VESICLES WITHIN COLLAGEN I BIOMATERIALS FOR MUSCULOSKELETAL TISSUE REPAIR

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Musculoskeletal tissues possess limited potential for self-repair and large-scale injuries severely disrupt regeneration. Thus, great efforts have been made for the development of bioinstructive, extracellular matrix (ECM)-mimetic materials that guide mesenchymal stem cell (MSC) activity toward structural and functional tissue restoration. The design of these biomaterials relies on a comprehensive understanding of ECM components that confer its biomodulatory properties and direct wound healing; yet, it was only recently discovered that extracellular vesicles (EV) are natively embedded within ECM deposited by MSC in vitro (ECM-EV). This discovery has revealed a critically understudied mechanism of cell-environment interactions with significant implications for engineering cellular signals for regeneration. The bulk of current MSC EV research addresses those secreted into conditioned culture medium (liquid-EV), shown to be a primary mechanism of MSC paracrine signaling and exert regenerative functions within biomaterials in various tissues. Here, we characterize key differences in MSC liquid-EV and ECM-EV identity and compare their regenerative properties in collagen I biomaterials toward novel musculoskeletal tissue engineering strategies. We describe methods to isolate these two populations of EV from the same MSC culture in vitro and confirm EV enrichment by surface marker expression (Western blot), morphology (TEM), and size distribution (nanoparticle tracking analysis). We show that these EV differentially express tetraspanins (CD9, CD63, CD81) and possess unique proteomic profiles, evidenced by dSTORM microscopy and SDS-PAGE, respectively. Additionally, we incorporate these EV in collagen biomaterials to interrogate their ability to drive MSC proliferation, differentiation, and ECM deposition. Our results will contribute to a growing body of literature aiming to understand characteristic differences between liquid-EV and ECM-EV and elucidate the role of tissue-resident EV in cell sensing of the microenvironment.

F1016

OVERCOMING HOST MICROENVIRONMENT CHALLENGES IN RETINAL GANGLION CELL TRANSPLANTATION

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Glaucoma is a “silent creeper,” with a significant proportion of patients getting their diagnosis at the late stages of the disease when a structural loss already occurred and a significant portion of host retinal ganglion cells (RGCs) is gone. Available glaucoma treatments reduce intraocular pressure medically or surgically, but it can only slow or halt disease progression. No therapies exist to restore lost visual function and target RGCs directly to improve their survival. Functional replacement of retinal ganglion cells is needed to recover sight lost to Glaucoma and other optic neuropathies. Our roadmap to achieve functional integration of stem cell derived RGCs aims to recapitulate the normal development, which requires alignment of intrinsic and extrinsic signals necessary for donor cell survival, migration, structural and functional integration, dendrite and axon outgrowth, synapse formation and acceptance by innate and adaptive immune system. The advanced transcriptomic analysis of human retinal development allowed us to identify several factors in the developing retina microenvironment that are not present in the adult. These include known neurotrophins (GDNF, BDNF), migratory (SDF1, aFGF) and axon guidance cues (Netrin, Slit). By creating the chemokine gradient within

the host retina with recombinant SDF1 we were able to significantly improve the integration of donor RGCs following transplantation (2.7-fold) with no adverse effects. Our transplantation studies in severely immunodeficient NSG mice show that the viable donor neurons are primarily eliminated by host innate immune system. To further investigate this we have performed a timecourse transplantation study in reporter CX3CR1-GFP mice with in vivo imaging, histological and molecular readouts with and without inhibitors of microglia-RGC interaction. We have shown that it is possible to significantly increase donor cell survival (3.4-fold) by inhibiting the microglia-RGC interaction with Annexin V, which led to increased axon outgrowth into the optic nerve head. Overall, our studies confirm the feasibility of RGC replacement and strongly suggest the use of co-treatments to enable functional integration.

Funding Source: Gilbert Family Foundation, Department of Defense, National Eye Institute.

F1018

RECONSTRUCTING ORGAN-SPECIFIC VASCULATURE IN IPSC-DERIVED LUNG AND INTESTINAL ORGANOIDS

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The vasculature and mesenchyme exhibit distinct organ-specific characteristics adapted to meet local physiological demands. The microenvironment and cell-cell interactions are crucial in driving the adoption of organotypic features since the earliest developmental stages. To recapitulate this entire process, we co-differentiated mesoderm and endoderm lineages from iPSCs within the same spheroid to vascularize lung and intestinal organoids. The ratio of endoderm and mesoderm lineages was fine-tuned by BMP signaling during the initial stage of differentiation, a critical step in generating the appropriate proportions of endothelial and epithelial progenitors with tissue specificity for subsequent organoid patterning and vascularization. Single-cell RNA-seq analysis further demonstrated the organ-specific gene signatures of endothelium and mesenchyme, and identified key ligands driving endothelial specification in both lung and intestine. The organotypic endothelium exhibited tissue-specific barrier function, enhanced organoid maturation, promoted cellular diversity, and supported

alveolar structure formation on bioengineered scaffolds. Upon transplantation into mice, the vasculature retained its organ specificity and integrated with the host circulation, further enhancing the maturation and patterning of the organoids. Additionally, our model revealed abnormal endothelial-epithelial crosstalk in patients with FOXF1 deletion or mutations. Multilineage organoids provide a unique platform to study developmental cues guiding endothelial and mesenchymal cell fate determination, and investigate intricate cell-cell communications in human organogenesis and disease.

F1020

RECONSTRUCTING THE COMPLEX HUMAN AIRWAY NICHE: COMMUNICATIONS OF EPITHELIAL PROGENITORS, FIBROBLASTS, AND IMMUNE CELLS

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Human organoids derived from reprogrammed and tissue stem cells offer a valuable in vitro platform for studying human development, regeneration, and disease modeling. Current platforms mainly focus on generating airway epithelial cells from human ESCs and iPSCs but do not effectively translate complex communications across various cellular and tissue niches of the human airway. Moreover, there is a lack of consensus benchmarking standards across different organoid platforms and human samples. Addressing this knowledge and technological gap, we developed an improved method to create human airway organoids containing both epithelial and stromal lineages. These organoids exhibit functional motile ciliated cells and mucin-secreting submucosal gland cells within a well-organized pseudostratified epithelium, with transcriptomic profiles displaying a high similarity to human tissues. They contain a FOXF1+ fibroblast niche conserved in both fetal human and mouse airways. We introduced a co-culture approach incorporating blood-circulating monocytes, leading to enhanced mesenchymal-epithelial crosstalk, the emergence of fetal-specific pulmonary neuroendocrine cells, and the differentiation of monocytes into self-sustaining alveolar macrophages. Our multi-lineage organoid system, incorporating mesenchymal, immune, and epithelial cells, successfully recapitulates the cellular heterogeneity and organization of the human conducting airway and provides insights into the organized cell-cell interactions in the proximal airway. By presenting an integrated analysis using published single-cell RNA-seq datasets pertinent to the proximal airway of mice, humans as well as human organoids, we address current benchmarking progress and future challenges in the field. Our analyses underscore the importance of constructing a complex signaling niche for a better understanding of human tissue regeneration, highlighting the potential of this research in translating discoveries into clinical applications.

F1022

RETINOIC ACID CHANGES PROGENITOR CELL ELECTROPHYSIOLOGY VIA RCAN2-CALCINEURIN TO SCALE SIZE OF DEVELOPING/REGENERATING VERTEBRATE APPENDAGES BY CONTROLLING CA2+-CAMKK REGULATED SHH TRANSCRIPTION

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All organs require the controlled growth of stem and progenitor cells to scale the structure to the correct size with the body. While many growth factors and morphogens are known that control growth, it remains unclear how these mechanisms are coordinated to scale entire anatomical structures. Findings from several labs show that endogenous cell electrophysiology controls proportional growth of entire anatomical structures. We have uncovered how the K+-leak channel Kcnk5b (which decreases intracellular K+) scales proportional growth and how this electrophysiological mechanism can be regulated. The developmental mechanisms and the organization of gene expression patterns of all early embryonic mouse and human limbs are conserved in the development of the pectoral fin bud of the zebrafish embryo. Using fin bud as a model for early vertebrate fin/limb development, we observed coordinated decreases in endogenous intracellular K+ levels during bud outgrowth of the entire mesenchyme and ectoderm tissues, and overexpression of the different K+-leak channels is sufficient to increased bud size of the entire anatomical structure by enhancing the transcription of the morphogens that control bud development. We subsequently found that Kcnk5b-regulated scaling requires cell-autonomous IP3R-mediated Ca2+ release from the endoplasmic reticulum and CaMKK activity to regulate the transcription of shh, a morphogen that is itself required and sufficient to alter proportional growth. We also found that retinoic acid (RA), a hormone morphogen from the body, reduces intracellular K+ throughout the fin bud and that RA regulates Kcnk5b activity by dephosphorylation of serine 345 in its cytoplasmic tail via Rcan2-calcineurin signal transduction. Finally, we determined that this mechanism is also involved in scaling the growth of adult regenerating fins. Together, our findings show how a growth promoting hormone (RA) from the body decreases intracellular K+ and how this electrophysiological change controls a Ca2+-mediated signal transduction to regulate the transcription of shh in order to scale the proportions of entire anatomical structures.

Funding Source: ShanghaiTech University and National Science Foundation of China.

F1024

SIRT1 PROMOTES PANCREATIC PROGENITOR CELL DIFFERENTIATION BY ACTIVATING AUTOPHAGY-MEDIATED DEGRADATION OF THE NOTCH SIGNALING PATHWAY

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Differentiation of pancreatic progenitor cells (PPCs) into insulin-producing cells offers a promising approach for regenerating β cells and treating diabetes. Autophagy, essential for pancreatic β-cell development, plays a regulatory role in cellular differentiation. This study aims

to investigate the role of SIRT1 in the differentiation of human PPCs/islet-like cell clusters (ICCs) and the molecular mechanism by which SIRT1 regulates autophagy. Our results showed that SIRT1 translocated from the nucleus in PPCs to the cytoplasm in ICCs, accompanied by increased SIRT1 mRNA levels during differentiation. Overexpression of SIRT1 or SIRT1 activator SRT1720 significantly enhanced the levels of pancreatic endocrine differentiation markers (NGN3, NKX2.2, NKX6.1), maturation markers (INSULIN, PDX-1, GLUCAGON), and insulin content in ICCs. Conversely, SIRT1 knockdown or inhibition with EX527 had no such effects, indicating SIRT1 promotes PPCs/ICCs differentiation. Additionally, SIRT1 overexpression in ICCs enhanced their in vivo functionality to ameliorate progressive hyperglycemia in diabetic mouse model. Mechanistically, autophagy levels increased during differentiation, with SRT1720 elevating autophagy markers (LC3-II and p62) and phosphorylation levels of ULK1 and AMP-activated protein kinase (AMPK). Inhibition of AMPK signaling by Compound C abrogated the differentiation-promoting effects of SRT1720, reducing insulin levels, autophagosome-positive cells, and phosphorylation of autophagy-related proteins. Knockdown of autophagy-related gene ATG16L1 abolished the inhibitory effect of SRT1720 on NOTCH signaling, eliminating its differentiation-promoting effect. These findings highlight SIRT1 as a key regulator of PPCs differentiation via autophagy-mediated degradation of NOTCH signaling. This study advances our understanding of the transcriptional regulation of pancreatic stem cell differentiation by SIRT1 and provides new insights into its potential application in β-cell regeneration for diabetes therapy.

Funding Source: This work was supported by Medical Scientific Research Foundation of Guangdong Province (A2024424), National Natural Science Foundation of China (82000739) and Guangdong Basic Research Fund (2019A1515110128).

F1026

US2-MEDIATED IMMUNE EVASION: A NOVEL STRATEGY FOR GENERATING HYPOIMMUNOGENIC HIPSC LINES

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Mismatches in HLA haplotypes between donors and recipients significantly increase the risk of graft failure due to immune rejection. While knocking out beta-2 microglobulin (B2M) is the current standard for preventing HLA class I surface expression and protecting stem cell-derived products from allogeneic rejection, the complete ablation of HLA-I molecules can impair natural killer (NK) cell “self” recognition and disrupt critical immune-regulatory interactions. To address these challenges, we developed a novel hypoimmunogenic induced pluripotent stem cell (iPSC) line by inserting the cytomegalovirus-derived US2 encoding sequence into the AAVS1 safe harbor locus. Comparison of the transcriptomic profiles of B2M-deficient and US2-modified iPSCs to their parental counterparts, revealed that US2 expression does not affect stemness markers, including SMAD2, SMAD4, NANOG and SOX2, or differentiation potential into

endothelial cells (ECs) or kidney organoids, as confirmed by lineage-specific marker expression. Flow cytometry (FACS) analysis revealed that US2-expression abrogates HLA-A2 cell surface expression while retaining detectable levels of non-classical HLA molecules, measured at 10- to 15-fold lower than control levels. Co-culture assays revealed that US2 expression prevented HLA-A2 alloreactive T cells activation, as shown by the absence of difference in MIP-1β secretion compared to control. Moreover, US2 expression significantly reduced NK cell activation, with CD107a degranulation reduced by 5% and MIP-1β secretion by 20% to 40% compared to B2M KO ECs. These findings establish US2-mediated "stealth" technology as a refined alternative to B2M knockout, offering selective modulation of HLA expression to mitigate immune rejection while preserving critical immune-regulatory interactions.

Funding Source: This study was funded by LUF/Stichting Prof. Jaap de Graeff-Lingling Wiyadharma Fonds 2020-01 and the Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW, supported by Novo Nordisk Foundation grants (NNF21CC0073729)).

F1028

3D SCAFFOLD-BASED IN SITU STEM CELL TRANSPLANTATION STRATEGIES PROMOTING SPONTANEOUS TISSUE INTEGRATION AND VASCULARIZATION

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In emergency situations involving the loss of hard tissues, immediate treatment is crucial. Stem cell-based approaches have shown promise for improving tissue regeneration, but current treatments lack efficacy in urgent situations due to the limited transplantation methods available for the defect. Three-dimensional (3D)-printed scaffolds have been suggested to provide structural support for damaged tissue. However, poor cell affinity and limited encapsulation techniques hinder cell inoculation and integration. To overcome these challenges, this study prepared a 3D-printed scaffold loaded with high-density stem cells from the apical papilla (SCAPs) using an injectable hydrogel composed of carboxymethyl chitosan (CMCTS) and oxidized hyaluronic acid (oHA). The SCAPs were directly encapsulated in the CMCTS/oHA hydrogel through self-crosslinking and subsequently injected into/on the 3D-printed scaffold. The hydrogel-laden scaffold exhibited excellent mechanical properties. In vitro analysis showed that the hydrogel was fully degraded, leading to the formation of 3D tissue both within and outside the scaffold. After that, when implanted in mice without prior in vitro culture, the transplants were fully fused three weeks post-implantation, achieving strong tissue integration. Interestingly, mature blood vessels were histologically confirmed and resulted from the spontaneous degradation of the hydrogel and the interaction of the transplanted cells with native host tissue. Therefore, our research reveals potential applications in musculoskeletal tissue engineering, where immediate treatment is required, making these results suitable for volumetric tissue regeneration through stem cell transplantation.

Funding Source: This work was supported by the National Research and Development Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (RS-2024-00405574).

F1030

ADVANCED IPSC GENOME ENGINEERING PLATFORM FOR GENERATING NOVEL CELL TYPES IN REGENERATIVE MEDICINE

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Human induced pluripotent stem cells (iPSCs) offer powerful potential for applications in disease modeling and regenerative medicine. However, existing genome engineering tools fall short in enabling the large-scale, precise modifications required for these advanced applications. Our proprietary genome engineering platform, Geno-Writing™, overcomes these limitations by enabling (1) bi-allelic modification of endogenous genomic loci up to 100 kb and (2) integration and stable expression of up to 12 transgenes in human iPSCs within two months This unprecedented capability to perform swift and large-scale genomic alterations and complex functional enhancements represents a paradigm shift in iPSC engineering. As a proof of concept, we are applying the Geno-Writing™ platform to develop iPSCs for cell therapy in Type 1 Diabetes, focusing on two key aspects: (1) enhancing immune tolerance and (2) improving differentiation efficiency into pancreatic β cells. For immune tolerance, we precisely engineer the human leukocyte antigen (HLA) locus using Geno-Writing™, directly deleting only HLA genes while preserving the expression of adjacent protein genes. Conventional strategies, such as disrupting B2M or CIITA, can abolish HLA protein expression but may also impair other essential cellular functions due to the pleiotropic roles of these genes. Moreover, such approaches may not eliminate the potential re-expression of HLA molecules in specific organs, posing a risk of immune rejection after transplantation. Our HLA-specific editing avoids these pitfalls, generating truly HLA-null hypoimmune iPSCs. To enhance β cell differentiation, we identified candidate genes using our proprietary gene algorithm and generated their biallelic knockouts in iPSCs. These engineered clones having only single gene deletion demonstrated up to 10% improvement in β cell yield or a 6-fold increase in insulin secretion upon differentiation. These results highlight the power of the Geno-Writing™ platform as a highly effective technology for developing next-generation allogeneic iPSC-derived cell therapies.

Funding Source: This work was supported by internal funding.

F1032

COMPARISON OF MULTICELLULAR HEPATIC ORGANOID DIFFERENTIATION USING DIFFERENT CULTURE SYSTEMS AND INDUCED PLURIPOTENT STEM CELLS DERIVED FROM VARIOUS CELL SOURCES

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Stem cell-derived hepatic organoids are widely used across various research fields; however, current differentiation techniques face several limitations, including low efficacy, insufficient maturity, and challenges in replicating the in vivo microenvironment within an in vitro three-dimensional (3D) structure. Addressing these challenges requires careful selection of both the primary cell source and the culture system to optimize organoid differentiation from human induced pluripotent stem cells (hiPSCs). hiPSCs can be derived from various primary cells, such as blood cells, keratinocytes, and urine cells. However, differences in gene expression patterns, epigenetic modifications, and differentiation potential of primary cells influence their subsequent differentiation tendencies. Additionally, the choice of culture system plays a crucial role in shaping the growth environment and supporting the structural and functional development of organoids. For instance, 3D culture systems significantly influence the differentiation of specific cell types, including neurons, hepatocytes, and cardiac cells. In this study, hiPSCs were generated from cord blood mononuclear cells and differentiated into multicellular liver organoids (mHOs) under three experimental conditions: 1) without Matrigel, 2) with Matrigel added to the culture medium, and 3) embedded within a Matrigel dome. Additionally, hiPSCs derived from dermal fibroblasts and peripheral blood mononuclear cells were differentiated into mHOs. The findings reveal that both the choice of primary cell source and the culture conditions significantly affect the differentiation and development of hepatic organoids.

Funding Source: This work was supported by the National Research Foundation of Korea (NRF) and ministries including Science and ICT, Trade, Industry and Energy, Health and Welfare, and Food and Drug Safety (RS-2023-KH142779, RS-2024-00512348).

F1034

DESIGN, CHARACTERIZATION AND DEVELOPMENT OF TISSUE ENGINEERED CARDIAC VENTRICLES (TECVS) WITH CARDIAC-LIKE VALVES

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Diseases affecting the left ventricle pose a major challenge in cardiovascular research due to their high morbidity and mortality worldwide. Studying these conditions using animal models is limited by substantial electrophysiological and mechanical differences between animal and human hearts. To overcome these limitations, novel in vitro models of the left ventricle have been developed using 3D bioprinting techniques and human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), providing a promising platform to investigate disease mechanisms, as well as growth and remodeling responses. To address this challenge, we have developed tissue-engineered cardiac ventricles (TECVs) designed as truncated ellipsoids (10 mm length, 10 mm diameter, 0.5 mm thickness) optimized for bioprinting. A bioink of methacrylated gelatin and collagen embedded with hiPSC-CMs was used for fabrications. Constructs were cultured for six weeks, monitoring contractility and cardiac marker expression. However, TECVs showed weak contractions, with cardiomyocytes forming isolated beating

clusters instead of a connected network. To improve this, we incorporated human cardiac fibroblasts (hCFs), known to enhance cardiomyocyte maturity and contractility. We bioprinted cardiac patches (5 × 5 × 0.8 mm) with and without hCFs and monitored them at 2, 4, and 6 weeks. Patches with hCFs exhibited stronger contractions and better sarcomere organization, as shown by sarcomeric α-actinin and cardiac troponin T immunostaining. Additionally, we aimed to recreate pressure-volume loops in TECVs, key indicators of cardiac function not easily assessed in other 3D models. To achieve this, we are developing a novel closed-chamber pressure-generator TECV, for which we are also assessing cytocompatibility of different silicones used at the base. Our findings highlight the potential of bioengineered left ventricular models in cardiac disease research. Including hCFs significantly improved contractility and tissue organization, emphasizing the role of multicellular interactions. Further optimization, including pressure-volume assessments, will enhance their physiological relevance, paving the way for advanced disease modeling and therapeutic testing.

Funding Source: Funded by the European Union (ERC Starting Grant G-Cyberheart, agreement 101039349).

F1036

ELUCIDATING THE MECHANISMS UNDERLYING THERAPEUTIC EFFECTS OF HUMAN IPS CELL-DERIVED NEPHRON PROGENITOR CELLS FOR AKI AND CKD

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Acute kidney injury (AKI) and chronic kidney disease (CKD) are major clinical challenges with limited treatment options. Human induced pluripotent stem cell-derived nephron progenitor cells (hiPSC-NPCs) exert renoprotective effects in both AKI and CKD models. In this study, we investigated the mechanisms underlying these therapeutic effects, focusing on vascular endothelial growth factor A (VEGF-A). RNA-seq analysis revealed that VEGF-A, a key angiogenic factor known to protect renal function, is highly expressed in hiPSC-NPCs. Sustained VEGF-A delivery via a bioabsorbable hydrogel scaffold improved renal function in AKI mice by promoting angiogenesis. Conversely, VEGF-A knockout (KO) hiPSC-NPCs exhibited diminished therapeutic effects, leading to increased tubular apoptosis, peritubular capillary rarefaction, and fibrosis in both AKI and CKD models. Furthermore, mass spectrometry identified additional angiogenic factors in hiPSC-NPC-conditioned medium, suggesting that VEGF-A and other secreted factors play key roles in the therapeutic effects of hiPSC-NPCs in both AKI and CKD.

F1038

FEEDER-FREE 3D CULTURING FOR THE DIFFERENTIATION OF HEMATOPOIETIC ORGANOIDS AND RED BLOOD CELLS FROM INDUCED PLURIPOTENT STEM CELLS

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Blood transfusions play a crucial role in modern medicine, but donated blood comes with challenges such as donor shortage and risk of disease transmission. Therefore, the technology of producing red blood cells in vitro with the same quality and function as natural red blood cells (RBC) is an attractive concept and seems indispensable. Human induced pluripotent stem cells(hiPSCs) are emerging as an alternative to overcome donation-dependent transfusions because they have less limitations in cell supply and can differentiate into mature erythrocytes in vitro in a laboratory setting. However, the production and expansion of pluripotent hematopoietic stem/progenitor cells (HPSCs) for clinical application remains a challenge. In particular, the use of feeder cells, such as OP9 cells, to achieve high-efficiency RBC differentiation carries a risk of contamination by heterologous pathogens during culture or in medium, which consequently limits its clinical use. In this study, efficiency was directly compared and analyzed using two experimental methods in the process of producing RBC from hiPSC without stromal cells; dissociation and three-dimensional(3D) culture. Our results confirmed that 3D cultures had a relatively higher efficiency in differentiating RBCs compared to dissociation, and therefore, we established a relatively simple and highly efficient protocol for differentiating RBCs from hiPSCs without using stromal cells. Our findings suggest the possibility of artificial blood production for future clinical transfusions.

Funding Source: This work was supported by the National Research Foundation of Korea (NRF) and ministries including Science and ICT, Trade, Industry and Energy, Health and Welfare, and Food and Drug Safety (RS-2023-KH142779, RS-2024-00512348).

F1040

HUMAN GLIA REPROGRAMMING TO INTERNEURONS – SURVIVAL AND SYNAPTIC INTEGRATION IN THE MOUSE BRAIN

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Multiple neurodegenerative and neuropsychiatric diseases, including Alzheimer's disease, schizophrenia, and epilepsy, involve the loss or dysfunction of inhibitory interneurons, particularly parvalbumin (PV)-expressing GABAergic interneurons. These are critical for maintaining neuronal network balance. Neural reprogramming represents a potential approach for interneuron restoration by converting non-neuronal cell such as a glia into interneurons with forced gene expression. The process bypasses the stem cell state – that makes it applicable in vivo with minimized risk of tumorigenesis – i.e. in vivo reprogramming. In our group, we have

successfully reprogrammed human glial progenitor cells (GPCs) into PV interneurons and transplanted them into the prefrontal cortex of immunodeficient mice where they survive for up to 10 months, with mature neuronal markers and function. We have furthermore applied in vivo reprogramming of the human glia using doxycycline activated vectors. Preliminary studies demonstrate that, transplantation into the lateral ventricle—a neurogenic niche—of newborn mice provided superior survival and integration of human GPCs and enhanced their conversion to interneurons compared to the juvenile cortex. Ongoing studies investigate the long-term survival and neuronal subtype and function of these human in vivo reprogrammed neurons. Our strategy aims support the development of in vivo reprogramming strategies for interneuron-related disorders.

Funding Source: Knut and Alice Wallenberg foundation Olle Engkvist foundation Swedish research council.

F1042

INTERLEUKIN-8 OVEREXPRESSING MICROGEL-CELL MICROTISSUE FOR TREATING DIABETIC FOOT ULCER

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Diabetic foot ulcers (DFUs) affect around 25% of diabetic patients, posing a major clinical challenge due to impaired wound healing. While stem cell therapy has demonstrated potential in promoting tissue regeneration through paracrine signaling, its efficacy is limited by poor cell survival after transplantation. To address this, we developed collagen microgels (CMGs), micro-sized collagen gels that self-assemble with human adipose-derived stem cells (hASCs) to form CMG-cell microtissue (CCM). Nevertheless, the hyperglycemic microenvironment in diabetic patients disrupts angiogenesis and reduces the proliferation and migration of fibroblasts and keratinocytes, ultimately impairing wound healing. Interleukin-8 (IL-8), a well-characterized paracrine factor, possesses angiogenic, migratory, and proliferative properties, all of which are compromised in diabetic wounds. Therefore, to enhance the therapeutic efficacy of stem cell therapy, we identified IL-8 as a key target and overexpressed it in hASCs via adenoviral transduction. In this study, we optimized CMG:cell ratio (4:1) based on rheological properties, porosity, biocompatibility, and gene expression. Compared to conventional cell aggregates, CCMs exhibited increased IL-8 expression, attributed to enhanced integrin activation via CMG-cell interactions, which was accompanied by fibroblast growth factor receptor activation. In vitro transwell co-culture of CCMs with keratinocytes, fibroblasts, and endothelial cells demonstrated superior angiogenic, migratory, and proliferative effects compared to conventional cell aggregates. Furthermore, IL-8 knockdown impaired these properties, whereas IL-8 overexpression enhanced them, implying crucial role of IL-8 in wound healing. In a streptozotocin-induced diabetic rat model, IL-8-overexpressing CCMs significantly accelerated wound healing, as evidenced by increased granulation tissue formation and collagen deposition, whereas IL-8 knockdown impeded healing. Collectively, these findings highlight the advantages of the CCM platform over conventional cell spheroids in improving stem cell therapy outcomes. Moreover, IL-8 overexpression further enhances therapeutic effectiveness, suggesting IL-8 as a potential target for DFU treatment.

Funding Source: This work was funded by Korean Fund for Regenerative Medicine, the Ministry of Science and ICT and the Ministry of Health and Welfare of Korea.

F1044

MAPPING ECM STIFFNESS IN HUMAN PANCREAS DEVELOPMENT TO OPTIMISE IPSC DIFFERENTIATION INTO BETA CELLS

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Stem cell mechanobiology explores how physical factors, particularly extracellular matrix (ECM) stiffness, influence organogenesis and stem cell fate acquisition. As stem cell-based therapies advance, one being islet transplantation for diabetes, optimising beta cell differentiation and maturation remains a critical challenge. While biochemical signalling pathways are well understood and form the basis of current differentiation protocols, the role of mechanical properties in stem cell differentiation remains poorly understood. However, many current protocols rely on a transition from 2D to 3D culture at specific stages, and connections between mechanosensors like YAP1 and endocrine fate acquisition have been suggested, both underscoring the need for a better understanding of the underlying physical cues. Nonetheless, physiological stiffness ranges during human fetal pancreas development have yet to be reported. In this study, we present comprehensive stiffness maps of the developing human pancreas from 8 to 23 weeks post-conception, generated using contact-based Atomic Force Microscopy. These measurements suggest stiffness ranges of 50–250 Pa with ongoing stiffening over time, which we replicate in-vitro using a synthetic hydrogel system. We have optimised a fully tunable hyaluronic acid-based hydrogel for culturing iPSC-derived pancreas organoids which retain a progenitor phenotype and can be further differentiated to beta cells. With this setup, we aim to investigate whether tuning ECM stiffness during in-vitro beta cell differentiation can enhance the efficiency of iPSC differentiation into insulin-producing beta cells. This research wants to highlight the importance of mechanical factors for stem cell fate decisions and aims to offer new insights into how biophysical cues can improve regenerative medicine strategies for diabetes.

Funding Source: This work was supported by the Biotechnology and Biological Sciences Research Council [grant number BB/T008709/1].

F1046

PRECLINAL STUDIES ON A CELL THERAPY COMPOSITE FOR MTB BRONCHIECTASIS

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Tuberculosis, a highly infectious disease affecting globally is caused by Mycobacterium tuberculosis (MTb), a microbe characterized by resilient cell wall with intricate strategies for immune evasion. Bacille Calmette-Guerin (BCG) the most common anti-TB vaccine has demonstrated variable efficacy from 0 to 80%, decreasing over time from 10-20 years and uses live-attenuated antigen, that pose risk to immunocompromised patients. Bronchiectasis, an MTb complication that results to lung fibrosis and impaired respiratory performance caused deteriorating quality of life to patients In this study, a combination of in-silico and in-vitro approach to design and evaluate preclinically a cell therapy composite for MTb bronchiectasis, using immunoinformatic tools in mapping cytotoxic (CTL) and helper (HTL) T-cell epitopes, antigenic peptides were designed from the sequences of Mtb secretory proteins as potential multi-epitope vaccine. Identification of highly conserved sequences of the target protein was done using the Protein Variability Server (PVS). Epitopes predicted to be allergenic, cross-reactive, and toxic were discarded. The population coverages of CD4+ and CD8+ epitopes were estimated for the Southeast Asia region. The candidate epitopes were evaluated for binding thru molecular docking to their corresponding MHC molecules. In vitro and in vivo assessment of the peptides, matured DC and primed mesenchymal stem cells were utilized to evaluate efficacy of the cell therapy composite. Five secretory proteins associated with MTb pathogenesis and virulence were identified. The epitopes have IC50 scores ≤500 nM and are considered to be good binders. The CD4+ and CD8+ epitopes were not found to have similar human proteome and allergen hits in the databases. The peptides bind promiscuously with various HLA alleles while the estimated population coverage of other candidate epitopes identified reached up to 94% in Southeast Asia. In vitro evaluation showed favorable uptake responses from dendritic cells derived from healthy volunteers and significant interferon-gamma levels. Mesenchymal stem cell priming with bronchiectasis lung extracts as well as in vivo fibrotic mouse challenge results showed promising results. The cell therapy composite affords a potential for the management of Mtb bronchiectasis.

Funding Source: DOST-PCHRD and University of the Philippines-Manila.

F1048

SPACE-FILLING MEDIATED BY ATTRACTOR INDUCED VASCULAR BRANCHING EXPLAINS THE MORPHOGENESIS OF HEPATIC VASCULAR TREES

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Branching morphogenesis represents a fundamental biological process observed across multiple organ systems, including the lung, kidney, mammary gland, and vasculature. While core regulatory signaling pathways governing the individual branching event have been identified in various organs, how thousands of branches become coordinated at the organ scale remains incompletely understood. Here, we constructed and systematically analyzed the 3D hepatic vascular trees from mouse livers. Our data revealed that terminal branches of hepatic vascular trees exhibited a conserved 90-degree branching angle and uniform spatial distribution. To elucidate the principle of the morphogenesis of hepatic vascular trees, we developed a space-filling model mediated by attractor induced vascular branching. This framework successfully recapitulated the observed 90-degree terminal branching angle and uniform spatial distribution, validating space-filling as the governing principle of hepatic vascular patterning. Furthermore, we identified involvement of the Wnt-β-catenin signaling pathway in regulating vascular branching events. These findings collectively demonstrate that complex hepatic vascular trees develop through a space-filling mechanism, with Wnt signaling potentially serving as a candidate attractor molecule in the mouse liver.

F1050

THERAPEUTIC POTENTIAL OF HUMAN KERATIN-BASED BIO-INK WOUND DRESSINGS: A PRECLINICAL STUDY IN RAT AND MINIPIG MODELS

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This study aimed to evaluate the efficacy of a human-derived keratin-based bio-ink incorporated wound dressing in promoting wound healing using preclinical animal models. Full-thickness skin defect models were developed in both small animals (Sprague-Dawley rats) and large animals (minipigs) to simulate severe skin wounds. The wound dressings containing keratin-based bio-ink were applied to the wound sites, and their therapeutic effects were systematically assessed. In the rat model, full-thickness skin defects of 1 cm × 1 cm were created on the dorsal area, while defects of 4 cm × 4 cm were established in the minipigs. The wound size was measured weekly using digital imaging and ImageJ analysis to calculate wound closure rates. Histological analyses, including Hematoxylin and Eosin (H&E) staining and Masson's Trichrome staining, were performed to evaluate re-epithelialization, granulation tissue formation, and collagen deposition. In the rat experiments, we focused on evaluating whether an inflammatory response occurred during the early phase of implantation by analyzing the expression levels of pro-inflammatory cytokines, including IL-1β and TNF-α. In addition, the expression of genes associated with skin regeneration, such as VEGF, TGF-β1, PDGF, and EGF, was assessed. To investigate these factors, real-time PCR was performed using tissue samples collected at specific time points. Meanwhile, in the pig experiments, transepidermal water loss (TEWL) and skin hydration were measured as critical indicators of skin regeneration and barrier function recovery following the induction of full-thickness skin defects. The keratin-based wound dressing demonstrated enhanced wound healing by promoting re-epithelialization, reducing inflammation, and facilitating organized collagen matrix formation. These findings suggest the potential of human-derived keratin bio-ink as an effective material for advanced wound care applications.

Funding Source: This research was supported by the Korea Health Industry Development

Institute (KHIDI), funded by the Ministry of Health and Welfare, Republic of Korea (RS-2022-KH129883).

F1052

3D MORPHOMETRICS OF OLIGODENDROCYTES IN HUMAN BRAIN ORGANOIDS: A NOVEL APPROACH TO MYELIN DISORDERS

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Myelin disorders, including Pelizaeus-Merzbacher Disease (PMD), are marked by oligodendrocyte dysfunction and impaired myelination. Despite their severity, therapeutic progress is limited by the lack of human-specific models. Bioengineered neuronal organoids (BENOs) derived from human iPSCs provide a relevant platform for studying these disorders. BENOs are forebrain organoids with dorsal and ventral identities, containing interconnected networks of neurons, astrocytes, and oligodendrocytes. In this study, 120-day-old BENOs derived from a PMD patient iPSC line (PLP1-C33Y, c.98G>A) showed reduced gene expression of key oligodendrocyte markers (MBP, PLP1, OLIG2) and impaired neuronal network formation with increased excitability as demonstrated by multielectrode array (MEA) analysis (N=2 independent differentiations, n=6-8 BENO) in comparison to isogenic control PLP1-Y33C. Notably, this hyperexcitable phenotype represents a previously unreported feature of PMD. While functional and gene expression studies provided valuable insights into the PMD phenotype, a deeper understanding of the underlying pathomechanism required the investigation of oligodendrocyte morphology within the 3D environment of BENOs. Current imaging methods, limited to 2D analysis or sliced organoids, fail to capture the 3D complexity of oligodendrocyte architecture. To address this, we developed 3D Oligodendrocyte Morphometrics (3DOM), a machine-learning tool for whole-organoid analysis. Using 3DOM and a refined, oligodendrocyte-enhancing BENO protocol, we phenotyped PLP1-C33Y and isogenic control BENOs on Day 120 (N=2, n=10). BCAS1 immunostaining revealed reduced oligodendrocyte soma counts, larger and less circular somas, and less mature, fragmented processes compared to isogenic control. Results were validated in CRISPR knock-in lines carrying the c.98G>A mutation (N=2, n=8). To study dynamic oligodendrocyte behavior, we are adapting 3DOM for live imaging. With an established fluorescent reporter iPSC line (f-PLP1), 3DOM will enable real-time tracking of demyelination and remyelination processes. This work establishes BENOs and 3DOM as powerful tools for modeling myelin disorders, providing new opportunities to investigate disease mechanisms and identify therapeutic targets.

F1054

A BARRIER TO GENERATING PSC-DERIVED ORGANS THROUGH INTERSPECIES BLASTOCYST COMPLEMENTATION

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Regenerative medicine serves as a promising tool to address the shortage of organs for transplantation. However, constructing organs in vitro remains challenging due to the complex three-dimensional structures and the diverse cell types required. Interspecies blastocyst complementation using chimeric animals has garnered attention as a potential approach to generate complex organs in animals, thus helping to overcome the issue of organ shortage. Here, we report the generation of rat lungs and hearts in a mouse body via interspecies blastocyst complementation. For lungs, rat lung development was observed up to the P0 stage. However, the rat cells within the generated lungs unexpectedly retained their species-specific developmental timing in the mouse body, rendering the lungs non-functional after birth. Similarly, we successfully generated hearts using rat cell complementation in a heart-deficient mouse model, but the rat heart in the mouse only functioned until embryonic day 14.5 (E14.5). These findings highlight the functional limitations of xenogeneic lungs and hearts, presenting significant challenges in the development of mouse–rat chimeras. To address these issues, new strategies must be developed to overcome the challenges associated with interspecies blastocyst complementation.

Funding Source: Japan Society for the Promotion of Science KAKENHI KAC 40th Anniversary Research Grant NOVARTIS Foundation (Japan) for the Promotion of Science Nara Institute of Science and Technology.

F1056

A MODULAR STEM CELL PLATFORM FOR ENGINEERING ENDOTHELIAL CELL SPECIFICATION

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Endothelial cells (ECs) line the blood and lymphatic vessels and are essential for organ-specific metabolic, endocrine, and vascular functions. The high mortality of ischemic vascular diseases highlights the need for innovative strategies to promote neovascularization and generate functional blood vessels. Reprogramming pluripotent stem cells (PSCs) via transcription factor (TF) modulation offers a promising approach for regenerative medicine. In this study, we developed highly efficient, doxycycline-inducible embryonic stem cell lines expressing Etv2, Erg and Fli1(Etv2-ESCs, Erg-ESCs and Fli1-ESCs), three critical TFs for endothelial lineage specification, whose deletion causes embryonic lethality due to vascular defects. We demonstrate that Etv2, Erg, and Fli1 rapidly induce ESC differentiation into endothelial-like cells

within 12 hours by activating conserved gene regulatory networks (GRN), critical for EC specification. Time-course analysis revealed inter- and auto-regulatory feedback loops among these TFs, which suppress pluripotent enhancers, while activating endothelial enhancers. GATA and FOXO family members were identified as co-regulators, enhancing subtype-specific endothelial differentiation. Forced expression of the three TFs in naïve ESCs bypassed capacitation, silenced pluripotency genes, and upregulated mesodermal and endothelial markers driving differentiation. We addressed endothelial subtype plasticity, by mapping chromatin accessibility and gene expression in ~14,000 single endothelial cells from adult mouse liver and lung. This revealed organ-specific chromatin remodeling, with activation or repression of cis-regulatory elements, highlighting endothelial subtype heterogeneity. Using this data, we identified novel TFs that drive organotypic endothelial subtype specification via arrayed TF library screening. Additionally, we demonstrated that extracellular signals could direct progenitor cells toward specific subtypes, such as arterial endothelial cells (AECs), enhancing their identity and function. Collectively, our study provides a framework for endothelial cell engineering by elucidating how Etv2, Erg, and Fli1, along with specific TF partners, drive endothelial specification at the tissue and subtype levels.

Funding Source: This work is supported by NIH R01HL170286 and P01 HL160469 grants.

F1058

A NOVEL MARINE-DERIVED HYDROGEL WITH ANTIMICROBIAL AND ANTI-INFLAMMATORY PROPERTIES FOR THE MANAGEMENT OF PERIODONTAL DISEASE

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Periodontitis, affecting approximately 10% of the global population, is a plaque-induced inflammatory condition of the periodontium caused by pathogenic bacterial biofilms adhering to tooth surfaces. Current management strategies, including surgical procedures (e.g., root furcation treatment) and nonsurgical interventions (e.g., mechanical debridement), present significant limitations, such as difficulty accessing deep pockets, inadequate eradication of pathogenic microorganisms, leading to pain and discomfort, and a high rate of recurrence. Thus, there is a pressing need for innovative, minimally invasive, and effective treatment approaches. Although bacteria are necessary for periodontal disease to occur, a susceptible host is also required. The immune inflammatory response that develops in the gingival and periodontal tissues in response to the chronic presence of plaque bacteria results in the destruction of structural components of the periodontium, ultimately leading to the clinical signs of periodontitis. To address this issue, our study developed a novel hydrogel, termed Q-fCol hydrogel, by functionalizing marine-derived collagen, which resulted in enhanced antimicrobial and anti-inflammatory properties. Quality assessment indicated that the acid-based extraction

protocol successfully purified type I collagen from salmon skin, demonstrating high reproducibility and low endotoxicity. The inflammatory regulation properties were assessed via a cell-based periodontitis model using human gingival keratinocytes and macrophages. The Q-fCol hydrogel significantly shifted macrophage polarization from M1 to M2, indicating its anti-inflammatory potential. The application of Q-fCol resulted in a significant reduction in bacterial viability against Escherichia Coli and Porphyromonas Gingivalis. In conclusion, the Q-fCol hydrogel represents a promising, safe, and cost-effective treatment strategy for periodontitis, with substantial potential for clinical application.

Funding Source: Health@InnoHK.

F1060

A PROTOCOL FOR THE GENERATION OF FUNCTIONAL PANCREATIC BETA-CELL BY COMBINING SCFA SUPPLEMENTATION AND METABOLIC REGULATION THROUGH METHIONINE DEPRIVAL

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Transplantation of stem cell-derived β-cells (SC-β) presents a promising approach for treating diabetes, relying on high differentiation efficiency and robust functional maturity. Our previous research demonstrated that short-term deprival of reducing methionine and zinc before differentiation enhances pancreatic differentiation efficiency (Cell Reports, 2022; Star Protocols, 2023). Based on these findings, we developed an improved protocol that combines methionine modulation with supplementation of short-chain fatty acids (SCFAs), metabolites of intestinal microbiota that circulate systemically, to further enhance glucose-stimulated insulin secretion (GSIS) in SC-β cells. In this study, we evaluated the effects of SCFAs—specifically, acetic acid, propionic acid, and butyric acid—on SC-β differentiation during key stages, including endoderm formation (Stage 1), pancreatic progenitor differentiation (Stage 4), and endocrine maturation (Stage 6). We found that the addition of butyrate during Stage 1 inhibited endoderm differentiation and led to cell death. Moreover, its addition at Stage 4 allowed the cells to differentiate into β-cells, leading to a loss of glucose responsiveness. Notably, butyrate supplementation during Stage 6 substantially enhanced GSIS without compromising differentiation efficiency. In contrast, acetic and propionic acids did not demonstrate any beneficial effects on GSIS at Stage 6. RNA sequencing analysis revealed that butyrate addition during Stage 6 altered the expression of key genes associated with β-cell function and maturation. These findings highlight the synergistic potential of methionine modulation and SCFA supplementation in promoting the functional maturation of SC-β cells, providing a solid foundation for advancing stem cell-based therapies for diabetes.

Funding Source: JSPS Kakenhi to SK and NS.

F1062

A UNIQUE CHONDROCYTE STEM CELL POPULATION MAINTAINS MANDIBULAR BONE HOMEOSTASIS

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Degenerative joint disease (DJD) of the temporomandibular joint (TMJ) is a debilitating musculoskeletal disorder characterized by morphological and functional abnormalities of the TMJ, which poses a significant challenge in treatment due to the complex nature of the joint and the limited understanding of its underlying causes. Despite its high prevalence, there is currently a lack of effective long-term treatments targeting the root cause of this disease. Therefore, a new therapeutic approach through identifying and activating endogenous mandibular stem/progenitor cell populations is urgently needed for cartilage tissue regeneration. In this study, a cutting-edge single cell (sc) RNA-seq analysis was performed to show a unique in vivo chondroprogenitor cell (CPC) population in mandibular condyle and elucidate their role to contribute to whole mandibular, but not femoral, bone turnover by a Gli1Cre;Ai9 lineage tracing model. Interestingly, computational analysis indicate that mandibular CPCs can directly give rise to osteoblasts, which is similar as neonatal limb CPCs, but not CPCs from adult femoral condyle, indicating a unique Gli1+ CPC population maintains mandibular bone homeostasis. In vitro characterization of Gli1+ CPCs showed that mandibular CPCs have the higher proliferation and multi-lineage differentiation capacities than femoral CPCs, in which EGFR and downstream mTOR signaling is highly activated to maintain stemness of mandibular CPCs. By using a mandibular defect animal model in Gli1Cre;Ai9 reporter mice, our results indicate that Gli1+ CPCs significantly contribute to bone tissue regeneration to maintain mandibular bone homeostasis. Taken together, our findings reveal a unique endogenous chondrocyte progenitor population in mandibular condyle that maintains mandibular bone homeostasis and provides a new therapeutic avenue for regenerative medicine in the temporomandibular joint.

Funding Source: This work was supported in part by NIH/NIDCR-R21DE033128 and NIH/NCI-R01CA288366 to Dr. Chider Chen.

F1064

AN INTEGRATED CELL ATLAS OF HUMAN NEURAL ORGANOIDS AND MORPHOGEN SCREEN ON POSTERIOR BRAIN ORGANOIDS

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Human neural organoids, generated from pluripotent stem cells in vitro, are useful tools to study human brain development, evolution and disease. However, it is unclear which parts of the human brain are covered by existing protocols, and it has been difficult to quantitatively assess organoid variation and fidelity. We integrate 36 single-cell transcriptomic datasets spanning 26 protocols into one integrated human neural organoid cell atlas totalling more than 1.7 million cells. Mapping to developing human brain references shows primary cell types and states that have been generated in vitro, and estimates transcriptomic similarity between primary and organoid counterparts across protocols. We provide a programmatic interface to browse the atlas and query new datasets, and show that the atlas can be used as a diverse control cohort to annotate and compare organoid models of neural disease, identifying genes and pathways that may underlie pathological mechanisms with the neural models. We also find that the posterior brain regions are generally underrepresented and understudied in the brain organoid community, and therefore perform single-cell multi-omic and spatial characterization of posterior brain organoid development. We perform gene regulatory network inference and transcription factor perturbation which resolve mechanisms underlying neuronal differentiation. We perform multiplexed patterning screens, and identify morphogen concentration and combinations that expand existing organoid models.

F1066

ATACOMPASS: CROSS-SPECIES DECIPHERING OF CELL IDENTITES FOR SCATAC-SEQ USING LARGE LANGUAGE MODELS INDEPENDENT OF GENE ANNOTATIONS

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The single-cell assay for transposase-accessible chromatin using sequencing (scATAC-seq) technology enables the investigation of chromatin accessibility and epigenetic heterogeneity at single-cell resolution, providing critical insights into the regulatory mechanisms driving cellular heterogeneity and gene expression. However, the sparse and noisy nature of scATAC-seq data poses significant challenges in identifying features that accurately define cell states, complicating reliable annotation—one of the fundamental challenges in scATAC-seq analysis. Traditional gene activity score-based methods depend on accurate peak-to-gene mapping, which poses a substantial barrier for non-model organisms with incomplete or imperfect gene annotations. Furthermore, to our knowledge, no existing software currently supports cross-species cell type annotation for scATAC-seq data. In this study, we developed an end-to-end generative model, termed ATACompass, which leverages large language models to annotate cell types based on single-cell enriched peak sequences without relying on species gene annotation. By introducing systematic shifts and mutations to scATAC-seq peak sequences, we effectively expanded the training dataset, enhancing the model's annotation efficiency and enabling robust cross-species annotation capabilities. Finally, using datasets from five species, we established a comprehensive strategy for annotating scATAC-seq data across the majority of mammalian lineages. This represents a significant advancement over previous models. Overall, by leveraging peak sequence information, ATACompass achieves superior accuracy

for cell type annotation tasks across diverse scenarios and, for the first time, enables cross-species cell type annotation for scATAC-seq datasets.

F1068

BIOENGINEERED IPSC-DERIVED HEPATOCYTE ORGANOIDS: ADVANCING THERAPIES FOR ACUTE AND CHRONIC LIVER DISEASES

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Liver diseases caused by drugs, toxins, metabolic disorders, or pathogens can progress to liver failure, a life-threatening condition requiring transplantation. However, the demand for donor livers far exceeds the available supply, leaving many patients on waiting lists. As a result, many patients die while awaiting a transplant. Induced pluripotent stem cell (iPSC)-derived hepatocyte therapies offer a promising solution to address these challenges. Using iPSC technology, we developed a forward programming hepatocytes (FoP-Heps). These hepatocytes were culture in 3D suspension system to form hepatocyte organoids (HepO) which can be rapidly produced in large quantities. To evaluate their therapeutic potential, we investigated the engraftment, survival, safety, and functionality of HepO in vivo using various mouse models of liver injury, including the CCl4 model. At a later stage, the engraftment of HepO will be investigated in transplant-declined human livers ex vivo to confirm its feasibility for therapeutic application. Histological analyses confirmed the successful engraftment of HepO in vivo as single cells or cell clusters in various mouse tissues. We demonstrated that Hep organoids can survive for several weeks, as evidenced by the preserved cellular and nuclear integrity of the transplanted cells. HepO retained the expression of key hepatocyte markers, including human albumin, HNF-4α, and AAT. Notably, human albumin was expressed within the cytoplasm and secreted in the mouse bloodstream, highlighting functional protein secretion. Our findings demonstrate the robust engraftment of HepO while maintaining key human hepatocyte markers and functional characteristics. Their sustained survival over time represents a favourable feature for ensuring an adequate duration of the cell therapeutic effect. The secretion of albumin into the mouse circulation further reinforces their functionality in vivo. Altogether, these capabilities highlight the promising therapeutic potential of HepO for future transplantation into the livers of patients. Finally, we anticipate that this work will pave the way for the use of iPSC-derived FoP-Heps as a bridge therapy for patients awaiting liver transplantation and as a long-term solution to support liver regeneration in patients with chronic liver diseases.

Funding Source: This work was partly funded by the Wellcome Leap HOPE program.

F1070

CELLULAR SENESCENCE AND ORGAN DEGENERATION

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In response to a variety of stress factors, certain cells in our organs undergo a transition into a state of senescence, contributing to the accumulation of such cells in different organs as part of the aging process. These senescent cells play a role in the structural and functional decline of organs and are associated with degenerative diseases. Targeting these cells is a critical aspect of the broader strategy to combat aging and help to rejuvenate. However, the heterogeneity of senescent cells and their ambiguous characteristics in vivo pose challenges, exacerbated by the lack of efficient methods for their detection and targeted intervention within the body. Dr. Jing Qu is comitted to investigating both the driving causes and in vivo impacts of senescent cells. Her research interest is to uncover novel biomarkers and develop intervention strategies to manage cellular senescence and the degeneration in organ structure and functionality.

F1072

CHALLENGES IN APPLYING SPATIAL TRANSCRIPTOMICS TO ORGANOIDS: GENERATION OF A BENCHMARKING DATASET FOR A HIGH-RESOLUTION SPATIAL WHOLE-TRANSCRIPTOME, ACROSS MULTIPLE ORGANOID MODELS

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Spatial transcriptomics (ST) is a transformative technology enabling the exploration of gene regulatory networks with spatial and temporal resolution. The insight provided by ST platforms promises to shed new light on developmental biology across an array of tissue types including organoids. Organoids offer the opportunity to model development and disease while overcoming limitations of animal models or 2D cell cultures. As organoids can be cultured from genetically engineered stem cell and patient cell lines, they permit the analysis of the transcriptional perturbations that underlie disease pathogenesis. Organoids display 3D

organisation in vitro, making them prime candidates for exploration with ST. However, limitations of current ST technologies and the small size of organoids have led to relatively few studies using ST to investigate organoids, typically restricted to a single organoid type at a time. Here we present the use of Stereo-seq, currently the only ST platform to offer sub-cellular resolution while capturing transcriptome-wide information, to perform multi-organoid profiling of brain, heart, kidney, lung, cartilage, blood and engineered heart valve organoids. Our data demonstrates the versatility of Stereo-seq and its potential to determine the organisation of cell populations within this range of pluripotent stem cell derived organoids, with certain tissues requiring further optimisation. Furthermore, we provide examples of profiling multiple organoids from a single chip to maximise data capture. Benchmarking of quality metrics, such as the read depth and the number of genes and counts obtained in the different experiments, showed variable performance of Stereo-seq which were dependent on organoid types and sizes. While the sub-cellular resolution revealed limited gene expression within individual cells, by analysing gene expression at the regional level, rather than focusing on single cells, we were able uncover distinct gene expression patterns, particularly between the inner and outer regions of organoids. By providing a systematic overview of ST data across these varied organoid types, this study illustrates the power and limitations of ST to improve our understanding of organoids and their applications for modelling development and disease.

Funding Source: The Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW) is supported by a Novo Nordisk Foundation grant number NNF21CC0073729.

F1074

CHEMICALLY INDUCED EMBRYO FOUNDER CELLS ENABLE THE GENERATION OF HIGH-FIDELITY MOUSE EMBRYO MODEL WITH ADVANCED ORGANOGENESIS

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Embryo models open exciting opportunities for improving our understanding of development and advancing medicine. However, current models depend on intricate procedures and the integration of separately generated cell types. Here, we established a chemical-only strategy to induce cellular plasticity and generate embryo founder cells (EFCs) with full developmental potential both in vivo and in vitro solely from embryonic stem cells (ESCs). Within 60 hours, chemical-induced pluripotent stem cells activated the co-expression of early lineage specifiers: Cdx2, Gata6 and Oct4/Pou5f1, acquiring the 8-16-cell characteristics. In vivo, the EFCs have high and balanced contribution (>50%) to both embryonic and extraembryonic tissues in the chimeric assay. In vitro, the EFCs specified into three blastocyst fates and self-assembled into embryo-like structures, term ci-MOs (chemically induced embryo model). Then, 85% of the ci-MOs progressed into high-fidelity late-streak gastrulation stages (E7.5) within 4.5 days, displaying primitive streak formation via epithelial-to-mesenchymal transition, along with germ-layer tissues, amnion, and ectoplacental cone. More than 36% of the gastrulating ci-MOs

further developed into organogenesis stages containing high degree of morphology similarity with natural E8.5-E9.0 embryos, containing looping heart tube, 5-7 somite-pairs, prominent neural fold, anterior neuropore formation, clear foregut, and hindgut pocket, etc. Fate trajectories and molecular signatures from ci-EFCs to ci-MOs revealed remarkable similarity to natural development from E2.5 to E8.5~9.0 embryo. Summarily, ci-EFCs efficiently and accurately mimic embryo development, offering a game-changer, straightforward, rapid, small-molecule-only, and high-fidelity approach to study cell fate specification and model embryonic development from preimplantation to organogenesis.

F1076

COMPARATIVE THERAPEUTIC EFFECT OF MESENCHYMAL STEM CELLS DERIVED FROM ENDOMETRIUM AND UMBILICAL CORD ON ENDOMETRIAL REGENERATION

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Mesenchymal stem cells (MSCs) possess stem cell-like characteristics including self-renewal, multifunctional differentiation and low immunogenicity, making them an ideal cell source for regenerative medicine. MSCs can be derived from adult t(i.e. adipose tissue, bone marrow, endometrium) and neonatal tissues (i.e. placenta, umbilical cord). Despite displaying similar stem cell functions, different origins of MSCs present heterogeneous characteristics and components. However, studies on these differences remain largely unexplored. Intrauterine adhesions (IUA) significantly affects the physical and reproductive health of women. Endometrial injury and subsequent fibrous adhesions in this disease that are caused by various reasons, can severly disrupt the normal uterine physiological environment, leading to menstrual disorder and infertility. Although various treatments for IUA exist, their effectiveness is limited. Regenerative therapy using MSCs has demonstrated an outstanding and irreplaceable role in the treatment of IUA. Our previous study demonstrated that human endometrial MSC (eMSCs) can promote the regeneration of injured endometrium in an IUA mouse model. This was characterized by reduced fibrosis, increased cell proliferation and glands formation, which contributed to the restoration of the endometrium and improved fertility outcomes. Studies using umbilical cord MSCs (UC-MSCs) have also shown their therapeutic effects in endometrial regeneration. Nevertheless, direct comparison of their therapeutic differences require further exploration. Investigating their proliferation, migration and immunoregulatory properties will provide insight into selecting the optimal MSC source for restoring the injured endometrium in women with IUA.

F1078

CONTINUOUS PHYSIOLOGICAL OXYGEN INCREASES ATRIAL RATHER THAN VENTRICULAR SPECIFICATION OF HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED CARDIOMYOCYTES

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The human heart is unable to replace cardiomyocytes (CMs) lost following myocardial infarction, a major cause of death worldwide. In standard care, curative treatments for MI lack, but recent advances in generating CMs from induced pluripotent stem cells (iPSC-CMs) has renewed hope for a feasible therapy of MI patients. Despite this progress, current iPSC-CMs fail to fully reproduce the physiology of primary adult CMs, and one contributor might be a sub-optimal laboratory environment, including exposure to atmospheric O2 tension rather than the much lower physiological O2 tension experienced by cardiac stem cells during cardiac development. At present, we investigated the effect of physiological O2 tension on CM derivation as compared to iPSC-CM derived under atmospheric O2. For manufacturing purposes, we exploited a highly specialized GMP-compliant quad SCI-TIVE glovebox compartment with sensitive surveillance, enabling accurate monitoring of gas composition and temperature to ensure continuous and stable specified O2 tensions. We found that iPSC-CMs derived under theoretical physiological O2 levels displayed less ventricular specification, higher ploidy, and a lower binucleation rate as compared to iPSC-CMs derived at atmospheric O2. Single cell transcriptomics (scRNAseq) showed that iPSC-CMs at physiological O2 were more atrial-like, and this was confirmed by comparing to large scRNAseq datasets of atrial and ventricular CMs. Finally, in vitro functionality studies showed that iPSC-CMs at physiological low O2 exhibited less beating and calcium waves further supportive of an atrial phenotype. In perspectives, these data provide new biological insights essential for manufacturing of iPSC-CM cell products that may be used for regenerative or diagnostic purposes of the heart.

Funding Source: The Novo Nordisk Foundation (#NNF17OC0028764; #NNF19OC0055353) Danish Cardiovascular Academy (#PD2Y-2021004-DCA-67242) Danish Independent Research (# 8045-00019B and #4285-00027B) The Lundbeck Foundation (#R313-2019-573).

F1080

GENERATION OF HEPATOCYTE-LIKE CELLS FROM HUMAN EXPANDED POTENTIAL STEM CELLS

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The generation of functional human hepatocytes on a large scale is crucial for advancements in cell therapy and drug development. However, primary hepatocytes face challenges such as donor scarcity, unstable in vitro cultures, and genetic variability. The direct differentiation of human pluripotent stem cells into hepatocytes presents a promising avenue for regenerative medicine. In this study, we successfully generated hepatocyte-like cells (HLCs) from human expanded potential stem cells (hEPSCs) by replicating the developmental processes of the liver. These hEPSC-derived HLCs closely resembled primary hepatocytes in morphology, expressed specific hepatic markers at both transcriptional and protein levels, and exhibited essential liver functions, including albumin production. Notably, treatment with the THRB ligand T3 enhanced the expression of CYP3A4, a vital liver metabolism enzyme and a marker of mature hepatocytes, in hEPSC-HLCs. When transplanted into mice afflicted with metabolic liver disease stemming from fumarylacetoacetate dehydrolase (Fah) deficiency, hEPSC-derived HLCs not only restored liver function but also prolonged survival.

Funding Source: The Innovation and Technology Commission.

F1082

DECREASED GUT PERMEABILITY AND STEM CELL FUNCTION IN MENOPAUSAL MODELS USING INTESTINAL ORGANOIDS FROM OVARIECTOMIZED MICE

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Estrogen is an essential endocrine regulator for diverse physiological processes within the female body, including the maintenance of homeostasis. Postmenopausal estrogen deficiency is associated with clinical implications for the osteoporosis, female reproductive tract and gut health. Recent studies have elucidated that estrogen deficiency can impair the gut microbiome, characterized by altered microbial diversity. However, the precise impact by which estrogen deficiency influences gut function, maintaining intestinal stem cells differentiating abilities, and cell composition of the intestine remains unclear. By using intestinal organoids derived from ovariectomized (OVX) mouse intestinal stem cells, we investigated the effects of estrogen deficiency on gut permeability, epithelial regeneration, and stemness. Organoids from OVX mice exhibited increased gut permeability with upregulation of tight junction proteins (ZO-1, Occludin, Claudin-2, Claudin-4) and reduced expression of Lgr5 and CD24, indicating impaired stem cell and Paneth cell function. The administration of 10-9 mM of estradiol (E2) enhanced proliferation (increased budding and Ki67 expression), but it did not fully restore gut barrier integrity. These findings highlight the impact of estrogen deficiency on gut epithelial dynamics and provide better insights into gastrointestinal health in postmenopausal women.

F1084

DEVELOPMENT OF AN IN VITRO PARABIOSIS SYSTEM TO STUDY MUSCLE STEM CELL AGING

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Muscle stem cells (MuSCs) are the resident adult stem cells of skeletal muscles, where they play essential roles in tissue homeostasis and muscle regeneration. Previous heterochronic parabiosis studies noted that the aged mice receiving young blood showed enhanced capacity for muscle repair, suggesting a rejuvenating effect of young blood factors on MuSCs. Our high throughput mass spectrometric analysis of mouse plasma isolated from postnatal to geriatric mice revealed several distinct age-related expression patterns in the plasma protein composition. To investigate the influence of blood-derived proteins on MuSC functions during aging, here we developed an in vitro parabiosis system where MuSCs isolated from young and old aged mice are exposed to blood plasma harvested from mice of different ages in culture. By comparing the activation, proliferation, and differentiation of young and old MuSCs in response to aging blood, our research provides valuable insights into the crosstalk between MuSCs and the circulation, and contributes to a better understanding of adult stem cell aging.

Funding Source: This work was supported by the Hong Kong Research Grant Council (GRF16100322, GRF16102021, GRF16101524) and the Hong Kong Center for Neurodegenerative Diseases, InnoHK, and HKSAR.

F1086

DEVELOPMENT OF DECELLULARIZED EXTRACELLULAR MATRIX (DECM)-BASED CRYOGEL SCAFFOLDS FOR VASCULAR REGENERATION

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Vascular regeneration is a crucial process as the vascular system supports the provision of nutrients and oxygen to the engineered tissue. Even though a few tissues are supplied with nutrients and oxygen through diffusion, engineered tissue with distant capillaries over 200nm faces difficulties. Vascular endothelial growth factor (VEGF) plays a critical role in angiogenesis by stimulating cell proliferation and migration, but its therapeutic delivery remains challenging due to the need for precise dosing to avoid adverse effects. Here, we developed decellularized extracellular matrix (dECM)-based cryogel scaffolds with sustained VEGF release properties which are demonstrated capable of anchoring VEGF, thereby providing a sustained release and resulting in angiogenesis both in vitro and in vivo. Tissue-derived dECM contains structural elements such as collagen, glycosaminoglycans, and growth factors that can improve cell growth, proliferation, and attachment, and have an advantage in minimal immunogenicity. The neovascularization potential of a VEGF-loaded dECM/heparin cryogel was studied in a mouse hindlimb ischemia model. Blood perfusion was tracked until day 28, and the corresponding laser Doppler perfusion index (LDPI) was improved by 80%. This platform demonstrated is easily fabricated and has a sustained release, has the potential to be used not only for neovascularization but also in various tissue engineering fields with different growth factors.

Funding Source: This work was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government(MSIT) (RS-2023-NR077182(NRF-2023R1A2C2007283)).

F1088

DEVELOPMENT OF MILLIMETER-THICK CARDIAC TISSUE USING MULTILAYER FIBER SHEETS FOR MYOCARDIAL INFARCTION TREATMENT

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Human-induced pluripotent stem cell (hiPSC)-derived cardiac patches show promise for myocardial infarction treatment but are limited by insufficient thickness. Previously, we developed a fibrous scaffold that supported the formation of well-organized cardiac tissue constructs. In this study, based on the above technology, we developed a three-dimensional multilayer fibrous scaffold with dynamic perfusion, allowing the single-step seeding of approximately 20 million hiPSC-derived cardiomyocytes (CMs) to form 1 mm thick, viable tissue. This multilayer tissue exhibited enhanced contractility, increased cytokine secretion, and significantly improved functional recovery with reduced fibrosis in a myocardial infarction model. These findings indicate the need for precise evaluation of hiPSC-CM dosage in clinical therapy development, as higher cell doses significantly enhanced therapeutic outcomes.

F1090

DIFFERENT SPHEROID SIZE FROM HUMAN DENTAL PULP STEM CELLS ALTER THE OSTEOGENIC COMMITMENT

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It is well known that dental pulp stem cells (DPSCs) cultured in monolayers commit to the osteogenic lineage after 7 days under differentiation stimulus. This is defined by the peak of runx2 gene expression. When cultured in a three-dimensional conformation (3D), like a spheroid, this time could be different, furthermore, the spheroid diameter also seemed to influence this process due to the different metabolic zones inside. Our research group is studying the factors that could influence DPSCs commitment towards osteoblasts, including extracellular matrix, secretome from other cells, like macrophages and endothelial cells. Thus, the aim of the present study was to establish the period of osteogenic commitment of DPSC spheroids in different sizes in comparison to the monolayer. We cultured dental pulp stem cell monolayers (5.000 cells/cm2) and two sizes of spheroids, 100 µm (1.000 cells - fabricated in 2% agarose micromolds) and 500 µm (55.000 cells - fabricated in low adherent u-shape 96 wells). We then induced osteogenic differentiation on these cells, collected total RNA and performed histology at days 7 and 14, after spheroid formation. As a result, we observed that in monolayers and 100 µm spheroid, the peak of runx2 expression occurred on day 7, with accentuated differentiation and mineralization on day 14, shown by other RT-qPCR markers, like collagen-1, and alizarin red staining. The 500 µm spheroid, on the other hand, presented a later runx2 peak. At least on day 7, the 500 µm showed a β-catenin peak, usually seen before the runx2 peak, indicating a tendency towards differentiation. We conclude from this that the size of the spheroid is a determining factor for the period of cell osteogenic commitment and that the smaller size (100 µm) carries out the process more quickly, resembling the process in monolayer and in vivo. The delay in the largest size may be due to a difficulty in diffusing factors to all cell layers of the spheroid, or cell death, or secretome. With this, we suggest that the DPSCs spheroid smaller size (100 µm) has a similar osteogenic commitment than the monolayer.

Funding Source: CAPES and FAPESP.

F1092

DIFFERENTIATION OF NUCLEUS PULPOSUS-LIKE CELLS RELIES ON TGF-B1 AND MEK INTERFERENCE ACTIVITY

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The depletion of extracellular matrix-producing nucleus pulposus (NP) cells and long-lived progenitors leads to progressive degenerative changes in the intervertebral disc (IVD) over a lifetime. While pluripotent stem cells hold potential for deriving notochordal and NP cells, they face challenges in translational medicine. Conversely, adult MSCs may serve as a promising alternative for deriving NP progenitors, although an optimal differentiation protocol is still lacking. Tie2 and GD2 expressing adult NP progenitor cells have been reported to exhibit in vitro clonogenicity and in vivo self-renewal ability. Through microarray analysis of the transcriptome in rat primitive NP and costal cartilage, we identified preferential expressions of Cdh2, Krt19, and Car3 in rat primitive NP. Further in silico analysis of signaling pathways suggested that rat primitive NP exhibits low MAP kinase activity in TGF-β signaling. Mesenchymal stem cells (MSCs) have been demonstrated to differentiate into NP-like cells via TGF-β stimulation. Here, we hypothesized that human bone marrow MSCs could differentiate into NP progenitor-like cells through MAP kinase interference coupled with TGF-β1 mediated chondrogenic induction (MICCI). To this end, we confirmed the protein expression of CDH2 in Colonies Formation Units-Spherical (CFU-S) as a unique marker of human NP progenitors. Additionally, QPCR showed strong upregulations of CDH2 at Day 1 and NP-associated markers (COL2A1, ACAN, CD24 and KRT19) from Day 7 to Day 21 in MEK1/2 inhibitor-treated MSC micro-pellets. On the other hand, TGF-β1 could stimulate CFU-S formation in human NP cells. Interestingly, the ability to form CFU-S was reduced with TGF-β1 in the presence of a MEK inhibitor. This may suggest that TGF-β1 promotes the proliferation of dormant NP cells into progenitor colonies. MEK1/2 inhibition could couple with TGF-β1-induced nucleopulpogenic differentiation of progenitors into committed NP-like cells.

Funding Source: Health and Medical Research Fund (HMRF) 9202236.

F1094

EFFECT OF PERITONEAL DIALYSATE-DERIVED MESENCHYMAL STEM CELLS ON PHENOTYPE TRANSITION IN PERITONEAL MESOTHELIAL CELLS

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Peritoneal fibrosis (PF) is a critical complication of prolonged peritoneal dialysis (PD), driven by epithelial-mesenchymal transition (EMT), oxidative stress, and extracellular matrix accumulation. Current treatments focus on delaying damage rather than reversing fibrosis, highlighting the need for regenerative therapies. Mesenchymal stem cells (MSCs) have shown promise in preclinical models; however, clinical application is limited by challenges in cell harvesting. This study investigates mesenchymal stem cell-like cells derived from peritoneal dialysate (PD-MSC) as a novel, autologous, and clinically accessible cell source for mitigating PF. PD-MSCs were isolated from the dialysate of early-stage PD patients and characterized for MSC markers (positive: CD29, CD44, CD73, CD90, CD105, CD166; negative: CD34, CD79a, HLA-DR) using real-time PCR and FACS analysis. Their differentiation potential into adipocytes, chondrocytes, and osteocytes was assessed. The therapeutic effects of PD-MSCs on TGFβ-induced EMT in peritoneal mesothelial cells (HPMC) were evaluated by real-time PCR, Western blotting, and reactive oxygen species (ROS) assays (DCF-DA and MitoSOX). Anti-fibrotic protein expressions (HGF and BMP-7) were also measured. PD-MSCs expressed characteristic MSC markers and demonstrated trilineage differentiation potential. Treatment with PD-MSCs significantly alleviated transforming growth factor-β-induced EMT in HPMC by restoring epithelial markers and suppressing mesenchymal markers. PD-MSCs also reduced ROS production, as detected by DCF-DA and MitoSOX assays, and restored the expression of HGF and BMP-7, which were downregulated by TGFβ. Compared to T-MSCs, PD-MSCs exhibited comparable or superior efficacy in mitigating EMT, reducing oxidative stress, and promoting anti-fibrotic protein recovery. PD-MSCs derived from early-stage PD patients represent a unique, autologous, and clinically accessible cell source with significant potential for treating peritoneal fibrosis. By alleviating EMT, reducing oxidative stress, and restoring anti-fibrotic factors, PD-MSCs offer a promising therapeutic strategy for preserving peritoneal membrane function in PD patients.

Funding Source: This work was supported by the Korean Fund for Regenerative Medicine (KFRM, 23A0201L1) grant funded by the Korea government (MIST).

Clinical Trial ID: IRB No. 2019-10-014 in KOREA.

F1096

EFFICIENT IPSC-DERIVED ENDOTHELIAL PROGENITOR CELL GENERATION AND TARGETED SMALL-MOLECULE MODULATION

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Endothelial progenitor cells (EPCs) are integral to neovascularization and vascular repair, offering considerable therapeutic promise for ischemic disorders. Here, we present a streamlined protocol to generate and expand EPCs from induced pluripotent stem cells (iPSCs) without strict reliance on a detailed time-course. The procedure begins with iPSCs plated on Geltrex®-coated surfaces, followed by an initial mesoderm-inductive phase mediated by the GSK-3β inhibitor CHIR-99021. After mesodermal specification, FGF2 is introduced to reinforce lineage commitment, and VEGF plus BMP4 guide the transition toward early EPCs. Once cells reach an appropriate differentiation stage, the culture is supplemented with the Rho-associated kinase inhibitor Y-27632, the TGF-β inhibitor A83–01, and additional CHIR-99021 to bolster proliferation and maintain the EPC phenotype. Subsequent TrypLE™ replating eliminates non-EPC contaminants, yielding a highly enriched and functionally competent EPC population.

Gene expression analyses reveal a clear progression from pluripotency to the endothelial lineage, as evidenced by downregulation of Oct4, transient upregulation of the mesoderm marker TBXT, and an increase in MMP1, indicative of angiogenic activity. Phenotypic assessment confirms that early EPC markers (e.g., CD105) are eventually superseded by late-stage markers (e.g., CD133) as the cells mature. By leveraging targeted small-molecule supplementation, this protocol consistently produces high-purity EPCs capable of robust expansion. The resulting cells exhibit strong angiogenic potential and hold significant promise for scalable therapeutic applications in regenerative medicine, particularly in managing ischemic conditions and enhancing vascular repair.

Funding Source: NSTC112-2636-M-006-010,NSTC112-2321-B-006-012, NSTC113-2320-B-006-047, NSTC113-2321-B-006-012.

F1098

ENGINEERING A TENDON-MIMETIC HYDROGEL TO FACILITATE STEM CELL-DRIVEN TENDON REPAIR

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Tendon injuries, caused by overuse or age-related degeneration, are a prevalent clinical problem The slow healing process of tendons is attributed to insufficient cellularity and vascularity, often leading to the formation of fibrotic scarring and adhesion. To date, mesenchymal stem cells (MSCs) have emerged as a promising candidate for tendon regeneration and repair; however, the clinical application of MSC-based therapies is impeded by obstacles such as acute cell death, low functional engraftment yields, and off-target tissue formation. To address these challenges, there is a need to develop strategies that can precisely induce MSCs tendon-specific differentiation within a biomimetic microenvironment prior to implantation. In this study, we aimed to develop a hydrogel (TenoGel) that is biochemically and biomechanically co-stimulated to establish a functional tenogenic niche for stem cell pre-conditioning and delivery. The efficacy of TenoGel in promoting tendon healing was evaluated through two approaches: (1) In vitro characterization, which involved evaluating the effects of human adipose-derived stem cells (ASCs) tenogenic differentiation via established tenogenic markers. (2) In vivo evaluation using a rat patellar window defect model to examine tendon healing outcomes. Our results indicate that TenoGel has high toughness and supports human ASCs with strong viability, early spreading, and rapid proliferation. When stimulated by tendon-specific factors and tensile loading, ASCs displayed an organized cytoskeletal structure and increased tenogenic marker expression. In a rat patellar tendon defect model, TenoGel combined with rat ASCs enhanced tendon regeneration, showing a wavy, organized matrix and improved biomechanical properties similar to the uninjured control group. These findings suggest that bioactive TenoGel could be a promising strategy for pre-conditioning and delivering stem cells to improve tendon repair, with further research planned on biomaterial-stem cell interactions using bioinformatics analysis. TenoGel exhibited strong mechanical properties, high viability, and rapid spreading of ASCs. It also enhanced tenogenic differentiation of ASCs, enabling precise and functional tendon healing when combined with preconditioned rat ASCs in a rat model.

Funding Source: Research Grants Council of Hong Kong (GRF14118620; 14121121, DMW) National Natural Science Foundation of China/Research Grants Council of Hong Kong (N_CUHK409/23, DMW) Innovation and Technology Commission (Health@InnoHK, DMW).

F1100

ENGINEERING FUNCTIONAL BLOOD VESSEL ORGANOIDS VIA OPTIMIZED 3D BIOPRINTING OF HUMAN PLURIPOTENT STEM CELL-DERIVED EARLY VASCULAR CELLS

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The development of physiologically relevant vascularized tissue models remains a critical challenge in human vascular research and regenerative medicine. Here, we report a novel approach combining 3D bioprinting technology with GelMA-based hydrogel to generate stable blood vessel organoids (BVOs) from human pluripotent stem cell-derived early endothelial cells (hPSC-ECs) and smooth muscle cells (hPSC-SMCs). Single-cell transcriptomic analysis indicated that the enhanced maturation of ECs and SMCs in the BVOs is likely facilitated by complex cell-cell interactions, augmented mechanical support, increased tension, and hypoxic conditions within the 3D bioprinted structure, resulting in the development of a more robust and stable vascular network. When transplanted into a murine hindlimb ischemia model, these BVOs demonstrated robust integration with host vasculature, significantly improving blood perfusion and promoting vascular reconstruction in the ischemic tissue. Our work establishes a scalable and automatable platform for generating transplantable BVOs, highlighting their therapeutic potential for ischemic disease treatment.

Funding Source: This work was supported by the National Key Research and Development Program of China Grants 2022YFA1103103 and 2023YFA1800302 and the National Natural Science Foundation of China (NSFC) Grants 32270784 and 31970819 to Jie Na.

F1102

ENHANCED EFFECTS OF SLOWLY CORELEASED TGFB3 AND BMP2 FROM BIOMIMETIC CALCIUM PHOSPHATECOATED SILK FIBROIN SCAFFOLDS IN THE REPAIR OF OSTEOCHONDRAL DEFECTS

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Bioactive agents have demonstrated regenerative potential for cell-free bone tissue engineering. Nevertheless, certain challenges persist, including ineffective delivery methods and confined therapeutic potency. Here, we demonstrated that the biomimetic calcium phosphate coating system (BioCaP) could effectively uptake and slowly release the incorporated bioactive agents compared to the surface absorption system via osteoclast-mediated degradation of BioCaP coatings. The release kinetics were determined as a function of time. The release rate was stable without remarkable burst release during the first 1 day, followed by a sustained release from day 7 to day 19. Then, we developed the bi-functional BioCaP-coated silk fibroin scaffolds enabling the effective co-delivery of TGF-β3 and BMP-2 (SFI-T/SFI-B) and the corresponding slow release of TGF-β3 and BMP-2 exhibited superior potential in promoting chondrogenesis and osteogenesis without impairing cell vitality in vitro. The SFI-T/SFI-B scaffolds could improve cartilage and bone regeneration in 5mm×4mm rabbit osteochondral (OC) defect. These findings indicate that the biomimetic calcium-phosphate coated silk fibroin scaffolds with slowly co-released TGF-β3 and BMP-2 effectively promote the repair of OC defects, hence facilitating the future clinical translation of controlled drug delivery in tissue engineering.

Funding Source: Jiangsu Provincial Medical Youth Talent [QNRC2016115], Natural Science Foundation of Jiangsu Province [SBK2021021787], and Top Young and Middle-aged Innovative Talents Program of Nanjing [ZKX20048].

F1104

ENHANCED RECELLULARIZATION OF DECELLULARIZED XENOANTIGEN FREE SCAFFOLDS WITH HUMAN MESENCHYMAL STEM CELLS AND HUMAN UMBILICAL ENDOTHELIAL CELLS

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Removal for major immunogenic xenoantigens of the Galα1-3Gal (α-Gal) epitope and the non-human sialic acid N-glycolylneuraminic acid (Neu5Gc) are essential to eliminate xenoimmunogenicity and optimize recellularization for cardiac xenografts. The aim of this study was to evaluate the safety and efficacy of α-galactosidase for removal of α-Gal xenoantigen and PNGase-F for removal of non-α-Gal xenoantigen combined with optimal decellularization, and the potential of in vitro recellularization was assessed with co-culturing human adipose-derived stem cells and human umbilical vein endothelial cells for xenoantigen free cardiac xenografts. We investigated the mechanical properties, and efficacy for xenoantigen removal with expression of carbohydrate-binding lectins in porcine pericardium decellularized and treated with α-Gal and PNGase-F. In the H&E stain, there were no histological changes

depending on α-Gal and PNGase-F treatment. There was no difference in tensile stress, tensile displacement, tensile strain at break, and permeability test following enzymatic treatments. Both enzyme-treated xenografts were stained with all lectins, and showed synergistic effects for low fluorescence qualitatively and quantitatively. The enzymatic treatments for decellularization significantly reduced lectin expression, demonstrating the synergistic effect of both enzymes and decellularization. During recellularization, both enzyme treated xenografts with decellularized were entirely repopulated in 28 days and detected DAPI-positive cells. In addition, they were observed expression of fibronectin as an important factor in remodeling of ECM. In vitro recellularization for decellularized and both enzymes-treated xenografts was assessed with vimentin, calponin, fibronectin and CD31staining. Stronger signals were detected in decellularized xenografts, and decellularized xenografts treated with both enzymes showed significantly faster mesenchymal cell infiltration into the tissue, leading to accelerated recellularization. We have successfully produced xenoantigen-free scaffolds by demonstrating the safety and the synergistic effect of α-Gal and PNGase-F treatments, and proved effective recellularization for the xenoantigen-free scaffolds.

Funding Source: This study was supported by SNUH Lee Kun-hee Child Cancer and Rare Disease Project, Republic of Korea (Grant Number: 23C0220100).

F1106

ENHANCING THE MATURATION OF CHEMICALLY INDUCED CARDIOMYCYTE-LIKE CELLS VIA MITOCHONDRIAL DELIVERY

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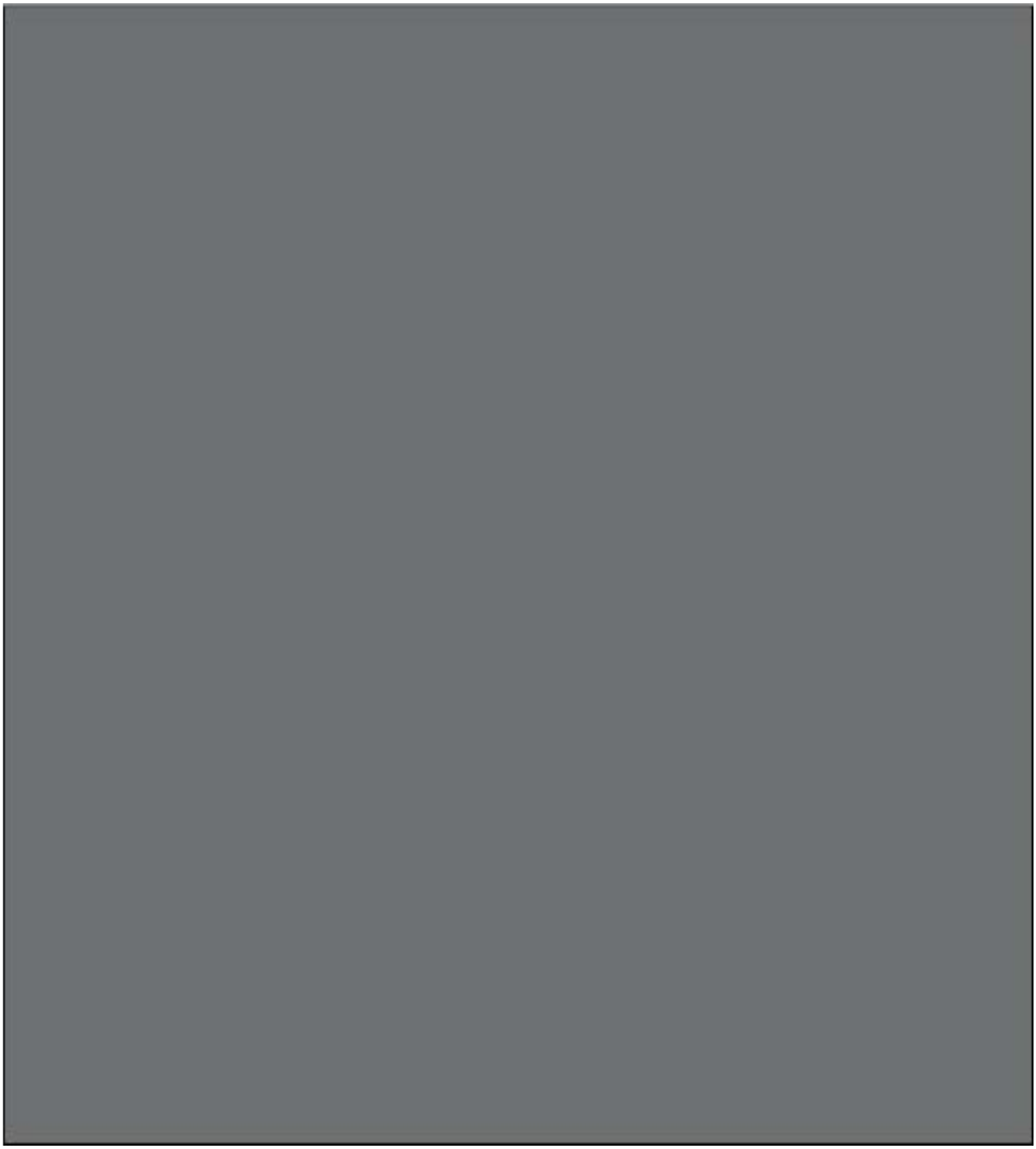
Given the heart's inherent inability to regenerate, cardiovascular diseases remain a formidable challenge due to the limited regenerative potential of native cardiac tissue. Small-molecule-based direct cardiac reprogramming has emerged as a promising strategy for cardiac regeneration, offering a rapid and non-invasive alternative to genetic modification. However, clinical translation of this approach is hindered by obstacles such as low reprogramming efficiency and functional immaturity of the reprogrammed cells. This study investigates the role of mitochondria delivery in enhancing the metabolic activity and maturation of chemically induced cardiomyocyte-like cells (CiCMs). By leveraging mitochondria from high-energy organs (heart, brain, liver), we achieved significant improvements in structural and metabolic maturation. Notably, heart-mitochondria(h-mito) induced CiCMs to exhibit elevated oxygen consumption rates, improved electrophysiological properties, and heightened sensitivity to hypoxic stress. These functional advancements are attributed to metabolic reprogramming driven by enhanced protein internalization and increased bioenergetic activity in CiCMs. In conclusion, our findings underscore the potential of h-mito delivery to markedly enhance the metabolic activity and maturation of CiCMs, thereby advancing their applicability in regenerative medicine and disease modeling. Furthermore, our results highlight the broader therapeutic implications of tissue-specific mitochondrial transplantation.

Funding Source: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (NRF-2021R1C1C2009131) and the

Brain Korea 21 Project for Medical Science, Yonsei University.



F1110

EVALUATING ASCL1, LMX1A, AND NURR1 IN COMPARISON TO PTBP1 KNOCKDOWN FOR HUMAN GLIA-TO-DOPAMINERGIC NEURON REPROGRAMMING ACROSS CELLULAR CONTEXTS

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Parkinson's Disease (PD) is characterized by the progressive degeneration of dopaminergic (DA) neurons in the substantia nigra, resulting in debilitating motor symptoms. While stem cell-based therapies have reached clinical trials, direct reprogramming of resident glial cells into functional DA neurons presents a promising alternative. However, critical questions remain regarding the efficacy and specificity of reprogramming strategies in human cells. In this study, we compared two reprogramming approaches: Ascl1, Lmx1a, and Nurr1 with REST inhibition (ALNRi) versus polypyrimidine tract-binding protein 1 (PTBP1) knockdown, for their ability to generate DA neurons from human stem cell-derived glial progenitor cells (GPCs) in vitro. Reprogrammed cells were evaluated using immunocytochemistry to assess neuronal and DA-specific markers, RNA sequencing for transcriptional profiles, and electrophysiology for functional maturation. ALNRi-induced neurons exhibited distinct electrophysiological and transcriptional signatures of induced DA neurons, whereas PTBP1 knockdown failed to generate neurons with the desired specificity or functionality. To investigate whether extrinsic microenvironmental cues provided by different brain regions could influence or improve DA neuron reprogramming, human GPCs were transplanted into 3D organoid models mimicking the forebrain and midbrain. Single-nuclei transcriptomic analyses showed that ALNRi activation post-transplantation consistently generated DA neurons within these organoid environments, while PTBP1 knockdown remained ineffective and did not yield DA neurons. These findings suggest that intrinsic mechanisms engaged by ALNRi are critical for generating DA neurons and that extrinsic microenvironmental cues alone cannot overcome the limitations of PTBP1 knockdown as a reprogramming strategy. Our results establish ALNRi as a robust strategy for glia-to-neuron reprogramming, capable of reliably generating DA neurons. By demonstrating efficacy in vitro and after transplantation into regionalized organoid models, this approach provides a strong foundation for advancing to in vivo studies, with the goal of developing innovative therapies for PD.

F1112

EXPLORING MASH-DERIVED ECM IN VIVO AND IN VITRO TO INVESTIGATE ITS USE AS AN ALTERNATIVE TO INCREASE THE LIVER DONOR POOL

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The large number of discarded marginal livers offers a promising source of organs to expand the donor pool and reduce global organ shortages. However, the post-transplantation use of a marginal liver derived - extracellular matrix (ECM) has not been demonstrated. In this context, we performed in vitro and in vivo analyses to determine whether the Metabolic dysfunction-associated steatohepatitis (MASH)- derived ECM from rat and human livers could be used to reduce organ shortage. For in vivo assays, Wistar rats (n=24) were submitted to MASH induction model based on a high-fat diet (45% fat, 20% fructose, 2% cholesterol) followed by 6% sucrose in water for 16 weeks and intraperitoneal injections of CCl4 (1mL/Kg), 3x a week for the last 3 weeks (Animal Care approval-UFRJ 69/24). Biochemical and histological analyses were performed before, and after the MASH induction and post-transplantation. MASH rat livers (n=5) were submitted to perfusion-based decellularization to obtain MASH-derived acellular liver scaffold (MASH-ALS). Macroscopic and microscopic analyses revealed cell-free, dense, opaque, and rigid ECM. After characterization, MASH-AHA were partially and orthotopically transplanted into MASH receptor rats (n=8). Macroscopic analysis revealed a well-distributed and connected MASH-AHA 30 days after transplantation. Inflammation, absence of steatosis and intense recellularization confirming the presence of liver cells, including liver stem/progenitor cells were detected into the MASH-ALS after transplantation. No significant differences were observed in body weight, glucose levels, total cholesterol, triglycerides, very high-, high- and low-density lipoproteins 30 days after transplantation. For in vitro assays, MASH-derived human livers cubes (n=20) were submitted to a decellularization process to generate human-derived MASH ALS (hMASH-ALS). HepG2 cells (500k) were cultured in hMASH-ALS for 14 days and then, submitted to histological analysis and an intracellular Ca2+ signalization assay (n=5). Sequential confocal images identified an increase in cytosolic Ca2+ after ATP stimulation and revealed that HepG2 cells acquired a synchronized signal in hMASH-ALS. Taken together, these data show that MASH-derived ECM can be transplanted, allowing for cell survival both in vivo and in vitro.

Funding Source: CNPq (170653/2023-4), FAPERJ, Capes, INCT-Regenera, INCT-Nanobiofar, Ministério da Saúde.

F1114

EXTRACELLULAR VESICLES FROM HUMAN UMBILICAL MESENCHYMAL STEM CELLS TRANSFECTED WITH MIR-7704 PROMOTE CARTILAGE REPAIR AND SUPPRESS MATRIX METALLOPEPTIDASE 13 ACTIVITY

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We investigated the therapeutic potential of miR-7704-modified extracellular vesicles (EVs) derived from human umbilical cord mesenchymal stem cells (HUCMSCs) for osteoarthritis (OA) treatment. In vitro studies confirmed the successful transfection of miR-7704 into HUCMSCs and the subsequent isolation of EVs. In vivo experiments utilized an OA mouse model to evaluate the effects of intra-articular injection of these miR-7704-modified EVs. Outcomes

assessed included walking capacity (rotarod test), cartilage morphology, histological scores, and the expression levels of type II collagen, aggrecan, interleukin-1 beta (IL-1β), and matrix metalloproteinase 13 (MMP13) in cartilage tissue. Characterization of the EVs verified their suitability for therapeutic application. Treatment with miR-7704-overexpressing EVs led to increased type II collagen and decreased MMP13 expression in IL-1β-treated chondrocytes in vitro. In vivo, the injection of miR-7704-modified EVs significantly enhanced walking capacity, preserved cartilage structure, and improved histological scores compared to controls. Additionally, the downregulation of MMP13 in treated cartilage suggested a potential mechanism underlying the therapeutic benefits observed. These findings highlight miR-7704-modified EVs derived from HUCMSCs as a promising therapeutic approach for OA. Future research should focus on optimizing dosing strategies, elucidating underlying mechanisms, ensuring safety and efficacy, improving delivery systems, and conducting early-phase clinical trials to establish the potential of HUCMSC-derived EVs for OA treatment.

Funding Source: Hualien Tzu Chi Hospital.

F1116

FROM DECELLULARIZATION TO PROTEOMICS: EXPLORING LUNG EXTRACELLULAR MATRIX ACROSS SPECIES

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The extracellular matrix (ECM) is a fundamental, yet often overlooked, component of tissue architecture. Beyond serving as a passive scaffold, the ECM dynamically regulates cellular behavior and tissue homeostasis through complex biochemical and biomechanical signals. Its continuous remodeling enables adaptation to environmental changes, while dysregulation of ECM dynamics can profoundly impact organ function and contribute to a range of pathological conditions such as fibrosis, tumor growth, metastasis, and impaired wound healing. While extensive studies have explored the ECM in individual species, there is a lack of comprehensive cross-species comparisons that can reveal both conserved and divergent aspects of ECM composition. Such comparisons are vital for understanding the evolutionary conservation of ECM components and for validating the relevance and translatability of animal models to human health and disease. In this study, we performed a detailed proteomic analysis of lung ECM from mouse, pig, and human tissues. By employing the method of decellularization, we isolated the ECM from the lung tissues of each species, ensuring the removal of cellular components and genetic material while preserving the intricate ECM

composition and structure as confirmed through histological, immunofluorescent, biochemical assays, and electron microscopy. This approach allows for an accurate proteomic analysis of the ECM itself by eliminating predominant cellular proteins that might otherwise mask the detection of less abundant ECM components. These findings enhance understanding of the lung ECM matrisome, providing insights for tissue engineering, regenerative medicine, and therapeutic strategies for ECM-related diseases. This cross-species approach also underscores the importance of evaluating animal models for their relevance to human biology, paving the way for more effective translational research in stem cell and regenerative medicine.

Funding Source: Resources for tissue engineering (MUNI/A/1738/2024).

F1118

GENERATION OF ANIMAL FETAL KIDNEY SCAFFOLDS THROUGH CONDITIONAL ABLATION OF STROMAL CELLS FOR HUMAN KIDNEY REGENERATION

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Organ transplantation is the definitive treatment for end-stage organ failure. However, the global rise in kidney failure and severe organ shortages necessitate alternative approaches. While organ regeneration using pluripotent stem cells holds promise, generating fully functional three-dimensional organs remains a major challenge. This study focuses on regenerating human nephrons using fetal animal kidneys as scaffolds. We previously achieved interspecies nephron regeneration by eliminating host Six2-positive nephron progenitor cells (NPCs) and Foxd1-positive stromal progenitor cells (SPCs) using a diphtheria toxin-based Cre-loxP system, followed by transplanting rat progenitor cells. However, this method was unsuitable for human applications due to the non-specific toxicity of diphtheria toxin to human cells. To address this issue, we developed a mouse model incorporating an inducible caspase-9 (iC9) system to selectively eliminate fetal kidney progenitor cells without harming human cells. Foxd1-inducible caspase-9 mice (Foxd1-iC9) were created using CRISPR-Cas9 to knock in iC9 and a Venus reporter at the Foxd1 C-terminus. Homozygous mice were bred, and E13.5 fetal kidneys were harvested and cultured in transwell plates. AP20187 was administered daily to induce cell elimination. Immunostaining was performed to evaluate SPC elimination efficiency after four days. Cell death began within 24 hours of AP20187 administration. By 48 hours, fluorescence microscopy showed a significant reduction in Venus-positive SPCs. On day 4, immunostaining confirmed successful elimination of mouse SPCs. This elimination caused hyperproliferation of NPCs at the ureteric bud tips, consistent with previous reports of NPC dysregulation after SPC removal. We developed a novel mouse model using the iC9 system for selective elimination of kidney progenitor cells, compatible with human applications. We plan to create double-knock-in mice that eliminate both NPCs and SPCs. This will allow transplantation of human iPSC-

derived NPCs and SPCs, potentially enabling the generation of advanced three-dimensional human nephron structures within mouse fetal kidneys. This represents a major step toward organ regeneration using human stem cells.

F1120

GENERATION OF FUNCTIONAL HEPATOCYTES WITH HUMAN EMBRYONIC STEM CELLS FOR REGENERATIVE MEDICINE

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For decades, the treatment of end-stage liver diseases has been hampered with donor liver shortage. Stem cell therapy can potentially overcome the limitation by supplying functional hepatocytes for cell therapy and tissue engineering. For example, the transplantation of human embryonic pluripotent stem cells (hESCs)-differentiated functional hepatocyte-like cells (HLCs) can contribute to liver regeneration by restoring damaged liver tissues. This can alleviate donor shortage burdens for liver transplantation treatments. However, many of the protocols available currently tends to be time and cost-consuming. Besides, these differentiated cells still possess an immature and heterogenous phenotype compared to primary human hepatocytes with low cell yields. In this study, we have differentiated hESCs in 2D monolayers, 3D spheroids, and microspheres, into HLCs utilizing human hepatic growth factors and Oncostatin M molecules. These cells were evaluated for their differentiation and maturation efficiency. Our present data demonstrated that, with our established protocol, these ESCs have been differentiated into HLCs with progressively maturing characteristics. An ESC-hepatocyte differentiation platform has then been established as a foundation for future explorations.

F1122

GENERATION OF HUMAN VITREOUS FROM PLURIPOTENT STEM CELLS

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As the largest intraocular tissue, the vitreous occupies approximately 80% volume of the human eyeball and providing the eye with its spherical shape, a transparent light pathway as well as the homeostasis of intraocular humor. Reconstruction of the vitreous in vitro remains an unmet biomedical challenge. Here, through a stepwise differentiation from human pluripotent stem cells (hPSCs), we generated vitreous-like organoids (VOs) with vitreoretinal interface, displaying remarkable sameness to human vitreous in terms of transparency, molecular composition, biological profiles and transplantability. Using VOs as a developmental model, we revealed that fibroblasts, rather than vasculature, are essential for human vitreous

development, thereby settling the longstanding debate in vitreous formation. Additionally, VOs with a transthyretin (TTR) mutation recapitulated the vitreous phenotypes of human patients with vitreous amyloidosis. Together, VOs open avenues to future studies of human vitreous development, disease modeling and regenerative therapy for a broad spectrum of human vitreoretinal diseases.

Funding Source: This project was supported by fundings from the National Natural Science Foundation of China (82125007,92368206), Beijing Natural Science Foundation (5242005, Z200014).

F1124

GENERATION OF MATURE EPICARDIUM DERIVED FROM HUMAN-INDUCED PLURIPOTENT STEM CELLS VIA INHIBITION OF MTOR SIGNALING

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Reactivating the human epicardium post-cardiac injury holds promise for cardiac tissue regeneration. Despite successful differentiation protocols yielding pure, self-renewing epicardial cells from induced pluripotent stem cells (iPSCs), these cells maintain an embryonic, proliferative state, impeding adult epicardial reactivation investigation. We introduce an optimized method that employs mammalian target of rapamycin (mTOR) signaling inhibition in embryonic epicardium, inducing a quiescent state that enhances multi-step epicardial maturation. This yields functionally mature epicardium, valuable for modeling adult epicardial reactivation. Furthermore, we assess cardiac organoids with cardiomyocytes and mature epicardium, probing molecular mechanisms governing epicardial quiescence during cardiac maturation. Our results highlight iPSC-derived mature epicardium's potential in investigating adult epicardial reactivation, pivotal for effective cardiac regeneration. Additionally, the cardiac organoid model offers insight into intricate cardiomyocyte-epicardium interactions in cardiac development and regeneration.

F1126

GENERATION OF VASCULARIZED RETINAL ORGANOIDS FROM HUMAN PLURIPOTENT STEM CELLS

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Human retinal organoids (ROs), derived from human pluripotent stem cells (hPSCs), have been widely applied in disease mechanism researching, drug screening, and cell therapy. However, current ROs exhibit slow photoreceptor maturation and lack a vascular system, preventing them from accurately recapitulating retinal development and limiting their utility in studying retinal vascular diseases and drug screening. Here, we present a novel approach for generating human vascularized retinal organoids (vROs) that exhibit a typical ordered, stratified retinal architecture, comprising multi-layered neurons, and incorporate a functional blood vessel network that remains viable for over 6 months, establishing a robust model for vascularized retinal tissue. We found that, the inclusion of pericytes in vROs facilitated the development of a more elaborate and longer-lasting vascular network, enhancing endothelial cell survival and network stability. Moreover, timely addition of extracellular matrix (ECM) was critical for the proper integration of the vascular network with ROs and ensured the coordinated development of retinal neurons across different layers, with photoreceptor cells being able to extend typical outer segments. Notably, pericytes in vROs surrounded endothelial cells, forming lumen-like structures, and collagen IV deposition was observed around the vascular network. Importantly, single-cell RNA sequencing and immunostaining analyses indicated that vascularization accelerated the maturation of bipolar cells and photoreceptors, which was further supported by electrophysiological assessments. CellChat analysis suggested that the vascular network may promote photoreceptor and bipolar maturation through the IGF signaling pathway. Taken together, we have successfully developed vROs that exhibit typical multi-layered neuronal architecture and earlier photoreceptor maturation, providing a more accurate model for studying the pathogenesis of retinal vascular diseases and advancing drug development.

Funding Source: The National Natural Science Foundation of China (82125007, 81970838, and 82201225), Beijing Municipal Public Welfare Development and Reform Pilot Project for Medical Research Institutes (PWD and RPP-M RI, JYY2023-6).

F1128

HEPATOCYTE GROWTH FACTOR AND EPIDERMAL GROWTH FACTOR DELIVERED VIA MRNA IN LIPID NANOPARTICLES IMPROVE ENGRAFTMENT OF HUMAN PRIMARY AND IPSC-DERIVED HEPATOCYTES IN MICE

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Thousands of patients die each year awaiting liver transplantation due to end-stage liver failure. To address the scarcity of donors, transplantation of primary human hepatocyte (PHH) or induced pluripotent stem cell (iPSC) derived hepatocyte-like cells (iHeps) is explored as alternative therapies to restore liver function. However, cell therapies are constrained by limiting factors such as low efficiency of engraftment and rejection. Our lab addresses these issues by the establishment of a clinically relevant injury mouse model and stimulation of key liver

regeneration pathways in transplanted cells. Our mouse model is the NSG-PiZ mouse which recapitulates human alpha-1 antitrypsin deficiency (AATD)-associated liver disease, a genetic disease that can lead to liver failure. We precondition NSG-PiZ mice with AAV8-TBG-p21 to express the cell-cycle inhibitor p21 under the hepatocyte specific thyroxine binding globulin (TBG) promoter to recapitulate p21-induced hepatocyte senescence observed in virtually all AATD patients. Survival and proliferation of transplanted cells are significantly promoted by the delivery of hepatocyte mitogens such as human hepatocyte growth factor (HGF) and epidermal growth factor (EGF) using safe and non-integrative nucleoside-modified mRNA encapsulated in lipid nanoparticles (mRNA-LNP). We demonstrate that the combination of HGF and EGF mRNA-LNP leads to 30% repopulation of the NSG-PiZ/p21 mouse livers by PHH and significantly reduces AATD liver disease burden. iHep cell therapy is an attractive alternative to PHH therapy as it eliminates the need for a donor and generation of iHep from human iPSC has the advantage to provide an unlimited supply of patient-specific hepatocytes. Therefore, we established an efficient iHep differentiation protocol which results in a near 100% of FOXA2+ HNF4a+ hepatic endoderm derivatives with up to 94% expressing AFP by day 10. Transplantations of luciferase-expressing iHeps show sustained engraftment in HGF+EGF mRNA-LNP-treated NSG-PiZ/p21 over 4 weeks as assessed with bioluminescence compared to that in control mice injected with control mRNA-LNP and AAV-TBG-Null. These findings improve our knowledge of the mechanisms that underlie liver repair and advance the search for donor-free cell therapies for liver failure.

Funding Source: R01DK124361 and Alpha one foundation research award to Valerie Gouon-Evans, and F31DK135378 to Anna Smith.

F1130

HUMAN STEM CELL-DERIVED EXOSOME-EMBEDDED HYDROGEL REJUVENATES JOINT MICROENVIRONMENT IN OSTEOARTHRITIS

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Osteoarthritis (OA) is characterized by progressive cartilage degradation and a disrupted joint microenvironment, necessitating innovative therapeutic strategies. While human stem cell-derived exosomes offer promising regenerative potential as disease-modifying osteoarthritis drugs (DMOADs), their clinical application is hindered by rapid clearance and insufficient retention within the joint. To address these limitations, we developed an exosome-embedded adhesive injectable hydrogel (Exo-aiGel), designed to enhance exosome localization and therapeutic efficacy. This biomimetic hydrogel, formulated using natural biocompatible components, was administered intra-articularly and demonstrated superior outcomes, including the reversal of OA pathology, improved cartilage regeneration, and enhanced recruitment of joint-resident MSCs. Compared with hyaluronic acid or exosome-HA solutions, Exo-aiGel significantly amplified therapeutic effects by restoring the joint microenvironment and supporting sustained tissue repair. These findings highlight the transformative potential of Exo-aiGel as a durable and clinically applicable treatment, offering a novel approach to rejuvenating cartilage and mitigating OA progression.

Funding Source: This work was supported by the National Science and Technology Council, Taiwan (Reference No. 111-2320-B-039-070-MY3) and China Medical University Hospital

(EXO-113-004, DMR-113-106, DMR-114-092) to C.W. Lee.

F1134

IMPACT OF SARS-COV-2 INFECTION ON NEUROGENESIS AND NEURODEGENERATION

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Since the emergence of COVID-19 in 2019, many patients have reported long-term neurological symptoms, known as Long-COVID syndrome. Evidence suggests that SARS-CoV-

2 can infect the human brain, potentially contributing to these symptoms. In addition, COVID-19 patients have been linked to a threefold increased risk of Alzheimer's disease (AD) and a twofold increased risk of Parkinson's disease (PD). However, the mechanisms by which the virus reaches the brain remain a critical area of ongoing investigation. In contrast to adult neurons, it has been shown that neuronal progenitor cells (NPCs) are permissive to SARS-CoV-2 infection in vitro, which might have detrimental consequences even years after initial infection. Given that NPCs are the primary regenerative cells after central nervous system (CNS) injury, we hypothesize that SARS-CoV-2 infection might disrupt neurogenesis and lead to neurological anomalies which pave the way to neurodegeneration. Using 2D and 3D human cell cultures (organoids) and cryo-electron microscopy (cryo-EM) strategies, we will study SARS-CoV-2 neuroinvasion and its role in neurodegenerative diseases. Interestingly, preliminary data showed that SARS-CoV-2 transfer from primary human nasal cells to human neuroprogenitors through Tunneling Nanotubes (TNTs), actin-membrane-based structures that connect distant cells. TNT-like structures positive for the SARS-CoV-2 N antigen were also observed, supporting the possibility of a cell-to-cell transmission route to neural cells. This study provides critical insights into the neuropathogenesis of SARS-CoV-2 and its potential contribution to long-term neurological symptoms.

Funding Source: Pasteur Paris University (PPU) International Doctoral Program.

F1138

IN VIVO PERTURB-SEQ SYSTEMATICALLY IDENTIFIES BARRIERS TO HEART REGENERATION AND REPAIR VIA DIRECT REPROGRAMMING

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Direct induction of cardiomyocytes (iCMs) from cardiac fibroblasts after myocardial infarction is a promising strategy for heart repair and regeneration. However, compared to in vitro settings, in vivo transdifferentiation remains significantly less efficient, with key barriers—particularly microenvironmental cues—remaining incompletely understood. Here, we present an in vivo Perturb-seq system to systematically identify potential barriers to cardiomyocyte induction. Using 143 hub-centered genes from the in vivo cardiomyocyte induction gene regulatory network as candidates, we investigated their roles in cardiac reprogramming. Cardiac myofibroblasts were transduced with shRNAs targeting these candidate genes, along with reprogramming factors, and transplanted into the infarcted heart to evaluate their effects on in vivo reprogramming. Our analysis revealed seven transcriptionally distinct fibroblast subpopulations, including Thbs4+ fibroblasts predisposed to fibrosis and Tnnt2+ iCMs indicative of successful transdifferentiation. Interestingly, we observed a population of Sca1+ quiescent fibroblasts emerging during late-stage reprogramming, which suggests that many fibroblasts evade excessive activation but fail to fully transition into cardiomyocytes. The fidelity of our Perturb-seq approach was confirmed by identifying known reprogramming barriers, such as IFNAR2. Among newly identified candidates, Calreticulin (CALR) emerged as a critical barrier. CALR knockdown significantly enhanced fibroblast-to-cardiomyocyte transdifferentiation both in vivo and in vitro. Mechanistically, CALR knockdown elevated intracellular free calcium levels, which activated MEF2C, a key transcription factor for cardiomyocyte development. Moreover, treatment with the L-type Ca2+ channel agonist FPL64176 further activated MEF2C and promoted iCM generation, highlighting the role of calcium signaling in overcoming reprogramming barriers. Collectively, our study reveals the pivotal role of CALR and calcium signaling in in vivo cardiac reprogramming and provides a systematic framework for identifying barriers to heart repair and regeneration via direct reprogramming approaches.

F1140

INVESTIGATING THE ROLE OF MITOPHAGY ON THE MATURATION OF PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTE

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Cardiac regenerative medicine has provided promising solutions to cardiac diseases as researchers can now generate cardiomyocytes (CMs) from pluripotent stem cells (PSCs). Nevertheless, the immature state of PSC-derived CMs (PSC-CMs) has become the major difficulty of their utilization. Multifaceted methods, including hormonal induction and tissue engineering, have been used to enhance PSC-CMs' maturity, but a comprehensive maturation status has not yet been achieved. The perinatal phase has been recognized as a crucial time point for the in vivo maturation of mammalian CMs. Shortly after birth, CMs undergo a short period of starvation which leads to autophagy and mitophagy, ultimately resulting in structural and metabolic alterations. Mitophagy is the selective autophagy of mitochondria; it is a critical process in mediating metabolic transition and is pivotal for the maturation of CMs. Mitophagy in mammalian cells can be divided into two major pathways. One is receptor-mediated mitophagy, the other one is PINK1-Parkin-mediated mitophagy. Notably, Parkin has been shown to be associated with CMs' maturation. In the current study, starvation and bafilomycin A1 (Baf A1) were used to increase and inhibit mitophagy, respectively, in PSC-CMs. Interestingly, increase in mitophagy in CMs demonstrated an increased in maturity as reflected by multiple parameters, including an increase in calcium transient kinetics, cell size and sarcomere length. On the other hand, Baf A1-treated CMs exhibited a decreased in maturity in various aspects. The preliminary data revealed that mitophagy positively regulates PSC-CMs maturation, however, the underlying pathway of how mitophagy regulates PSC-CMs maturity remains elusive. Further study would be conducted to elucidate this. By understanding how mitophagy contributes to the maturation of CMs, this study aims to delineate an understudied molecular pathway that holds great promise for enhancing PSC-CM maturity.

F1142

IPSC-DERIVED ALPHA6 INTEGRIN-POSITIVE CELLS RESTORE CONVENTIONAL OUTFLOW IN GLAUCOMA EYES

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Decreased trabecular meshwork (TM) cellularity is a critical pathogenic cause for primary open-angle glaucoma (POAG), yet therapies to regenerate the decellularized TM are very limited. Induced pluripotent stem cell-derived TM cells (iPSC-TM) can efficiently restore aqueous humor outflow and maintain intraocular pressure homeostasis. Here, we conducted a multi-modal RNA sequencing analysis to characterize the molecular mechanisms underlying TM regeneration. Our clustering analysis based on single-cell RNA sequencing identified a group of iPSC-derived alpha6 integrin-positive (iPSC-ITGA6+) cells with a distinct transcription pattern that was not observed among primary TM (pTM) cells. These iPSC-ITGA6+ cells not only stimulated cell proliferation of pTM much more efficiently than other iPSC-TM subtypes but are also capable of

restoring aqueous humor outflow in glaucoma. Furthermore, iPSC-ITGA6+ cells repopulated the TM and reversed damage to the inner wall of Schlemm's canal. The repair process in these cells primarily relied on the abundance of paraspeckles within iPSC-ITGA6+ cells, which triggered changes in cell cycle dynamics and cell fate of pTM, as elucidated by bulk RNA sequencing. Meanwhile, enhancing paraspeckle assembly by menRNA transfection triggered pTM proliferation and rejuvenation, becoming an alternative approach for TM regeneration. This study advances our understanding of the mechanism of cell therapy and facilitates the development of new treatments for POAG.

Funding Source: This study was supported by the National Key Research and Development Program (2022YEF0132500) and Taishan Scholar Youth Expert Program (tsqn202103055).

F1144

IVABRADINE-DRIVEN INSIGHTS INTO ENGINEERING SINOATRIAL NODE MICROENVIRONMENTS

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This study characterizes the differentiated sinoatrial node (SAN) microenvironment using induced pluripotent stem cells (iPSCs) enhanced by adenoviral vector (AdV)-mediated transcription factor (TF) delivery. By mimicking the SAN's complex cellular and molecular landscape, we aim to provide insights into its function and structure. Using AdV to overexpress key TFs - Tbx18, Shox2 and Isl1 - we directed iPSCs to differentiate into SAN-like cells. Proteomics and electrophysiology studies, together with publicly available single-cell RNA sequencing were used to assess and compare extracellular matrix components, ion channels and TFs critical for SAN development. Differentiated SAN cells exhibited distinct electrophysiological properties, including shorter action potential durations and higher contraction frequencies than ventricular cardiomyocytes. Immunocytochemical staining confirmed the presence of SAN-specific subpopulations. Additionally, fibroblasts and extracellular matrix proteins such as collagen III and IV supported the structural and functional integrity of the SAN microenvironment. The pacemaker-like properties of SAN-derived cells were validated by assessing the effect of ivabradine (IVA) on cycle length. SAN cells displayed

intrinsic pacemaker activity with shorter cycle lengths than ventricular myocytes (VMs). IVA treatment significantly prolonged the cycle length of SAN cells, confirming If current inhibition, while minimally affecting VMs. The complete protocol showed robust pacemaker activity, while intermediate responses in the SAN-Tbx18 group highlighted subpopulation diversity and potential immaturity. This study highlights the critical role of the SAN microenvironment in maintaining pacemaker activity and establishes AdV-mediated TF expression as an efficient approach for generating functionally distinct SAN cells. These findings advance regenerative medicine strategies targeting cardiac dysfunction and provide a powerful platform for exploring cardiac biology.

F1146

LABEL-FREE MONITORING OF HUMAN INTESTINAL ORGANOIDS USING HOLOTOMOGRAPHY AND VIRTUAL STAINING

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Human intestinal organoids (HIOs) are invaluable in regenerative medicine and disease modeling due to their ability to mimic the architecture and function of the human intestine. The use of HIOs is growing rapidly, driven by advancements in stem cell-derived organoid technologies. A key challenge in studying and utilizing HIOs is the non-invasive monitoring of their development and cellular status. Most existing imaging techniques rely on genetic or molecular labeling, which can disrupt organoid integrity and require extended sample preparation times. In this study, we demonstrate label-free monitoring of three-dimensional (3D) subcellular structures in HIOs using holotomography (HT) enhanced by artificial intelligence (AI). HT is a label-free imaging method for measuring the refractive index (RI) of cells and has recently proven effective for imaging organoids. By combining HT with AI, we fully exploit HT's potential to profile HIOs at the subcellular level. This integration overcomes the molecular ambiguity of RI; the AI translates HT images of HIOs into fluorescence-equivalent images of subcellular structures, including nuclei and cell membranes, typically achieved through labeling. Our results show that AI-assisted HT enables virtual staining of organoids without any genetic or molecular intervention, providing a safe and efficient way to monitor the temporal dynamics of HIOs in applications such as disease modeling and drug screening.

Funding Source: This work was supported by grants from NRF Korea (RS-2024-00442348, 2015R1A3A2066550, 2022M3H4A1A02074314), IITP (2021-0-00272), MOTIE/KIAT (P0028463), and KFRM (21A0101L1-12).

F1148

LIMB DIGIT ORGANOIDS AND MATHEMATICAL MODEL REVEAL A NOVEL PRINCIPLE OF THE EMERGENCE OF TISSUE MORPHOLOGIES

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Tissue morphogenesis is one of the emergent phenomena, which is referred to as a property that cannot explained merely as the sum of its components, such as functions of genes and cellular behaviors. Understanding the mechanisms of morphological emergence is essential for accurately predicting or designing tissue shapes in regenerative medicine and tissue engineering. In physics, emergence is defined as a qualitative property that can only occur in the limit that the number of microscopic constituents tends to infinity. Applying this principle to biology requires mathematical descriptions of genetic and cellular behaviors. This supports the need for multicellular in vitro models such as 3D organoids and micropatterning, as they allow the extraction of tissue morphogenesis in a controlled environment. Indeed, the multicellular in vitro models have advanced our understanding of epithelial self-organization, such as eyes and gut. However, mesenchymal tissues like limbs and tails remain poorly understood due to their complex 3D cellular dynamics. To address this, we developed a novel limb organoid system. When we cultured limb mesenchymal cells derived from mouse embryos with Fgf and Wnt, these organoids exhibited symmetry breaking and self-organized digit-like structures, even without directional cues. Agent-based modeling revealed that cell sorting, self-organized morphogen gradient, and cell polarity drive these processes. By applying continuum limits to the equations describing the cellular behaviors in the agent-based model, we derived partial differential equations, showing a type of mathematical instabilities as a potential mechanism explaining the emergence of digit tissue morphologies. Capitalizing on these insights, our objective extends to reconstructing limb morphogenesis using limb mesenchyme derived from human ES cells. Our approach highlights the potential of organoid and mathematical models, paving the way for precision tissue engineering and functional limb regeneration strategies.

Funding Source: Funded by JSPS and ASHBi fusion grant.

F1150

MATURATION DELAY IN HUMAN IPS-DERIVED CARDIOMYOCYTES DEPENDS ON PRDM16'S CONTROL OVER PROLIFERATIVE GATEWAYS

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Human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) hold significant potential for clinical applications, including disease modeling, regenerative therapy, and drug screening. However, they lag behind in acquiring the structural, physiological, and metabolic phenotypes characteristic of functional maturity. This immaturity presents a major obstacle to their full utility in clinical settings. Whereas adult cardiomyocytes are generally postmitotically arrested in the G0 (quiescent) phase, hiPSC-CMs retain residual proliferative activity. Chemical inhibition of mTOR signaling is known to enhance hiPSC-CM maturity through p53-induced

quiescence. However, p53 is not the sole factor mediating complete cell cycle withdrawal in cardiac cells, as proliferative signaling, such as phosphorylated AKT, remains active. Among the genes implicated in promoting cardiac quiescence postnatally, we have identified PRDM16 as a key regulator of both proliferation and maturation. Increased PRDM16 expression is inversely correlated with proliferative states, suggesting that elevated PRDM16 levels promote maturation while restricting proliferation. In contrast, proliferative hiPSC-CMs exhibit low PRDM16 expression and reduced maturity, whereas decreased PRDM16 levels enhance cardiac proliferation and allow cells to bypass G0 entry. Ectopic downregulation of PRDM16 in hiPSC-CMs impedes maturation under both basal and mTOR-inhibited conditions. Conversely, preliminary data indicate that ectopic overexpression of PRDM16 enhances transcriptomic maturation. These findings highlight PRDM16's dual role in balancing proliferation and maturation in cardiomyocytes, providing insights into cardiac developmental biology and potential avenues for regenerative therapies. Further exploration of PRDM16 modulation could lead to novel strategies for enhancing cardiac repair and function in disease contexts.

F1152

METTL3-MEDIATED M6A MODIFICATION PROMOTES AXON DEVELOPMENT OF RETINAL GANGLION CELLS

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Retinal ganglion cells (RGCs) are the key neurons responsible for transmitting visual information to the brain, and their degeneration in retinal diseases such as glaucoma leads to severe vision loss. N6-methyladenosine (m6A) modification plays a crucial role in gene expression and cellular functions in eukaryotes, including nervous system development. However, the specific functions of m6A modification in RGC remain unclear. Our previous research has demonstrated that the transcription factors Ascl1, Brn3b, and Isl1 can efficiently induce fibroblasts into RGC-like neurons (iRGCs), providing a robust experimental platform for studying RGC development and physiology. Using this iRGC model, we investigated the functions of Mettl3, the key catalytic component of the m6A-writer complex, in RGC. Our results showed that METTL3 knockdown significantly impaired axon growth and reduced iRGC induction efficiency, while METTL3 overexpression significantly promoted iRGC axon growth. RNA sequencing and m6A sequencing revealed downstream target genes regulated by METTL3 and its mediated m6A modification, which play complex roles in RGC axon growth. Thus our study demonstrated that METTL3-mediated m6A modification positively regulates axonogenesis and neurogenesis during RGC reprogramming. Our findings deepen our understanding of the molecular networks governing RGC axon development and regeneration and identify new targets for potential therapies for RGC degenerative diseases.

Funding Source: The National Natural Science Foundation of China (32470848).

F1154

MITOCHONDRIA TRANSFER FROM DENTAL PULP STEM CELLS RESCUES APOPTOTIC ENDOTHELIAL CELLS UNDER HYPOXIA

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The objective of this study is to elucidate the presence and directionality of mitochondria transfer between dental pulp stem cells (DPSCs) and human umbilical vein endothelial cells (HUVECs) under normoxic and hypoxic conditions and test whether they are transferred through intercellular tunneling nanotubes (TNTs), as well as its roles in HUVECs survival and apoptosis. Mitochondria were labeled by MitoTracker within either DPSCs or HUVECs, then cocultured under normoxia or hypoxia (1% oxygen) for 12 or 24 hours. Mitochondria transferred to recipient cells were observed using laser scanning confocal microscopy (LSCM), and its transfer rate was quantified through flow cytometry. Intercellular TNT structures were visualized via F-actin immunofluorescent staining. After TNT formation was disrupted via Boyden chamber indirect coculture assay or the F-actin polymerization inhibitor Cytochalasin B, the mitochondria transfer rate was quantified through flow cytometry. Rotenone and Antimycin A (ROT/AA) were used to inhibit mitochondrial respiration of DPSCs before cocultured with HUVECs; its effects on HUVEC proliferation and apoptosis were assessed through Ki67 and AnnexinV/7-AAD staining. Besides, MitoSOX and MitoProbe TMRM staining was performed to detect mitochondrial superoxide levels and mitochondrial membrane potential. Our results found a significantly higher mitochondria transfer rate from DPSCs to HUVECs ($p< 0.05$), with a significantly higher transfer rate under hypoxia ($p< 0.05$). Mitochondria transferring via TNTs was successfully observed, and Boyden chamber indirect coculture and Cytochalasin B treatment significantly decreased mitochondria transfer rate from DPSCs to HUVECs ($p < 0.05$). Compared with the control group, ROT/AA treatment of DPSCs has no significant effects on Ki67, MitoSOX, and MitoProbe TMRM expression in HUVECs. However, it significantly decreased the apoptosis rate of HUVECs ($p < 0.05$) under hypoxia. In conclusion, mitochondria preferentially transfer from DPSCs to ECs, with an increased transfer rate under hypoxia. Mitochondria transfer from DPSCs to ECs was partially mediated via TNTs, and it rescued apoptotic ECs under hypoxia, indicating a critical role of mitochondria transfer in DPSC-EC interactions.

Funding Source: This research was supported by the Research Grants Council, Hong Kong (General Research Fund; 17119524).

F1156

MODULATION OF MYC-DRIVEN CELL COMPETITION ENHANCES INTERSPECIES CHIMERISM

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Blastocyst complementation is a promising strategy to generate transplantable organs. However, the low efficiency of the complementation process and the limited ability of human

cells to generate interspecies chimeras significantly constrain the potential of this technology. In this study, we tested the effect of modulating cell competition mechanisms through c-Myc levels, first in a mouse-rat interspecies model and then in human-mouse settings. As a starting point we have microinjected rat embryonic stem cells (ESCs) in mouse host embryos expressing Myc at varying levels, according to their genotype. We found that reduction of Myc expression in the host increases both the frequency of chimera generation and the contribution of rat cells to the chimera embryo at E9.5, in a dose-dependent manner. Applying this approach to an interspecies heart complementation model, it boosted the efficiency of heart complementation by rat cells three folds, by embryonic day E10.5. Finally, we have extended this approximation to human-mouse chimera generation. Reducing Myc levels in host mouse embryos or artificially enhancing them in human naïve cells significantly improves the colonization of mouse embryos by human iPSCs 72 hours post-injection in vitro. However, following embryo transfer into pseudopregnant mouse recipients, human cell contribution is minimal as analyzed at E6.5, highlighting the need for innovative strategies to enhance human cell survival and integration in xenogeneic embryos

Funding Source: This work was supported by PID2021-122589OB-I00 funded by MCIN/AEI/10.13039/501100011033 and FEDER a way to make Europe, UE; Gob. Navarra Bioheart 0011-1411-2022-000092; “La Caixa” Foundation LCF/PR/HR24/00152.

F1158

MULTIFACETED ACTIVITIES OF HUMAN PLURIPOTENT STEM CELL-DERIVED ECTOMESENCHYMAL STROMAL CELLS FOR THE TREATMENT OF HYPOXIC-ISCHEMIC ENCEPHALOPATHY

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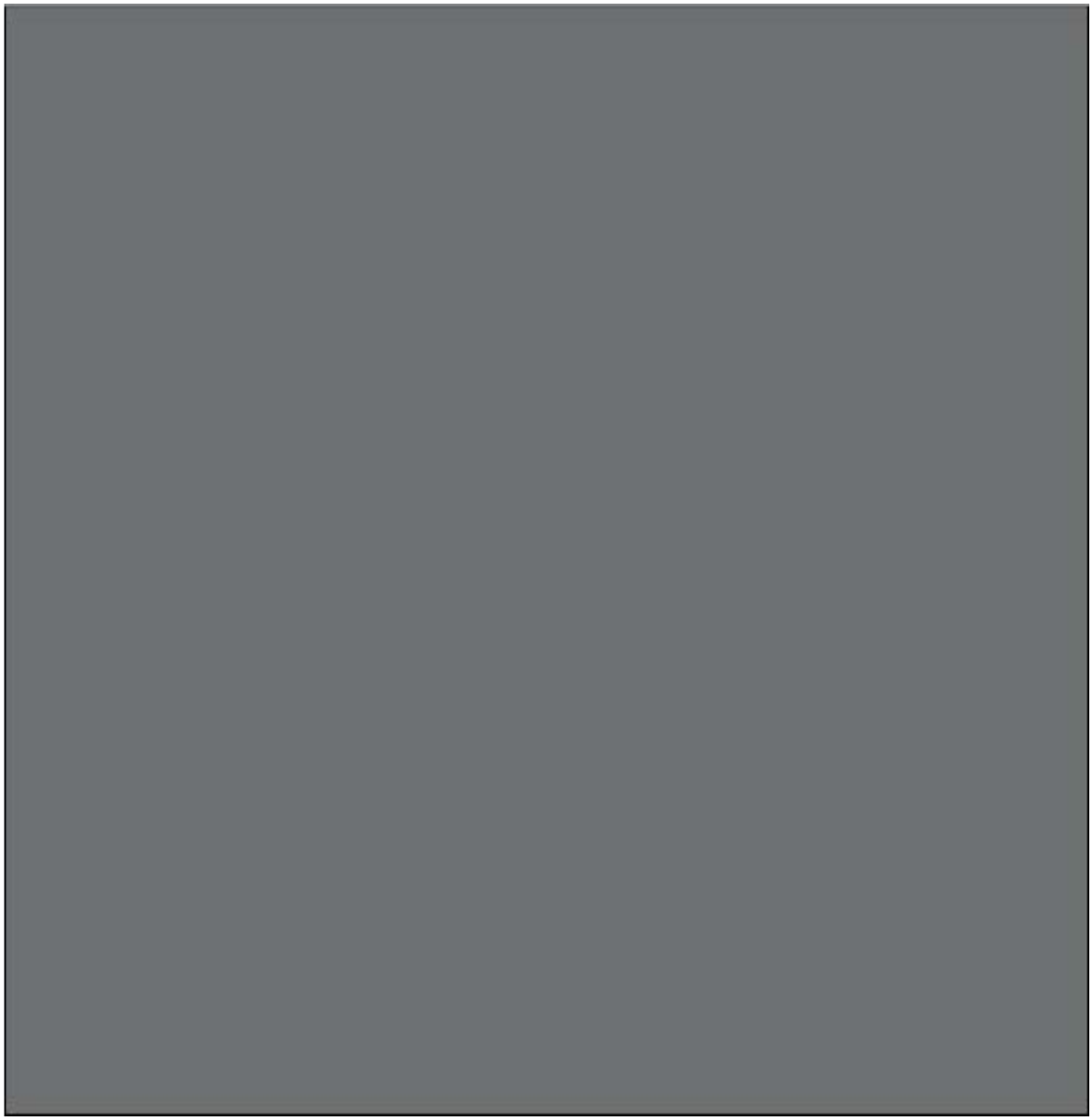
Hypoxic-ischemic encephalopathy (HIE), affecting 0.1–0.8% of newborns, can lead to severe outcomes such as cerebral palsy and neurobehavioral impairments. The primary pathological changes in HIE involve gray and white matter injuries characterized by axon degeneration and a deficiency of mature oligodendrocytes (OLs). Although neural progenitor cells (NPCs) and oligodendrocyte precursor cells (OPCs) increase in response to injury, their differentiation toward a mature state is often hindered in HIE, highlighting the necessity for treatments that promote endogenous repair through neurogenesis and oligodendrogenesis. Mesenchymal stromal cell (MSC) therapy is emerging as a promising treatment for HIE. However, clinical application is limited by cell heterogeneity, low isolation efficiency, and unpredictable effectiveness. This study investigates the therapeutic effects and underlying mechanisms of human pluripotent stem cell-derived ectomesenchymal stromal cells (hPSC-EMSCs) in a rat model of HIE. Our findings demonstrate that hPSC-EMSCs provides greater neuroprotective potential and better functional recovery compared to hUC-MSCs in rats following hypoxia-ischaemia (HI). Intranasal delivery of the secretome derived from hPSC-EMSCs significantly reduced brain lesion size, mitigated inflammatory responses, promoted endogenous neurogenesis, improved remyelination， and improved functional recovery in rats with HIE. Mechanistically, the secretome derived from hPSC-EMSCs was found to potentiate NGF-

induced neurite outgrowth and the neuronal differentiation of NPCs via the ERK/CREB pathway, while also promoting OPC differentiation mediated by the Periostin/Integrin-β3 axis. In summary, hPSC-EMSCs not only repairs gray matter injury but also addresses white matter injury, offering promising avenues for new treatment strategies aimed at restoring neuronal and myelin function in patients with HIE and other neurological diseases.



F1162

OLIGODENDROCYTE DERIVED R-SPONDIN3(RSPO3): KEY ROLE IN WHITE MATTER DEVELOPMENT AND INJURY REPAIR THROUGH OLIGODENDROCYTE PROGENITOR CELL DIFFERENTIATION

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White matter injury (WMI) and repair frequently happens during central nervous system degeneration and regeneration processes. Current therapeutic interventions for WMI are very limited. Demyelination is a pathological process characterized by myelin loss and oligodendrocyte damage. While remyelination involves the proliferation, migration, and differentiation of oligodendrocyte progenitor cells (OPCs) into mature oligodendrocytes, this process is often insufficient to restore axonal function or improve neurological deficits in humans following WMI. Thus, novel, effective and targeted therapies aiming to promote endogenous oligodendrogenesis and remyelination are urgently needed. The R-Spondin (Rspo) cytokine family plays important roles in development and acts as powerful stem cell regulators; however, their roles in brain development and neurological diseases remain largely unknown. We observed that Rspo3 expression in brain white matter tracts increased during postnatal development and correlated spatially and temporally with OL lineage markers. Loss of Rspo3 in Cspg4 positive cells both in-vivo and in-vitro, resulted in hypomyelination and remyelination failure due to OL production. In-vitro, Rspo3 promoted oligodendrogenesis via enhancing differentiation. Both focal and systemic administration of recombinant RSPO3 significantly increased OL maturation and remyelination, and improved motor function in injured animals following lysolecithin (LPC) and hypoxia-ischemic brain damage. Together, these data indicate that Rspo3 acts as a positive regulator of OL development and remyelination, highlighting its potential as a therapeutic strategy for promoting remyelination to combat WMI.

Funding Source: GRF/UGC, 14116622, Hong Kong Basic research funding of Guangdong Province, 2024A1515012929, China.

F1164

PENUMBRAL RESCUE FOLLOWING PERINATAL HYPOXIC-ISCHEMIC BRAIN INJURY: CHARACTERIZING "FETAL REVERSION"

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Perinatal hypoxic-ischemic injury (HII) is the most common cause of neurologic disability in children. HII initiates a molecular cascade that results in widespread neurodegeneration,

characterized not only by neuron death, but also impaired glial function, neuroinflammation, neural network disruption, vascular reorganization, and glial scarring. The penumbra of the infarct is characterized by injured but salvageable cells. If the penumbra is rescued, inter-neuronal communication not only within the penumbra but from one brain region to the other as fibres de passage are preserved; if not, they are axotomized. We've published that the penumbra (though not the necrotic core, where the cells are already dead) can be rescued by transplanted human neural stem cells (hNSCs) that migrate to the penumbra, promoting histological, MRI, and functional improvements. Though great progress has been made in using single cell 'omics to characterize normal mammalian brain development, little, if any, work has been published using the same techniques to characterize the injured developing brain spatially at a single-cell level. We are comparing the brains of intact rat pups with age-matched pups that have been subjected to HII and have subsequently received: no treatment (HII-only), hNSC transplantation, or hNSC-conditioned media. The infarcts of these rats are being characterized using single-nuclei RNA-sequencing (snRNAseq) as well as spatial transcriptomics and proteomics. We are also characterizing the axons within and passing through the penumbra, identifying molecular mechanisms by which neural networks are preserved. Data-to-date suggest that trans-penumbral neural connections may be preserved by promoting endogenous astrocytes to shift their fate from reactive to trophic (reminiscent of their fetal cerebrogenic phenotype). Our snRNAseq data suggest – unexpectedly – that astrocytes, not solely neurons, are impacted by HII. Both snRNAseq and spatial analysis are being used to build a novel library of HII-associated molecules – both with and without rescue from hNSCs. We have begun identifying the cytoarchitectural topography and 'omics profile that hNSCs induce to promote trophic astrocyte resurgence and inhibit reactive astrogliosis and microgliosis, consequently promoting neural network integrity in an injured brain.

F1166

PRUSSIAN BLUE NANOZYME IMPREGNATED MESENCHYMAL STEM CELLS FOR HEPATIC ISCHEMIA-REPERFUSION INJURY TREATMENT

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One of the stem cell therapies using mesenchymal stem cells (MSCs) has a great possibility for treating innumerable diseases because of their capacity to heal injured tissue/organ through the production of paracrine factors and immunomodulation. Even with high expectations, the elevated oxidative stress levels and low viability of transplanted cells in the injured tissue remain the biggest obstacles in the MSC-based cell therapy. To achieve the desired treatment efficiency, transplanted MSCs must be guaranteed to survive in a high oxidative stress environment. In this research, ROS-scavenging nanozyme is suggested to protect transplanted MSCs from the self-destruction of oxidative stress-mediated cells and improve the treatment effect. Prussian blue (PB) nanoparticles were impregnated as biocompatible ROS-scavenging nanozymes and incorporated into MSCs by endocytosis, which did not affect MSCs' main characteristics and differentiation potential. Nanozyme impregnation not only greatly improved MSCs survival under high oxidative stress conditions but also strengthened paracrine effects, anti-inflammatory properties and presented deep therapeutic effects in hepatic ischemia-

reperfusion damaged animal model. The results indicated that impregnating PB nanozymes into MSCs is a simple but efficient way to improve the potential of MSC-based cell therapy. Also, it could be a promising candidate for diseases associated with high oxidative stress and inflammation that need ROS-scavenging treatments.

F1168

RBFOX2-DEPENDENT ALTERNATIVE SPLICING OF NUMB REGULATES NOTCH SIGNALING DURING MUSCLE STEM CELL ACTIVATION

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Satellite cells (SCs), or muscle stem cells (MuSCs), are the resident somatic stem cells responsible for skeletal muscle regeneration. Most SCs remain quiescent in resting adult muscles but can activate rapidly upon receiving a stimulus. Whereas post-transcriptional regulation by microRNAs or RNA-binding proteins has been reported to regulate SC quiescence and activation, the role of alternative splicing during SC activation remains elusive. Using an in vivo fixation approach to preserve QSCs in situ, we revealed rapid and extensive splicing changes upon SC activation. Genes regulated by alternative splicing during SC activation are enriched in fundamental pathways, including RNA splicing, transcription regulation, chromatin organization, and the cell cycle. We revealed that RNA Binding Fox-1 Homolog 2 (Rbfox2) regulates alternative splicing during SC activation. The loss of Rbfox2 delays SC activation and muscle regeneration. We further demonstrated that Rbfox2 regulate the splicing of Numb, a well-known Notch regulator during SC activation. The inclusion of exon 6 of the Numb transcript is Rbfox2-dependent and required for SC activation. The skipping of Numb exon 6 delays SC activation and upregulates the Notch signaling pathway. Altogether, our study provides an alternative splicing landscape changes during SC activation and demonstrates Rbfox2 as a key splicing regulator in MuSCs. Moreover, we demonstrate that manipulating the splicing of a single gene is sufficient to affect SC activation and essential signaling pathways such as Notch signaling, highlighting the importance of alternative splicing in regulating SC fate decisions.

Funding Source: This study was supported by Hong Kong Research Grant Council (GRF16100322, GRF16102021, C6018-19G, AoE/M-604/16 and T13-605/18W) and partly supported by the Innovation and Technology Commission (ITCPD/17-9).

F1170

RECONSTRUCTION AND FUNCTIONALIZATION OF SINUSOIDAL VESSELS IN PLURIPOTENT STEM CELL-DERIVED HUMAN LIVER BUD ORGANOIDS

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Liver-specific capillary plexus runs between radiating hepatic cords and is comprised of liver sinusoidal endothelial cells (LSECs) which are the most potent cell type in coagulation factor VIII secretion and scavenger system. Recently, several directed differentiation protocols generated LSEC-like cells from pluripotent stem cells. However, the induction of three-dimensional liver-specific vessels in in vitro living tissue systems remains challenging. Here, we directly differentiated human pluripotent stem cells into CD32b+ putative liver sinusoidal progenitors (iLSEP) by dictating developmental pathways. By devising an inverted multilayered air-liquid interface (IMALI) culture, hepatic endoderm, septum mesenchyme, arterial and sinusoidal quadruple progenitors self-organized to generate and sustain hepatocyte-like cells neighbored by iLSEP-derived CD32+ LSEC. Single cell RNA-seq analysis including comparison with human liver and other organoid technologies uncovered in vivo relevant unique molecular profiles of divergent endothelial subsets composed of CD32blowCD31high, LYVE1+F8+CD32bhighCD31lowTHBD-, and LYVE1-THBD+ICAM1+ cells. WNT2 mediated sinusoidal-to-hepatic intercellular crosstalk potentiates hepatocyte differentiation and branched endothelial network formation. Intravital imaging revealed iLSEP developed fully perfused human vessels with functional sinusoid-like features. Organoid-derived hepatocyte- and sinusoid-derived coagulation factors enabled correction of in vitro clotting time with Factor V, VIII, IX, and XI deficient plasma and rescued the severe bleeding phenotype in hemophilia A mice upon transplantation. Advanced organoid vascularization technology allows for interrogating key insights governing organ-specific vessel development, paving the way for coagulation disorder therapeutics.

F1172

REDEFINING NEURAL PATTERNING OF THE DEVELOPING HUMAN CENTRAL NERVOUS SYSTEM

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The heterogenous human neural progenitor cells (hNPCs) are the origins of specific neurons and glial cells. Their identity in the human developing spinal cord can be defined by neural patterning (NP) - a process by which hNPCs acquire distinct identities based on their specific spatial positions due to their exposure to the gradient of different signaling pathways secreted in the dorsal and ventral area. However, the unique regulations of each domain during human spinal cord NP is not fully understood yet. Recent single-cell and spatial omics show potentials to characterize such domain-specific regulations, but standard analyses method often fail to do so, due to the challenge of distinguishing "subtypes" and "transient cell states" of stem cells. Here we used early human developing neural tubes (postconceptional week 3 to 8), performed high-resolution spatial transcriptomics (Visium HD and in situ sequencing), and introduced a new bioinformatic pipeline to capture unique domain regulations in the human developing spinal cord. We identified improved outcomes of spatially organized neural stem cells during neural patterning, and achieved lineage tracing by computational methods for human spinal cord development. Therefore, our work can 1) identify the origins of different neuronal and glial subtypes during development and their distinct regulations; 2) provide new insights into mechanisms underlying neurodevelopmental disorders like neural tube defects or spinal bifida;

and 3) improve stem cell therapy by guiding hNPCs toward desired neuronal or glial cell types for regeneration.

F1174

REPETITIVE STIMULATION MODIFIES NETWORK CHARACTERISTICS OF NEURAL ORGANOID CIRCUITS

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Neural organoids form complex networks but lack external stimuli and hierarchical structures crucial for refining functional microcircuits. In this study, we modeled the hierarchical and modular network organization by connecting multiple organoids and tested if the connection enhances the external stimuli-induced network refinement. We cultured networks of one, two, or three organoids on high-density microelectrode arrays, applied repetitive stimulation at two input locations from the microelectrodes, and monitored emergence of output signals that can decode the stimulus locations with machine learning algorithms. After two weeks of daily stimulation, networks of three organoids showed significantly higher stimulus decoding capability compared to the simpler one- or two-organoid networks. Long-term stimulation induced pronounced changes in the three-organoid network's response patterns, spontaneous activity, and inter- and intra-organoid functional connectivity. These findings underscore the importance of hierarchical network organization, e.g. creating distinct subnetworks with specialized roles, for stimuli-induced formation of circuits with robust input-output functionality.

F1176

REVEALING BLOOD-BASED BIOMARKERS FOR ASSESSING MUSCLE PATHOLOGIES

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Blood-based biomarkers are gaining prominence in clinical research for disease prognosis, diagnosis, and monitoring of disease progression. Sarcopenia and muscular dystrophies are diseases that result in the loss of muscle mass and functional decline, leading to frequent falls

and reduced mobility. Currently, there are no definitive sets of biomarkers for muscle-related diseases. This project aims to identify blood-based biomarkers to stage muscle disease progression and muscle regeneration. We profiled the blood proteome across different timepoints following injury and regeneration using an induced hindlimb muscle injury mouse model. In addition to observing the upregulation of DNA damage and repair mechanisms at 1dpi (day post-injury), muscle-associated proteins were upregulated, correlating to muscle structure development and myogenesis. Also, proteins correlated to oxidative phosphorylation are enriched at 1 and 3 dpi, whereas proteins associated with neurogenesis and epithelial-mesenchymal transition are enriched at 5 and 7 dpi. We will then focus on determining the origin of these circulating proteins, identifying the role of adult muscle stem cells (MuSCs) and fibro-adipogenic progenitors (FAPs) in secreting signaling factors into the bloodstream and facilitating the muscle repair process. Finally, we strive to verify those biomarkers during physiological and pathological conditions by profiling the muscle strength and blood proteome in physiologically aged and dystrophic mice. Using a blood proteomics approach to stage muscle pathologies, we can investigate the functional status of MuSCs and FAPs in aging and diseased mice, allowing for the non-invasive assessment of disease progression and the regenerative capacity of skeletal muscle.

Funding Source: Work was supported by the Hong Kong Research Grant Council (GRF16100322, GRF16102021, GRF16101524), the University Grants Committee, the Croucher Foundation, and the Hong Kong Center for Neurodegenerative Diseases, InnoHK, HKSAR.

F1178

REVOLUTIONIZING PEDIATRIC CRANIOPLASTY WITH THE INTEGRATION OF STEM CELL-BASED THERAPEUTICS AND BIOMIMETIC SCAFFOLDS: A SCOPING REVIEW

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Cranioplasty options for critical-sized cranial defects in pediatric patients are limited and often associated with disadvantages, especially to accommodate developing brain structures. Tissue engineering using mesenchymal stem cells (MSCs) on biomimetic, biodegradable scaffolds offers promising solutions. This scoping review evaluates and compares the osteoconductive potential, effectiveness, and clinical practicality of three types of human-derived MSCs—adipose-derived stem cells (ADSCs), amniotic fluid-derived stem cells (AFSCs), and stem cells from exfoliated deciduous teeth (SHEDs)—for cranial bone regeneration in non-human subjects. Following PRISMA for Scoping Reviews guidelines, a database review across MEDLINE, Scopus, Embase, Web of Science, and Cochrane library was conducted. In-vivo preclinical trials investigating human-derived-MSCs seeded onto scaffolds to regenerate calvarial defects were included. Among 340 articles reviewed, 24 studies were selected. The MSCs were seeded onto 5 major categorised scaffold groups: poly lactic-co-glycolic acid composites, calcium-phosphate based composites, silk fibroin, collagen-based hydrogels, and polycaprolactone-based composites. Successful osteogenic potential was demonstrated for all involved stem cells, resulting in calvarial bone regeneration and subsequent scaffold degradation with new bone formation in preclinical subjects, though at varying bone regeneration rates. ADSCs, AFSCs, and SHEDs emerge as promising stem cell sources for treating calvarial defects in pediatric patients. Their demonstrated osteogenic potential on

biodegradable scaffolds implies feasibility for skull bone regeneration, underscored by factors such as minimal immunogenic risks, diminished harvest site morbidity, and the ample availability of stem cells within the pediatric age group of newborns to 12 years old. The high proliferation and differentiation rates of in-vivo expanded stem cells further support their therapeutic potential. Scaffold material composition compatibility and its influence on osteogenic growth rates hold promise for enhancing treatment efficacy.

F1180

SCALABLE AND CLINICAL-GRADE XENOGENEIC-FREE CULTURE OF HUMAN INTESTINAL STEM CELLS ON FUNCTIONAL POLYMERS FOR INTESTINAL REGENERATION

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Intestinal stem cells (ISCs), located at the crypt base of the intestinal epithelium, hold significant promise as a cell source for regenerative medicine targeting gastrointestinal diseases. While human Intestinal organoid (hIO) cultures have emerged as a powerful tool for studying ISCs in vitro, efficient and scalable expansion of ISCs remains challenging due to their limited portion within the diverse cellular population of hIOs. To overcome these limitations, we previously developed a novel 2-dimensional (2D) culture method for ISCs derived from hIOs (ISCs3D-hIO), enabling rapid expansion, long-term maintenance, and cryopreservation through enriched ISC populations and enhanced luminal accessibility via in vitro differentiation into 2.5 dimensional (2.5D) intestinal epithelium. However, the reliance on undefined basement membrane extract (BME) like Matrigel posed a critical barrier to the development of clinical-grade cell therapies. Here, we introduce the xenogeneic-free dish for ISC (XF-DISC), a scalable and clinical-grade culture platform deposited by using initiated chemical vapor deposition (iCVD) process on cell culture substrates. The XF-DISC supported the rapid expansion of ISCs3D-hIO, achieving a 24-fold increase in 30 days, while maintaining long-term expansion over 30 sequential passages. Additionally, the XF-DISC enabled robust stock banking, with ISCs3D-hIO cryopreserved for over 3 years, and facilitated efficient differentiation into intestinal epithelium comparable to Matrigel-coated surfaces. Furthermore, ISCs3D-hIO cultured on XF-DISC demonstrated their clinical potential through successful engraftment and regeneration of the intestinal epithelium in EDTA-induced injury and DSS-induced colitis mouse models via direct transplantation. This innovative xenogeneic-free culture method offers a scalable and clinical-grade solution, underscoring its potential as a therapeutic tool for regenerative medicine in human intestinal diseases.

F1182

SELF-ORGANIZATION OF CHAMBERED AND VASCULARIZED HUMAN CARDIAC ORGANOIDS

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Cardiac organoids are three-dimensional, self-organized structures that spontaneously adopt the shape and function of heart tissue. They contain key cardiac cell types. Recent advancements in self-organizing cardiac organoids have provided a powerful tool for uncovering human cardiac development, studying cardiovascular diseases, testing drugs, and facilitating transplantation. In our study, we established a protocol to generate cardiac organoids from human iPSCs. Our system enables the high-throughput development of chambered and vascularized cardiac organoids in cell culture dishes, allowing for spontaneous self-organization. These organoids exhibit consistent size and features, demonstrating characteristics similar to normal heart tissue. Single-cell RNA-seq analysis revealed that the induced cardiac organoids include various cardiac cell types, such as cardiomyocytes, cardiac fibroblasts, epicardial cells, and endothelial cells. We believe these cardiac organoids hold significant potential for assessing the safety and efficacy of drugs. We also plan to enhance the differentiation system to improve both the quality and quantity of the cardiac organoids. Additionally, we aim to decipher the underlying regulatory mechanisms that govern the self-organization of these organoids and further explore their potential applications.

Funding Source: This work was supported by A-Smart Donation, NSFC/RGC JRS (N_CUHK428/22) and NSFC-Young Scientists Fund (82200314).

F1184

SKELETAL CELL NICHE FORMATION IN VITRO AND IN VIVO FROM HPSCS

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Skeletal muscle regeneration is made possible by the muscle's endogenous stem cells (SCs); which are supported by a dynamic microenvironment and immediate SC niche. Without the SC niche, SCs rapidly activate and lose their regenerative potential both in vitro and in vivo. Cell therapies which commonly rely on amplifying progenitors detached from their niche would greatly improve if we could replicate SC niches in vitro or understand how niches form following transplantation. Directed differentiation of human pluripotent stem cells (hPSCs) to skeletal muscle is among the few robust in vitro systems able to increase PAX7 expression by 1,000-fold. We thus performed bioinformatic lineage tracing using single nucleus RNA seq of hPSC undergoing directed differentiation to identity how muscle progenitor cells arise commitment and are supported by their unique in vitro niches. Analysis using CellRank identified the myogenic lineage commitment signature and supportive cell types that arise shortly after mesoderm induction, which we traced through several stages of in vitro myogenesis. We then transplanted hPSC-derived muscle progenitors into NSG mice and performed spatial transcriptomics at 5 time points post engraftment to determine how progenitors form and interact with their niches. We found that within a subset of muscle progenitors the expression of the stem cell marker PAX7 increases between days 1-5 in vivo and concurrently the stem cell niche receptor gene MEGF10 was highly expressed by these cells. We thus engineered a tetracycline inducible MEGF10 system in hPSCs, differentiated hPSCs to muscle progenitors,

and induced MEGF10 at several time intervals following transplantation in NSG mice with doxycycline. We found that the first 10 days of MEGF10 overexpression in vivo significantly increased niche formation and PAX7 numbers, and demonstrate that MEG10 activates Notch signaling as a potential mechanism of action. Just as adult stem cells are dynamically regulated by their niche, hPSC-derived niches formed in development control cell fate decisions to mature progenitor cells. Identifying the unique ligands and targets can help transplanted progenitors colonize available niches to enable effective long term cell therapies skeletal muscle regeneration.

Funding Source: NIH NIAMS.

F1186

SMALL-MOLECULE COCKTAIL 5SM PROMOTES CARDIAC REGENERATION BY ENHANCING EPICARDIUM AND CARDIOMYOCYTE INTERACTION VIA FGF-FGFR1-CAV1 SIGNALLING

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Adult mammalian hearts have limited regenerative capacity, unlike zebrafish and neonatal mouse hearts, which can fully regenerate after cardiac injury. In adults, heart regeneration primarily relies on cardiomyocyte proliferation, but the low proliferative capacity of adult cardiomyocytes hinders heart regeneration after myocardial infarction (MI) and limits our understanding of the regulatory mechanisms involved. Our previous work demonstrated that the small-molecule cocktail 5SM promotes cardiomyocyte proliferation and cardiac regeneration in adult rats post-MI. Based on these findings, we hypothesize that 5SM can help identify proliferating cardiomyocytes and uncover key signaling pathways involved in cardiac regeneration. To investigate the molecular mechanisms of 5SM, we employed single-cell RNA sequencing and spatial transcriptomics. Our results revealed that 5SM treatment significantly increased cardiomyocyte proliferation in the infarct border zone and Wt1+ non-myocytes in the epicardium, indicating a dual effect on both cell populations. Further analysis identified two primary mechanisms through which 5SM promotes cardiomyocyte proliferation. First, 5SM indirectly promoted cardiomyocyte proliferation by activating FGF signaling from Wt1+ non-myocytes to cardiomyocytes, as evidenced by elevated Fgf1 expression in Wt1+ cells and Fgfr1 in cardiomyocytes. Overexpression of Fgf1 in Wt1+ epicardial cells amplified the proliferative effects of 5SM, while Fgfr1 knockdown in cardiomyocytes impaired cardiac recovery. Second, 5SM directly increased Caveolin-1 (Cav1) expression in cardiomyocytes, and Cav1 knockdown inhibited cardiomyocyte proliferation. These two mechanisms are interconnected, as 5SM enhanced the interaction between Cav1 and FGFR1 in cardiomyocytes, activating the ERK and PI3K-AKT signaling pathways to synergistically promote cardiomyocyte proliferation. In conclusion, the 5SM-induced FGF1-FGFR1-CAV1 signaling pathway between Wt1+ cells and cardiomyocytes plays a crucial role in heart regeneration. This study highlights the importance of intercellular signaling in cardiac repair, offering a promising therapeutic strategy for MI and

new insights into heart regeneration mechanisms.

F1188

SP1-FUNCTIONALIZED CX-SIS SCAFFOLDS FOR ADVANCED STEM CELL RECRUITMENT AND ENHANCED WOUND HEALING

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Regeneration of skin tissue is vital for wound healing, and endogenous stem cell-based in-situ regeneration has drawn significant attention due to its potential to enhance tissue repair by recruiting the body's own stem cells. However, existing wound dressings often fail to effectively control the release of bioactive molecules or provide sustained structural support for cellular recruitment. To address these gaps, this study developed SP1-functionalized cross-linked small intestinal submucosa (Cx-SIS) scaffolds to enhance stem cell recruitment and tissue regeneration. SP1, a chemoattractant peptide analog of Substance P, was incorporated into Cx-SIS scaffolds optimized for porosity, surface roughness, and degradation rate by adjusting cross-linker concentrations. Non-cross-linked (N-SIS) scaffolds exhibited rapid SP1 release, resulting in uncontrolled diffusion, while Cx-SIS scaffolds provided a controlled burst release and maintained their structural integrity for 14 days, unlike N-SIS, which degraded within 2 days. In vitro, SP1-functionalized Cx-SIS scaffolds significantly enhanced rat mesenchymal stem cell (rMSC) migration compared to non-functionalized scaffolds and controls. In vivo experiments demonstrated that SP1+Cx-SIS scaffolds accelerated rMSC recruitment, improved tissue regeneration, and reduced scar formation. Histological and immunofluorescence analyses confirmed enhanced collagen deposition, tissue remodeling, and angiogenesis. This study demonstrates that SP1+Cx-SIS scaffolds effectively enhance stem cell recruitment and wound healing by integrating the chemoattractant properties of SP1 with the structural advantages of cross-linked scaffolds. These findings provide a novel approach for regenerative medicine and advanced wound care, offering a clinically relevant platform to improve patient outcomes and support endogenous tissue repair.

F1190

STEM CELL GRAFTS ELICIT CHARACTERIZED NEURON REGENERATION BY REGRESSING SPINAL CORDS TO DEVELOPING STATE TO RECOVER WALKING AFTER PARALYSIS

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Promoting axonal regeneration to fully restore functions after spinal cord injury (SCI) is a considerable challenge. Given that the regeneration in a complete SCI can realized for developing spinal cord (SC), we propose that SCI may achieve ideal repair if stimulating signal is enough to regress SCI to the developing state. To achieve substantial therapeutic outcome, here we develop a biodegradable hydrogel to co-encapsulate GMP-produced human neuroepithelial stem cells (NESCs) at neural tube stage and mesenchymal stem cells (MSCs)

(NESC+MSC) and then transplant them into completely transected SCI rats at 4th thoracic vertebra and quarter-sectioned SCI adult monkeys at 10th thoracic vertebra. NESC+MSC grafts reverse injured SC into developing state resembling to postnatal day 1-3 along with inflammatory inhibition. Instead of cell direct replacement, NESC+MSC grafts elicit directed regrowth of reticulospinal neuron and propriospinal neuron axons to cross lesions, span at least ten spinal segments caudally to lumbar segments, and relay the supraspinal command to restore walking in paraplegic rats. Thus, reversing developing state forms an important strategy of axon regeneration to restore lost neurological functions.

Funding Source: This work was supported by grants from National Key Research and Development Program of China Major Basic Research Project of Science and Technology of Yunnan, Spring City Plan the High-level Talent Promotion and Training Project of Kunming.

F1192

STEPWISE DIRECTED DIFFERENTIATION OF HUMAN EXPANDED POTENTIAL STEM CELL FOR CARTILAGE REGENERATION

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Osteoarthritis (OA) is a chronic degenerative disease characterized by the progressive erosion of articular cartilage. Current OA treatments fail to restore damaged cartilage, highlighting the urgent need for regenerative strategies. Pluripotent stem cells have emerged as a promising solution for cartilage regeneration, as they can be patient-derived and allow for directed differentiation. Developmentally, articular cartilage originates from the lateral plate mesoderm and matures within the long bones. However, most existing articular cartilage regeneration studies focus on chondrocytes derived from the paraxial mesoderm, leading to a gap in leveraging the authentic developmental lineage to replenish the joint chondrogenic loss. To address this gap, our project applies the expanded potential stem cell (EPSC) to recapitulate the developmental trajectory of lateral plate mesoderm-derived cartilage. Our goal is to establish a robust and scalable chondrogenesis platform for in vitro cartilage regeneration research. We have established an in-house protocol to generate cartilage-like pellets and employed single-cell transcriptomic analyses to evaluate how the protocol recaptures key in vivo chondrogenic pathways. To improve differentiation homogeneity, we are using CRISPR-Cas9-edited reporter cell lines to enable precise comparative analyses between well-differentiated chondrogenic cells and other cell types. Our work will contribute to the development of personalized, stem cell-based therapies for OA patients.

F1194

SUPPRESSING THE FIBROSIS-DETERMINING FACTOR MEOX1 ENABLES EFFICIENT IN VIVO REPROGRAMMING OF CARDIAC MYOFIBROBLASTS INTO CARDIOMYOCYTE-LIKE CELLS

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Direct reprogramming of cardiac fibroblasts (CFs) into induced cardiomyocyte-like cells (iCMs) is a promising regenerative medicine strategy for myocardial infarction (MI) patients. CFs are activated and transition to myofibroblasts (MFs) after MI, which are abundant in the injured

area. However, MFs show extremely lower cardiac reprogramming efficiency. We identified Meox1, a TGF-β1 downstream transcription factor, which involved in cardiac fibrosis, as a barrier to cardiac reprogramming. In MFs induced with MGT (Mef2c, Gata4 and Tbx5) or MGT plus Myocd (MGTM), Meox1 knockdown results in significant increase in reprogramming efficiency. Single-cell analysis revealed meox1 as a central regulator in antagonistic transcriptional networks between fibrotic myofibroblasts and induced cardiomyocytes. Mutations disrupting the MEOX1 DNA binding domain or fusing it to a transcriptional repressor domain attenuate its inhibition of cardiac reprogramming. Furthermore, RNA-seq and CUT&Tag analysis revealed that Meox1 stabilizes a fibrosis-associated transcriptomic program and modulates the inflammatory response. Dual recombinase tracing showed that Meox1 knockdown enhances the efficiency of in vivo iCM reprogramming and restores cardiac function following myocardial infarction. Finally, we found that Meox1 knockdown improved reprogramming efficiency in human MFs. These findings provide a potential target for direct cardiac regeneration.

F1196

THE COMPLEX INTERPLAY BETWEEN ENDOTHELIAL CELLS AND OSTEOBLASTS DURING OSTEOGENIC DIFFERENTIATION

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The processes of angiogenesis and osteogenesis are intrinsically linked, with a complex interplay between endothelial and osteogenic cells governing the delicate balance of bone homeostasis and mineralization. The aim of this study was to investigate and expand our understanding of the paracrine and juxtacrine mechanisms that mediate crosstalk between endothelial cells (EC) and osteoblasts (OB) in the context of osteogenic cell differentiation in vitro. To distinguish these two types of interaction, we co-cultured OB and EC directly to activate juxtacrine signaling (direct co-culture) or separated them by a membrane penetrable to paracrine factors, but not to cells (indirect co-culture). We have determined that the presence or absence of direct contact between OB and EC during the induction of osteogenic differentiation can lead to either osteoinductive or osteosuppressive effects. Our proteotranscriptomic analysis showed that the osteosuppressive effect is associated with the action of paracrine factors secreted by EC, while the osteoinductive properties of EC are mediated by the Notch signaling pathway, which can only be activated by physical contact of EC with OB. Indeed, upon direct co-cultivation, the knockdown of Notch1 and Notch3 receptors in EC has an inhibitory effect on osteogenic differentiation of OB, whereas activation of Notch by the intracellular domain of either Notch1 or Notch3 in EC has an inductive effect on osteogenic differentiation of OB. This represents a new and important discovery, as it reveals previously unknown crosstalk between EC and OB mediated by dynamic regulation of Notch1 and Notch3 signaling.

Funding Source: The work was carried out with financial support from the Russian Science Foundation (project number 23-15-00320).

F1198

THE EFFECTS OF 3D PRINTED DECELLULARIZED AMNIOTIC MEMBRANE-BASED LENSES ON CORNEAL DEFECTS

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The therapeutic effect of amniotic membrane (AM) is a proven treatment method used in the field of medicine, especially in wound healing. Its skin-like properties, angiogenic effect, and ability to promote cell proliferation and differentiation make AM an important material for therapeutic applications. In ophthalmology, AM transplantation is widely used, including corneal, conjunctival and retinal pathologies. Early wound healing deterioration means repeated transplants. Therefore, the use of more easily applicable, durable and off the shelf transplantation products can improve the treatment approach. 3D printing technology has made it easier to fabricate tissue-like structures on demand. In this study, the effect of lyophilized amniotic membrane-based lenses produced via 3D printing, on wound healing purpose in corneal epithelial-stromal defects in rabbits was investigated. For this purpose, AM collected from donors was first decellularized and then lyophilized. In order to make it suitable for 3D printing, powdered AM was mixed with alginate and antibiotics to form a hydrogel. The resulting hydrogel was printed with a 3D bioprinter in the shape of a lens of appropriate size for the rabbit eye. Polycaprolactone (PCL) in the form of a cage, which allows the lenses to be fixed in the damaged eyes, was also printed with the 3D bioprinter and sewed to the eyes of the rabbits to secure the lenses. At the end of the 7th day, there was no statistically significant difference between rabbits which received 3D printed AM and conventional AM transplantation in terms of histologic epithelial formation and keratocyte presence, inflammation and wound healing results. Compared to the control group, the 3D printing group showed 50% more moderate-intensity keratinocyte formation and 16.7% more 2-row epithelial formation. The results indicated that rabbits had similar responses to treatment after both 3D printed AM and conventional AM transplantation. Furthermore, the antibiotic secreted through the hydrogel eliminated the need for regular topical drops. Since AM was decellularized, the proposed strategy reduces the risk of immunological response and enables long-term storage. In conclusion, considering these advantages, 3D printed AM lenses offer an alternative treatment to conventional AM transplantation.

F1200

THE ROLE OF TGF-BETA SIGNALING IN REVIVAL STEM CELL INDUCTION IN THE MOUSE INTESTINE

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The outermost layer of the small intestine, termed the intestinal epithelium, is critical for regulating digestion, nutrient absorption, and microbiota in the gut. As such, this epithelial layer shows remarkable regenerative capacity, completely restoring its cellular architecture within a few days following damage. For a long time, the cellular mechanisms driving this regeneration were unclear as the stereotypical intestinal stem cells, called crypt base cells (CBCs), are lost following irradiation and chemical injury. Our lab previously identified a novel ‘revival stem cell’ (revSC) population in the mouse intestinal epithelium, marked by the expression of the Clu gene. RevSCs, while normally rare in the homeostatic intestine, are strongly induced by irradiation damage and give rise to new CBCs. However, the cellular signaling mechanisms driving revSC induction were still unclear. We find that the TGF-b signaling pathway is strongly activated in the mouse intestinal epithelium following irradiation. Consistent with this, treatment of mouse intestinal organoids with the TGF-b1 ligand results in strong Clu upregulation. These TGFb1-induced organoids also upregulate other revSC marker genes, F3 and Anxa1, that we previously indentified. Additionally, when isolated and replated, TGF-b1 induced Clu-expressing cells give rise to new organoids confirming their regenerative capacity. Next, using a combination of small molecule modulators of the Notch and Wnt signaling pathways, we generated specialized organoids enriched for Enterocytes, Paneth, or Goblet cells, the three most abundant cell populations in the intestinal epithelium. By treating these enriched organoids with TGF-b1, we found that each of these populations could give rise to Clu+ stem cells, demonstrating that revSCs appear from pre-exisiting differentiated epithelial cells. Together, these data help uncover the complex cellular and molecular mechanisms driving the emergence of revSCs, a critical injury-induced stem cell population in the intestinal epithelium. Further, we identify the TGF-b signaling pathway as a potential therapeutic target to drive revSC-mediated regeneration in the intestine.

Funding Source: CIHR Postdoctoral Fellowship (FRN 193957).

F1202

THE THERAPEUTIC EFFECT OF LOW PH-PRECONDITIONED TENDON-DERIVED STEM CELLS EXOSOMES ON TENDINOPATHY

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Tendinopathy, a common musculoskeletal disorder, is a chronic disease of an unknown etiology and associated with inflammation. Currently, conservative and surgical treatments often prove to be insufficient for the complete resolution of symptoms, with some patients unfortunately needing to undergo surgery at a later stage. Exosomes are nano-sized extracellular vesicles (30-150 nm in diameter) secreted by various cell types, including mesenchymal stem cells (MSCs). These vesicles play a crucial role in intercellular communication by transferring bioactive molecules such as proteins, lipids, and nucleic acids (mRNAs and miRNAs) between cells. Exosomes derived under acidic preconditioned tendon-derived stem cells (TDSCs) may carry a distinct cargo of proteins, lipids, and genetic material that is specifically tailored to promote tissue repair. Therefore, the objective of this study was to investigate the pro-tenogenic bioactivity of low pH exosomes on TDSCs, and to develop GelMA-loaded exosomes for the repair of Achilles tendinopathy. Our results demonstrated that: Exosomes derived from acidic conditions (pH 5.0) enhanced the proliferation and migration of rATSCs. At day 3, rATSCs treated with pH 5 exosomes exhibited decreased expression of pro-inflammatory cytokines and increased expression of the anti-inflammatory cytokine IL-10. At days 6 and 12, rATSCs treated with pH 5 exosomes demonstrated increased expression of tenogenic markers. Compared to pH 7.35 exosomes, pH 5 exosome loading implantation in rat Achilles tendon resulted in significantly enhanced histological and functional tendon repair at week 4. Our study suggested that low pH-preconditioned tendon-derived stem cell exosomes exhibit potent tenogenic bioactivity in vitro and enhance tendon repair in vivo in rat Achilles tendinopathy. Further optimization of exosomes will be necessary to improve its tendon regeneration efficacy. Additionally, investigations into the underlying mechanism of action will also be needed to justify its potential clinical translation for tendon repair.

F1204

TONSIL-DERIVED MESENCHYMAL STEM CELLS AMELIORATED PERITONEAL EPITHELIAL-TO-MESENCHYMAL TRANSITION AND FIBROSIS VIA A MITIGATION OF OXIDATIVE STRESS

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Mesenchymal stem cells (MSCs) are multipotent adult stem cells with regenerative capabilities and exert paracrine actions on damaged tissues. The epithelial-to-mesenchymal transition (EMT) of mesothelial cells (MCs) is an early mechanism of peritoneal dysfunction in peritoneal dialysis (PD). MSCs have recently attracted attention for their ability to prevent organ fibrosis by inhibiting EMT. This study investigates the role of T-MSCs in TGFβ-induced EMT of human peritoneal mesothelial cells (HPMCs) and its underlying mechanism. An animal model of PD was established in Sprague-Dawley rats by daily infusion of glucose-based dialysate with methylglyoxal for three weeks. T-MSCs (5 x 10^6) were administered intraperitoneally on day 14. Expression of markers of EMT and oxidative stress were evaluated in peritoneal membrane with the peritoneal equilibrium test (PET) and histologic analysis. HPMCs were co-cultured with

MSCs or MSC-conditioned medium (CM) using a transwell co-culture system to evaluate EMT, reactive oxygen species (ROS) generation, and the expression of antioxidant enzymes and anti-fibrotic proteins. Comparative analyses were performed using T-MSC, adipose-derived MSCs (AD-MSC) or bone marrow-derived MSCs (BM-MSC). PD+T-MSCs rats had improved D2/D0 glucose and D/P creatinine ratios compared to PD rats. Anti-human nuclei staining revealed scattered positive signals along the peritoneal mesothelial layer in PD +T-MSCs rats. In PD+T-MSCs, EMT and peritoneal fibrosis were alleviated with the restoration of oxidant-antioxidant balance as evidenced by decreased expression of 8-OHdG, NT, and 4-HNE, along with increased GSH and SOD2 in peritoneal membrane. Co-culture of HPMCs with T-MSCs or T-MSC-CM inhibited TGFβ-induced EMT and ROS generation. T-MSCs expressed higher levels of antioxidant enzymes (catalase, GPx, and SOD) and anti-fibrotic proteins (HGF and BMP-7) compared to AD-MSCs and BM-MSCs. TGF-β-induced reduction in expressions of antioxidant enzyme and antifibrotic peptides in HPMCs were restored by T-MSC-CM. These findings highlight the anti-fibrotic and antioxidant effects of T-MSCs, suggesting their therapeutic potential in preventing peritoneal dysfunction and fibrosis in PD patients.

Funding Source: This work was supported by the Korean Fund for Regenerative Medicine (KFRM, 23A0201L1) grant funded by the Korea government (MIST).

F1206

TRANSPLANTATION OF RETINAL CELLS DERIVED FROM RETINAL ORGANOIDS INTO RD10 MICE WITH RETINITIS PIGMENTOSA

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Cell therapy, a cornerstone of regenerative medicine, involves transplanting cells to replace damaged tissues. Recent advancements in treating retinal degeneration through RPE cell replacement have shown promise, but these cells can only provide early intervention or nutritional support. Photoreceptor cells, which are specialized for light-sensing, face challenges in surviving and integrating after transplantation due to the host microenvironment, limiting their therapeutic efficacy. To address this, the concept of photoreceptor cell co-transplantation was proposed. In this approach, two types of cells are utilized: the primary cell source-photoreceptor cells, which replace the degenerated photoreceptor cells of retinal degeneration; sub-cell source -X cells, that support the primary cell source for efficient transplantation and improved post-transplant survival. Therefore, whether the combined transplantation of well-functioning X cells can enhance the subretinal microenvironment, thereby promoting the survival and integration of transplanted photoreceptor cells and improving overall transplantation efficiency, is a critical scientific question that this study aims to address. In our previous studies, we have developed techniques to efficiently differentiate human pluripotent stem cells (hiPSCs) into X cells and retinal organoids (ROs) to obtain photoreceptor precursor cells. We also established an humanized immunodeficient mouse model of retinal degeneration (Rd10-MRG) suitable for X cell transplantation. Significant progress has been made in treating Rd10-MRG through the co-transplantation of X cells and photoreceptor cells. Experimental results indicate that co-transplantation enhances the survival and integration of photoreceptor cells compared to single-cell type transplantation, leading to improved functional and structural outcomes in the host retina. However, further molecular biology experiments are required to elucidate the underlying mechanisms. It is anticipated that this combined cell transplantation approach will offer novel

insights and strategies for the treatment of retinal degenerative diseases.

F1208

USING NATIVE PIG KIDNEY AS A STANDARD MODEL TO DEVELOP AN AFFORDABLE AND EFFICIENT EX-VIVO PERFUSION SYSTEM FOR RECELLULARIZED ORGANS

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In our laboratory, the primary objective is to engineer a transplantable human organ using a patient's stem cells and decellularized pig organs as a scaffold, with the goal of addressing the growing demand for organ transplants. A critical aspect of this work is normothermic ex-vivo perfusion (NEVP) culture, which supports organ growth and maintenance. The challenge lies in the high cost and complexity of current clinical NEVP systems, which rely heavily on external oxygen tanks, red blood cells, and expensive single-use components. Once the NEVP system is fully developed and optimized, the next phase will focus on humanizing pig kidneys with patient-derived induced pluripotent stem cells (iPSCs), advancing us toward functional, transplantable organs. To address these issues, we have designed an affordable and efficient normothermic ex-vivo perfusion (NEVP) system using a native pig kidney model. This system is based on a mouse NEVP system developed in our lab. Key innovations include the elimination of an external oxygen source and red blood cells, reusable accessories, and optimized medium flow. All of these leads to reducing costs and enhancing accessibility. Additionally, we implemented contamination prevention strategies and fine-tuned osmolarity to reduce edema and therefore prevent cell death. Pressure, flow, and oxygen sensors were incorporated to ensure physiologically relevant conditions . Our results demonstrate successful maintenance of a native pig kidney for 24 hours using this new system, with kidney health assessed by urine analysis, including total protein, albumin and creatinine levels in the ultrapurfusate (urine). Moving forward, we plan to optimize oncotic pressure and investigate the use of the oxygen carrier perfluorocarbon (PFC) to extend the kidney’s viability. This work represents a significant step toward making ex-vivo perfusion more cost-effective and scalable for both organ transplant applications and research studies that require organ culture.

F1210

ZYGOTIC GENOME ACTIVATION GENES IN GENOME STABILITY OF AGED STEM CELL

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Assisted reproductive technology (ART) has gained increasing significance amidst declining birth rates. However, its efficacy is challenged by aging, particularly the decline in oocyte quality observed in individuals in their mid-30s. Our research focuses on the molecular mechanisms that underlie the age-related deterioration of stem cell function, aiming to pave the way for improved outcomes in ART for older individuals. Mouse embryonic stem cells (ESCs) were isolated from parthenogenetically developed blastocysts. Induced pluripotent stem cells (iPSCs) were isolated from fibroblast of isogenic mice at three age groups: young (6–8 weeks), middle (6 months) and aged (12–30 months). RNA sequencing, radiation-induced genome stability assays, and telomere length quantification analyses were performed. Stem cells derived from all ages demonstrated robust self-renewal capacity and maintained pluripotency, as validated by immunohistochemistry and quantitative PCR. Hierarchical clustering of RNA-seq data revealed that aged iPSCs were transcriptomically distinct from young iPSCs and ESCs. Gene ontology analysis highlighted significant downregulation of genes involved in chromosome segregation, chiasma assembly, nuclear division and cell cycle processes in aged iPSCs. Intriguingly, transcriptomic profiling revealed upregulation of zygotic genome activation (ZGA) genes in aged iPSCs. Radiation-induced genome stability assays demonstrated a marked increase in γH2AX foci—an indicator of DNA damage—in iPSCs with aging, reflecting elevated genomic instability. Interestingly, knockdown of ZGA genes via small hairpin RNA modestly reduced γH2AX foci in aged iPSCs. The average number of foci decreased from 5.4 (control) to 4.8 (knockdown shRNA group) in young iPSCs. In aged iPSCs, the number decreased from 7.5 (control) to 6.5 (knockdown shRNA group). Notably, telomere length analysis did not reveal any significant differences between young and aged groups. Our findings suggest the presence of a compensatory ZGA gene cluster in aged stem cells that may mitigate the effects of aging. These insights pave the way for innovative strategies to enhance the developmental potential of aged embryos, potentially improving ART outcomes for older individuals.

TRACK: CLINICAL APPLICATIONS (CA)

Poster Session 3 (ODD)
4:00 PM – 5:00 PM

F1213

OPTIMISING STEM CELL DERIVED THERAPIES FOR DEGENERATIVE DISEASE: ASSESSING CLINICAL GRADE HUMAN EMBRYONIC STEM CELL LINES FOR AGE-RELATED MACULAR DEGENERATION ASSOCIATED RISK ALLELES

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Stem cell-derived therapies represent a cutting-edge frontier in regenerative medicine, offering innovative solutions for complex degenerative diseases by replacing damaged or diseased tissues with healthy cells. While significant progress has been made, ensuring the safety and efficacy of these therapies remains paramount. Critical considerations include teratoma risk, traceability, and genetic stability of pluripotent stem cell lines. Recent efforts have focused on understanding chromosomal abnormalities and functional alterations that could influence cell behaviour, survival, and proliferation. Despite these advancements, the impact of the genetic background of pluripotent stem cells on their therapeutic potential, including predisposition to the diseases they aim to treat, remains poorly understood. Here we have sequenced genetic risk scores for AMD in induced pluripotent stem cells from patients with neovascular AMD and clinical grade human embryonic stem cell lines, including ones currently being used as cell therapy clinical trials for AMD. All cell lines contained at least one risk allele for AMD, and various combinations of alleles associated with increased/decreased predisposition to AMD. Genetic risk scores, calculated for each cell line, ranged from 0.59-0.84, suggesting all lines examined have genetic backgrounds associated with increased risk of AMD. The impact of transplanting stem cell-derived therapies with a genetic predisposition to AMD into a compromised disease environment is unknown. The genetic background could influence donor cell viability, functionality, and therapeutic efficacy. Investigating how specific genetic variations affect stem cell-derived RPE will be crucial for guiding future research and optimising effective treatment strategies for AMD.

Funding Source: The London Project To Cure Blindness.

F1215

HIGH-DENSITY EXPANSION AND METABOLIC CHARACTERIZATION OF HUMAN ADIPOSE AND WHARTON'S JELLY MESENCHYMAL STEM CELLS IN SCALABLE MICROCARRIER SUSPENSION CULTURE

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Mesenchymal stem cells (MSCs) are widely used in regenerative therapies for various conditions but face challenges in large-scale production due to the limitations of conventional plate culture methods. This study proposes a scalable, lab-scale microcarrier culture system to

expand MSCs efficiently using commercial media supplemented with fetal bovine serum (FBS) or human platelet lysate (hPL) as a xenofree alternative. The metabolism of cultured cells was assessed by monitoring glucose, pyruvate, lactate, ammonia, and amino acid consumption/production. Human Wharton's Jelly MSCs (WJ-MSCs) and adipose-derived stem cells (ASCs) were successfully expanded in an agitated suspension culture using Cytodex beads. In FBS- or hPL-supplemented cultures, up to 200 million cells were obtained from ASCs in a single flask, representing a 28-fold increase in cell number. For WJ-MSCs, a significantly lower yield was observed with hPL (60 million cells), compared to FBS (over 200 million cells), likely due to the formation of spheroid-like structures instead of attachment to microcarriers. Despite this, culturing WJ-MSCs in xenofree suspension cultures was still possible. Metabolic analysis revealed high specific consumption/production rates (q_s) for glucose, pyruvate, lactate, glutamine, glutamate, and ammonia. Essential amino acids were consumed, except for lysine, which showed negative q_s values during the lag phase. No metabolites were depleted during cultivation, indicating efficient nutrient utilization. This culture system is scalable, producing up to 250 million cells in a single flask, equivalent to 40 adherent T175 flasks. It provides a cost-effective, contamination-resistant solution for MSC expansion. Further optimization of microcarrier adhesion, particularly for WJ-MSCs, and refined feeding strategies based on metabolic profiling will improve culture performance and reduce media usage. Additionally, the cells retained their pluripotency, as evidenced by phenotypic markers and differentiation potential, confirming the effectiveness of this method for MSC expansion and potential therapeutic applications.

Funding Source: Supported by ANID-FONDECYT #11230208, ANID-Basal funds for CeBiB #AFB240001 and #FB0001, and the ANID-National Master's Grant #22240802.

F1217

DEVELOPMENT OF AN AUTOMATED 3D PLATFORM FOR MANUFACTURING PLURIPOTENT STEM CELL-BASED CLINICAL CELL THERAPY PRODUCTS

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Human induced pluripotent stem cells were first produced over 15 years ago. Although this breakthrough in stem cell research has provided a novel platform for the development of individualized cell therapies, iPSC-based clinical products are yet to achieve wide global adoption. The biggest obstacle is the cost of cell manufacturing and quality control tests required to ensure safety of the cell therapies. Substantial reduction of the cost, while assuring safety of the cell therapies, requires major changes to the existing protocols of reprogramming and differentiation. We believe that development of the process in 3D system will simplify and streamline the workflows. The end goal is to create a customized proprietary system, but due to the complexity of the process consisting of reprogramming somatic cells, cloning, engineering and expansion of PSCs, and final differentiation, we are working with commercially available

instruments to evaluate and optimize all steps of the process. We have also learned that we still need to clarify certain aspects of stem cell biology, since established characteristics of the cells often seem to be dependent on specific parts of the culture system. Here we report our progress in development of an automated 3D platform for generation, expansion, cloning and differentiation of pluripotent stem cells for clinical applications. We have established a reprogramming protocol of hematopoietic progenitors in the Elplasia ultra-low attachment microplates (Corning). To manipulate cell aggregates we have utilized CellCelector platform (ALS/Sartorius). For single-cell cloning we have used either CellCelector or UP.SIGHT (Cellink/Cytena) with Elplasia microplates or SIEVEWELL slides (Tokyo Ohka Kogyo). Scaling up of 3D PSC culture was done on Elplasia system ranging from 96-well microplates up to 12K microcavities flask as a stationary culture, followed by 100- and 500 mL MAG vessels driven by PBS-MINI MagDrive Bioreactor (STEMCELL Technologies). We are currently working on the differentiation protocols development starting from cell aggregates in Elplasia plates, as well as utilizing microcarriers. Regarding cell quality control, the biggest issue is genetic stability. To address this problem in the most cost-effective manner, we are working on the multi-omics automated approach.

Funding Source: Japan Agency for Medical Research and Development (AMED), grant JP24bm1323001h0102.

F1219

ADVANCED HPSC BIOPROCESSING VIA 3D INOCULATION FROM CRYOPRESERVED CELL STOCKS

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Human pluripotent stem cells (hPSCs) have great potential for revolutionizing regenerative medicine. Despite recent progress in early clinical applications, differentiation and production of hPSC-progenies has been mainly studied in laboratory scale adherence-dependent (2D) cultures. This approach has substantial limitations regarding bioprocess efficiency, control and scalability for cell production in clinically relevant quality and quantities. For overcoming these, we have established different 3D suspension culture platforms, focusing on fully controlled stirred tank bioreactors (STBRs). By leveraging the application of STBRs, the mass production of 5.25^9 pluripotent hPS cells in a 150 mL working scale has been achieved within a 7-day

process, resulting into a 70-fold expansion of the inoculated cell density. Nevertheless, pre-expansion of cryopreserved hPSC stocks via poorly controlled 2D culture was previously required before STBRs inoculation for 3D cultivation, creating challenges for good manufacturing practice (GMP)-compliant bioprocess development. Here we present a novel approach enabling the “high-density seeds” (HDS) for 3D bioprocess inoculation directly from cryopreserved hPSC stocks, thereby overcoming the need of any 2D culture. However, our novel strategy critically depends on the efficient recovery of viable hPSCs from cryopreservation followed by the impact of stirring shear stress in suspension. We have thus established screening assays for testing the efficiency of different “cell recovery reagents” with the HDS approach and will present solutions enabling efficient hPSC recovery and aggregation particularly during the critical phase post-inoculation. The resulting cultivation strategy is notably compatible with subsequent serial passaging of hPSCs combined with process upscaling, while all relevant hPSC characteristics such as exponential cell growth, pluripotency-associated marker expression, genomic stability and differentiation potential are maintained. Moreover, when combined with a directed differentiation protocol, the successful mass production of bona fide hPSC-cardiomyocytes in suspension culture has been demonstrated, fulfilling future clinical applications and high throughput screening platforms for drug testing as well.

F1221

OFF-THE-SHELF DOPAMINERGIC PROGENITORS DERIVED FROM IPSCS FOR IMPROVING SAFETY AND FUNCTIONAL RESTORATION IN PARKINSONIAN RATS

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Human induced pluripotent stem cell (iPSC) is a promising source of midbrain dopaminergic progenitor (mDAp) for Parkinson’s disease (PD) transplantation. However, current mDAp are unavailable to overcome the clinical challenges, including undifferentiated stem cell-associated tumorigenicity, limited efficacy of in vitro mDAp production, and delayed differentiation and maturation efficacy of mDAp in vivo. Here, we first applied n-butylidenephthalide, a chemical compound, to selectively reduce undifferentiated iPSCs and consequently promote neurite formation. Additionally, we introduced a novel set of patterning factors, including a transient high concentration of a GSK-3β inhibitor, to efficiently convert iPSCs into mDA neurons. With

these improved techniques, 75% of iPSCs robustly differentiated into mature mDA neurons, showing burst dopamine secretion and phasic electrophysiological activities with external stimuli. To evaluate the therapeutic potential, cryopreserved mDAp were transplanted into 6-OHDA PD rats. No teratoma or neural tumor was observed within 26 weeks post-implantation. Surprisingly, within just eight weeks post-transplantation, mDAp survived and robustly differentiated into mDA neurons in the injected striatum, leading to significant functional recovery. These preclinical findings demonstrate that our reproducible and innovative process produces high-quality off-the-shelf mDAp, potentially enhancing safety and reducing the nonreactive window in iPSC-treated PD patients.

Funding Source: National Science and Technology Council of Taiwan (MOST 110-2314-B-303-017-MY3 and NSTC 113-2314-B-303-024-, Dr. S.-Z.L.); (MOST 111-2314-B-303-027-MY2 and NSTC 113-2314-B-303-026-, Dr. C.-Y.C.).

F1223

DECREASED A-TO-I RNA EDITING AND HYPEREXCITABILITY ASSOCIATED WITH ENHANCED CHOLESTEROL BIOSYNTHESIS IN LOWER MOTOR NEURONS OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the degeneration and loss of motor neurons (MNs). Genome- and epigenome-wide association studies identify genetic predisposition to enhanced cholesterol biosynthesis as a risk factor for ALS. We previously reported that lower MNs derived from induced pluripotent stem cells (iPSC-LMNs) of patients with ALS exhibit upregulated expression of cholesterol biosynthesis enzymes relative to controls, and that this upregulation correlates with ALS-like phenotypes such as reduced neurite length. However, the mechanistic link between enhanced cholesterol biosynthesis in MNs and neurodegeneration in ALS remains incompletely understood. Here, we demonstrate that increased cholesterol biosynthesis in MNs is associated with reduced adenosine-to-inosine (A-to-I) RNA editing activity, as well as hyperexcitability. Under normal conditions, GRIA2, a subunit of the AMPA receptor, undergoes A-to-I RNA editing at the pre-mRNA level, thereby conferring calcium ion impermeability. In contrast, the unedited form remains permeable to calcium ions and contributes to excitotoxic damage. To accurately quantify A-to-I editing activity in iPSC-LMNs, we first confirmed that the A-to-I editing index, which is calculated based on editing activity for Alu sequences, correlates with the A-to-I editing ratio in GRIA2 ($R=0.4513$, $P< 0.001$). In iPSC-LMNs, overexpression of SREBF2, a key regulator of cholesterol biosynthesis, decreased the A-to-I RNA editing index and induced hyperexcitability, as measured by microelectrode array ($P< 0.001$). Conversely, siRNA-mediated knockdown of either SREBF2 or HMGCR (the rate-limiting enzyme in cholesterol biosynthesis), as well as treatment with ropinirole hydrochloride (which suppresses the expression of cholesterol biosynthesis enzymes in MNs), increased the A-to-I RNA editing index ($P< 0.004$) and mitigated hyperexcitability ($P< 0.001$). These findings suggest that

upregulated cholesterol biosynthesis in MNs may promote calcium ion-mediated excitotoxicity through reduced GRIA2 A-to-I RNA editing, potentially representing a crucial pathogenic mechanism in ALS. They further imply that inhibiting cholesterol biosynthesis in MNs could serve as a promising therapeutic strategy for ALS.

Funding Source: This study was supported by grants from the Japan Society for the Promotion of Science (JSPS) and the Japan Society for the Promotion of Science (AMED).

F1225

TRASIENT ROR-BETA KNOCKDOWN BY CELL-PENETRATING SIRNA ALLEVIATES RETINAL DEGENERATION CAUSED BY PROTEOTOXICITY

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Retinitis pigmentosa (RP) is characterized by rod photoreceptor degeneration-driven apoptosis, which secondarily triggers cone photoreceptor loss and eventual blindness. Preserving rod function remains a primary therapeutic objective. Inspired by prior studies demonstrating that neural retina leucine zipper (Nrl) knockdown conferred resistance to rod mutations and induced in vivo rod-to-cone conversion with therapeutic effects in inherited retinal degeneration (IRD) models, we hypothesized that knockdown of retinoid-related orphan receptor beta (RORβ), an upstream regulator of Nrl, could yield similar or superior outcomes. To test this, we developed a cell-penetrating asymmetric siRNA (cp-asiRNA) targeting RORβ (cp-asiRORβ), designed to overcome conventional siRNA limitations, including inefficient cellular delivery, off-target effects, and immune activation. Intravitreal administration of cp-asiRORβ in RhoP23H mice—an autosomal dominant RP model harboring the P23H rhodopsin mutation—successfully reduced RORβ expression in rod photoreceptors. Contrary to our initial expectation of observing an in vivo reprogramming effect, RORβ knockdown did not seem to induce rod-to-cone conversion. Instead, transient RORβ suppression effectively mitigated apoptosis by enhancing proteostasis, leading to improved rod survival, reduced degeneration, and preserved visual function. Furthermore, single-cell RNA sequencing of retinas treated with cp-asiRORβ in RhoP23H mice revealed significant upregulation of proteasomal subunits in RORβ-reduced rod photoreceptors. Consistent with these findings, in HEK293T cells under proteotoxic stress, RORβ knockdown reduced apoptosis, improved cell viability, and diminished aggresome formation. These results demonstrate that transient RORβ suppression alleviates RP progression by augmenting proteasomal activity to resolve proteotoxicity, offering a novel therapeutic avenue for RP patients.

F1227

IN VIVO SINGLE AND DUAL TRANSDUCTION OF AAV6 IN EXPANDED NON-MOBILIZED HAEMATOPOIETIC STEM CELLS FROM TRANSFUSION-DEPENDENT THALASSEMICS IN HUMANIZED MICE

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Hematopoietic stem cells (HSC) are an ideal target for gene modification therapies (GMT) as they reconstitute the entire haemopoietic and immune system, and HSC genetic diseases often cause severe lifelong morbidity. Ex-vivo GMT is established for major β-hemoglobinopathies, but limitations include accessibility, affordability and myeloablation and immunosuppression-associated morbidity. These barriers may be circumvented by in-vivo HSC GMT. Adeno-associated viral vector (AAV) hold numerous benefits for in-vivo GMT, but its small payload poses packaging challenges for large gene editors, preferred for their ability to correct mutations in situ. A dual-AAV strategy delivering split-intein editors directly addresses this limitation. We produced a humanized mouse (Humice) engrafted with in-vitro expanded human adult non-mobilised CD45+CD34+ HSC from transfusion-dependent β-thalassaemics to demonstrate the feasibility of dual-AAV serotype 6 (AAV6) targeting of human HSC in vivo. Humice (4-10% human cells, CD45+) were injected intravenously with 5E+12 vector genome (vg)/kg of ssAAV6 expressing GFP or mCherry (1:1 mixture). Peak transduction was 56.82±11.59%, achieved at one-week post-injection, with 31.77±26.21% dual transgene expression in peripheral circulating hCD45+. In Thal humice, single or dual transduction was observed in 0.2-98.2% of nested hCD45 in bone marrow, liver and spleen at four weeks, with AAV6 concentration ranging from 0.8-306017.3 copies per cell. Compared to non-thal humice (0.1–91.5% transduction and 0.0-9276.0 copies per cell), an increase in transduction efficiency and VCN was seen. Secondary transplantation of hCD45 initially transduced at 0.6% resulted in engraftment of hCD45 with 4.1-60.7% transduction, confirming AAV6 transduction of long-term repopulating HSC, with minimal activation of pro-inflammatory cytokines compared to non-AAV6 humice. This model demonstrates the feasibility of in-vivo targeting of circulating and nested HSC with AAV6, lending this strategy useful to gene or base editors amenable to split-intein designs packaged as dual AAV6, while benefiting from the promising safety profile of in-vivo AAV therapy and avoiding complications of ex-vivo HSC GMT.

Funding Source: This study was funded by the Singapore Ministry of Health National Medical Research Council NMRC/CSA INV/0012/2016. CM is supported by grants from the NMRC/TA/0003/2012, NMRC/CSA-INV/0012/2016, and NMRC/CSAINV20nov-0016.

F1229

POTENCY ASSAY MATRIX FOR DOPAMINERGIC NEURON PRECURSOR CELL REPLACEMENT THERAPY FOR PARKINSON'S DISEASE

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While the potential of cell replacement therapy for Parkinson's Disease was first demonstrated decades ago, extensive work was performed since then to develop stem cell derived neural progenitor cells with the optimal phenotype for an efficacious drug product. An additional challenge is to sufficiently characterize the potency of these progenitor cells that require in vivo maturation to perform functions critical to their mechanism of action. A novel suite of tests was developed to address these challenges. As Aspen Neuroscience advances an autologous iPSC-derived cell replacement therapy for Parkinson's Disease into clinical trials, we built a suite of analytical methods to serve as a potency assay matrix of iPSC-derived dopaminergic neuron precursor cells (DANPCs) product. Here, we show that a set of bioinformatics tests performed on bulk RNA sequencing (RNAseq) data of DANPCs, an analytical matrix which provides predictive analytics for the optimal maturity/identity, engraftment potential, and dopamine production potential. After utilizing a large, high-quality training dataset, these bioinformatics tests can reliably predict in vivo maturation, engraftment efficiency and dopamine production of DANPCs post transplantation. To overcome the limitation of bulk RNAseq not being able to capture the biochemical or physical function of DANPCs post-thaw, an enzymatic assay has been established to evaluate DANPCs functionality post-thaw. Dopa decarboxylase (DDC) was identified as an enzyme that is critical to the production of dopamine and is highly expressed in DANPCs. A DDC enzymatic activity assay was developed to assess the post-thaw function of DANPCs. Establishing the potency of stem cell derived therapies remains a challenge for many potential products, and novel work is required to address this early in the development of a therapy. This stability-indicating, conventional, enzymatic bioassay of DANPCs functionality in combination with the predictive bulk RNAseq-based bioinformatics tests provides a robust potency assay matrix strategy for Parkinson's Disease cell replacement therapy.

Funding Source: Aspen Neuroscience Inc., CIRM, Summit for Stem Cell.

F1231

INDUCED PLURIPOTENT STEM CELL-DERIVED MESENCHYMAL STROMAL CELLS: A SCALABLE AND VERSATILE SOURCE OF EXTRACELLULAR VESICLES FOR REGENERATIVE MEDICINE

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Induced pluripotent stem cell-derived mesenchymal stromal cells (iMSCs) represent an emerging approach for the production of functional extracellular vesicles (EVs) for regenerative medicine. Here, we emphasize the advantages of iMSCs as a reliable, highly expandable cell source of EVs (iEVs) and a platform for engineering EV cargo. These characteristics make iMSCs a promising alternative to tissue-derived mesenchymal stromal cells (MSCs), which are subject to donor variability. iEVs and EVs isolated from MSCs were characterized using transmission electron microscopy (TEM), flow cytometry, and interferometry to assess size, concentration, and morphology. iMSCs exhibited a 3X higher yield of iEVs, along with increased expression of exosome markers compared to MSCs. Proteomic analysis of EVs and iEVs provided valuable insights into their therapeutic cargo, including key molecules involved in immunomodulation and tissue repair. Additionally, glycan analysis identified distinct glycan signatures that play a critical role in cell recognition, adhesion, and internalization, important processes for the delivery of therapeutic cargo. Comparative functional assays conducted on fibroblasts, macrophages, and human umbilical vein endothelial cells (HUVECs) revealed that both iEVs and EVs supported key cellular processes essential for tissue repair. Notably, iEVs demonstrated greater consistency in their effects, particularly in their ability to modulate macrophage polarization from a pro-inflammatory M1 to an anti-inflammatory M2 phenotype. In vivo studies using a murine wound model showed that iEVs effectively reduced inflammation, promoted M1-to-M2 macrophage polarization, and accelerated wound healing. Furthermore, engineering approaches, such as genetic modification and preconditioning of iMSCs, further enhanced the immunomodulatory properties of iEVs. These findings support the use of iMSCs as a versatile and reliable option for EV-based therapies, offering a scalable and reproducible solution for regenerative medicine. Additionally, EV engineering provides an opportunity to extend their therapeutic potential to address a range of unmet medical needs.

F1233

A SCALABLE CULTURE SYSTEM FOR DIFFERENTIATION OF IMMUNE CELLS FROM PLURIPOTENT STEM CELLS

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Allogeneic cell-based immunotherapy (e.g. stem cell transplantation) has become increasingly relevant in the treatment of hematologic malignancies. Human pluripotent stem cell (hPSC)-derived allogeneic therapy allows for genetic manipulation and unlimited expansion of the starting material. However, the development of stem cell-based immunotherapy is limited by high costs of optimization and manufacturing challenges, and requires robust scalable protocols to produce high-quality immune cells in large quantities up to 6 x 10^9 per dose. We developed a robust serum- and feeder-free culture system for large-scale generation of multipotent hematopoietic stem and/or progenitor cells (HSPCs) from hPSCs. hPSCs from five cell lines maintained in 2D or 3D systems were aggregate passaged into a suspension culture system, such as the 6-well plate orbital shaker cultures (2 mL) or the PBS-MINI MagDrive bioreactor (e.g. 100 mL), where embryoid bodies (EBs) were spontaneously formed and cultured in STEMdiff™ Hematopoietic - EB Media. After 12 days of culture, CD34+ HSPCs suspended in culture were isolated from EBs using a cell strainer, yielding 1.3 x 10^6 ± 1.6 x 10^5 CD34+ HSPCs per 1 mL culture vessel volume, 7.0 ± 0.6 CD34+ HSPCs per input hPSC, with a frequency of 73.1 ± 6.9% CD34+ cells (mean ± SEM; n = 36). Bioreactor cultures of 100 mL performed similarly to small-scale 3D cultures, and produced 1.6 x 10^7 - 1.1 x 10^8 live CD34+ cells (n = 4), using three different PSC lines. Multipotency of the resulting HSPCs was demonstrated by successful 2D differentiation to natural killer (NK), T, B, microglia, erythroid, and megakaryocyte lineages. For immune applications, CD56+ NK cells were produced using STEMdiff™ NK reagents under 2D scalable conditions with a frequency of 76 ± 5% and a yield of 1.0 x 10^3 ± 3.0 x 10^2 NK cells per input hPSC (n = 8). Additionally, differentiation parameters can be optimized in a small 2 mL culture before scaling up to ≥ 100 mL bioreactor systems, significantly reducing costs and barriers associated with large-scale generation of multipotent CD34+ hPSC-derived HSPCs for allogeneic immunotherapies and other applications.

F1235

MONITORING SUCCESS: THE LONG-TERM SAFETY AND EFFICACY OF HEMATOPOIETIC STEM CELL GENE THERAPY

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Hematopoietic Stem Cell (HSC) gene therapy (GT) has the potential to provide lifelong reconstitution of the hematopoietic system with gene-corrected cells. However, the impact of underlying genetic diseases, replication stress, and aging on hematopoietic reconstitution and lineage specification remains incompletely understood. In this study, we analyzed hematopoietic reconstitution in 53 patients treated with lentiviral HSC-GT for conditions including metachromatic leukodystrophy (MLD), Wiskott-Aldrich syndrome (WAS), and β-thalassemia (β-Thal) over a follow-up period of up to eight years. Vector integration sites were used as markers of clonal identity. Our findings revealed that long-term hematopoietic reconstitution was supported by 770 to 35,000 active HSCs. While approximately 50% of transplanted clones demonstrated multilineage potential across all conditions, the remaining clones exhibited disease-specific patterns of lineage output and long-term commitment: predominantly myeloid for MLD, lymphoid for WAS, and erythroid for β-Thal, particularly in adult patients. These results suggest that HSC clonogenic activity, lineage output, long-term lineage

commitment, and rates of somatic mutations are shaped by several factors, including the underlying disease, patient age at the time of therapy, the extent of genetic defect correction, and the hematopoietic stress imposed by the inherited condition. This indicates that HSCs adapt dynamically to the pathological environment during hematopoietic reconstitution.

Clinical Trial ID: NCT01560182; NCT01515462; NCT02453477.

F1237

SCALING MANUFACTURING AND LOT RELEASE FOR OFF-THE-SHELF ALLOGENEIC HIPSC-DERIVED THERAPY FOR INFERTILITY

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In vitro maturation (IVM) is a fertility treatment that enables oocyte maturation outside of the body, greatly reducing the need for the high hormone doses typically required to retrieve fertilization-ready oocytes. We have recently demonstrated that supplementation of IVM media with hiPSC-derived ovarian support cells (OSCs) significantly improves oocyte maturation and euploid embryo formation by replicating the complex ovarian environment in vitro. To develop an off-the-shelf therapy capable of meeting the growing demand for assisted reproductive technologies, currently increasing 5-10% worldwide annually, manufacturing capacity must scale up to accommodate that need. In preparation for clinical and commercial manufacturing of this off-the-shelf ex vivo cell therapy, we scaled up OSC production to achieve up to 10,000 vials per lot. This scaling effort involved addressing key challenges, including high-throughput fill-and-finish processes, formulation optimization, and comprehensive lot release strategies to maintain cell therapy standards and ensure product quality across the entire lot. These strategies were designed to maintain the integrity of critical quality attributes such as identity, conformance, potency, and safety. We have demonstrated that our process can accommodate batch sizes from 1,000 to 10,000 doses, ensuring consistent quality across the lots. Our findings support the adoption of similar strategies for high-throughput manufacturing of hiPSC-derived products. Furthermore, this work represents the first description of a large scale hiPSC-derived therapy aimed at enhancing IVF outcomes and advancing women's health, paving the way for broader clinical applications.

F1239

AUTOMATION IN CELL THERAPY PRODUCTION: ENHANCING SAFETY, REPRODUCIBILITY, AND COST-EFFECTIVENESS OF HESC-DERIVED RPE CELLS

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Manual handling in cell therapy production, particularly for adherent cell types, poses challenges for scalability and increases operator variability. The scarcity of specialized Good Manufacturing Practices (GMP) production facilities further limits the accessibility to future cell therapies. This study investigates the use of automated systems to streamline cell expansion, differentiation, and the formulation of cryopreserved cell products for clinical administration. We used the CliniMACS Prodigy system with external CellSTACK to derive Retinal Pigment Epithelium (RPE) cells from human embryonic stem cells (hESCs), utilizing a GMP-compatible protocol. Additionally, we employed an acoustic-based, automated cell trapping system (AcouTrap) for washing our final, cryopreserved cell product from their freezing medium. Our results show that cell morphology, viability and marker expression of cells cultured in the CliniMACS Prodigy system were comparable to cells cultured manually. PEDF secretion levels and Trans-Epithelial Electrical Resistance measurements (TEER) further confirm the functionality of our RPE cells derived from both processes. The AcouTrap system demonstrated efficient contaminant removal, with post-wash viability and functionality levels of the resulting RPE cells matching that of manually processed cells. The use of automated systems can efficiently increase cell therapy scalability, reproducibility and safety, while decreasing production cost and operator variation. Employing an automated cell washing system to prepare cryopreserved cell therapy products for patient administration can furthermore enable bedside formulation, thereby improving accessibility in regions lacking access to specialized GMP facilities.

F1241

HOLOCLAR LESSONS: BIOLOGICAL AND CLINICAL INSIGHTS LEARNT FROM AUTOLOGOUS CULTIVATED LIMBAL STEM CELLS TRANSPLANTATION FOR REGENERATING CORNEAL EPITHELIUM IN LIMBAL STEM CELL DEFICIENCY PATIENTS

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The autologous cultivated human stem-cell based limbo-corneal epithelium is the first regenerative advanced therapy medicinal product in ophthalmology globally, and it is now a therapy with proven safety and efficacy across various regulatory frameworks available worldwide for limbal stem cell deficiency patients. In Europe is Holoclar□, applied in 4 studies compliant with current regulatory rules. These include two pan-European prospective studies (HOLOCORE and HOLOCORE FOLLOWUP) on 60 patients, monitored for 1 and 6 years, respectively; a real-world evidence PASS “registry like” 5-year follow up study on commercially-treated patients (HOLOSIGHT); and a 20-year study on patients treated previously (HOLOUP). The critical and integrate analysis of these study results has provided valuable biological insights. First, the expanded clinical experience has allowed for identification of the optimal biopsy to achieve an effective therapy. Then, optimization of biological and clinical procedures has enabled the reduction of the minimum required p63 bright stem cells within the cultivated autologous limbal graft (previously set at 3% based on statistical correlations with clinical efficacy found in real-world evidence) to 2%. Furthermore, despite the previously observed correlation between patient age and stem cell count, the positive treatment outcomes in elderly individuals (6 people treated aged 65 to 84 years) have shown that these residual stem cells were still capable of effectively regenerating tissue and maintaining it in the long term. Together with restoring tissue architecture and proper cellular localization, the transplanted cultivated epithelium produced multiple paracrine effects, steadily influencing the surrounding microenvironment with various outcomes. These included, among others, partial reabsorption of stromal opacities (suggesting an effect on keratocytes), promotion of physiological wound healing, as evidenced by full success following stromal substitution (perforating keratoplasty), regression of neovascularization, maintenance of lacrimal secretion, and improved visual acuity. FIn conclusion, this evidenced-based acquired knowledge is key for a deeper understanding of the system’s biology and for the advancement of future cell therapy

applications.

Funding Source: Holostem Terapie Avanzate S.r.l. ; Chiesi Farmaceutici S.p.A.

Clinical Trial ID: 2015-001344-11; 2014-002845-23.

F1243

CELL TRANSPLANT DELIVERY OF MAMBALGIN-1, AN ACID-SENSING ION CHANNEL INHIBITOR SNAKE PEPTIDE, COULD NUMB PAIN PERCEPTION

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Mambalgin-1, a non-toxic peptide derived from mamba snake venom, inhibits acid-sensing ion channels, which are implicated in pain perception. We engineered mouse embryonic stem cells to express mambalgin-1 using a doxycycline-inducible system to regulate secretion, along with our FailSafe™ cell system to control cell proliferation. When transplanted into mice, these cells produced mambalgin-1, leading to systemic pain relief. This study shows that the mambalgin-1 peptide, a known non-opioid pain reliever, can be effectively delivered in a controlled and continuous manner through cell transplantation. By using stem cells, we can create living systems for protein-based drug delivery, which could serve as an alternative and more advantageous to traditional methods. This strategy could lead to new and effective treatments for tissue-acidity-associated diseases, including chronic pain and other neurological diseases.

F1245

LARGE SCALE AND SERUM-FREE DIFFERENTIATION AND CULTIVATION OF HUMAN PSCS INTO ENDOTHELIAL CELLS USING A CLOSED SEMIAUTOMATED CULTIVATION PLATFORM

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The vascular system, lined by endothelial cells (ECs), provides a barrier to tissue and influences blood homeostasis. Among other processes endothelial cells are involved in neovascularization, which is essential for the growth and metastasis of tumors. In this context ECs transport nutrients and remove metabolic waste from tumor cells. To understand and influence this interactions in more detail as well as to engineer vessels and organ grafts, large amounts of ECs are required. Pluripotent stem cell derived endothelial cells (PSC-ECs) can be produced in unlimited number without ethical concern, under standardized environment and thus provide an optimal source for studying processes mentioned above. Endothelial differentiation of PSCs was performed within seven days using subsequently two different serum- and xeno-free cell culture media. Further cultivation for at least 14 days was performed under optimized xeno-free conditions. For scalable PSC-EC production we used the GMP compliant CliniMACS Prodigy® Adherent Cell Culture System. Resulting PSC-ECs revealed standard endothelial markers, showed DiI-acetylated LDL uptake and tube formation capacity. Described is a standardized, scalable and closed system to produce functional PSC-ECs in serum- and xeno-free condition.

F1247

FINDING HOPE IN UNCERTAINTY. PATIENTS' PERSPECTIVES ON CONSIDERING STEM CELL THERAPY FOR INBORN ERRORS OF IMMUNITY

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Emerging technologies such as stem cell-mediated gene therapies (GT) may provide a curative therapeutic option for patients with inborn errors of immunity (IEI). This condition presents diverse symptoms and may lead to frequent severe infections, immune dysregulation, autoimmunity, autoinflammation, allergy, and/ or malignancy. The development and evaluation of new GT interventions raise ethical challenges. To identify these challenges, insight into patients' perspectives on stem cell therapy, including hematopoietic stem cell transplantation (HSCT) and stem cell-mediated GT, is needed. These perspectives may inform the decision-making and consent process for clinical use. This qualitative study focused on the perspectives of patients with an IEI who had undergone HSCT to gain insights into how this cohort would view stem cell-based GT. Data was collected through in-depth interviews and analysed using a reflexive, inductive thematic approach. Currently, the sample consists of nine IEI patients who previously had treatment involving HSCT. Three main themes were observed in the analysis of the preliminary data. First, 'hoping for a positive future' reflects the primary motivation for considering curative therapy. Second, 'becoming prepared for the decision' highlights the critical processes patients underwent while considering a curative treatment option, such as adapting towards curation, weighing values, and needing to gain trust in the available options. Third, 'considering a hypothetical therapy' shows that while being hopeful about avoiding graft versus host disease, patients also recognized the experimental nature of GT which involves

unknown outcomes and risks that are difficult to assess. This last theme underscored the need for guidance from healthcare professionals because of the difficulty in balancing uncertainties about possible therapeutic options against patients' hope for a positive future. The patients' perspectives captured in our study highlight the complex challenges they faced when considering therapeutic options in IEI. As new GT and other novel stem cell-based interventions are developed, consideration of patients' hopes and the uncertainties regarding disease impact and therapeutic options is essential for patients to make treatment decisions autonomously.

Funding Source: Novo Nordisk Foundation Center for Stem Cell Medicine, reNEW.

F1249

HYPOIMMUNE IPSC-DERIVED MACROPHAGES ENGINEERED TO EXPRESS IL-12 ACTIVATE LYMPHOCYTES AGAINST PLATINUM-RESISTANT OVARIAN CANCER

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Immunotherapies, including immune checkpoint blockade (ICB), have demonstrated clinical success in certain solid tumor cancers such as melanoma. However, there are many cancers that immunotherapies have been unsuccessful in treating, including ovarian cancer. Ovarian cancers present immunosuppressive microenvironments, and ovarian cancer patients, particularly those with platinum-resistant tumors, respond poorly to ICB. Here we present engineered induced pluripotent stem cell (iPSC)-derived macrophages (iMacrophages) as a platform to repolarize the ovarian cancer tumor microenvironment. We generated an iPSC line with bi-allelic insertion of the human leukocyte antigen (HLA)-E transgene into the beta-2-microglobulin (B2M) gene. When co-cultured with PBMC T cells, B2M-HLA-E iMacrophages reduced the upregulation of the lymphocyte early activation marker CD69 in CD8+ T cells when compared to wildtype iMacrophages (1.72-fold versus 2.01-fold, p=0.02), suggesting that the B2M-HLA-E transgene mitigates cytotoxic T cell-mediated alloreactivity. B2M-HLA-E iPSCs were additionally engineered to express the potent immunostimulatory cytokine interleukin (IL)-12. The B2M-HLA-E/IL-12 iPSCs efficiently differentiated into iMacrophages that maintained expression of both transgenes throughout the differentiation, as measured by ELISA for IL-12 and flow cytometry for HLA-E. A feeder cell-free, xeno-free, bioreactor-based differentiation protocol amenable to clinical translation generated more than 500 million iPSC-derived macrophages in a 60mL working volume. B2M-HLA-E/IL-12 iMacrophages co-cultured with PBMC-derived T cells showed three-fold greater cell lysis of platinum-resistant SK-OV-3 ovarian adenocarcinoma cells (p=0.03) compared to B2M-HLA-E iMacrophages. In vivo, engineered iMacrophages injected intravenously trafficked to and infiltrated subcutaneous SK-OV-3 tumors while maintaining expression of the inserted transgene. These results suggest that iMacrophages could represent a novel treatment modality for immunosuppressive platinum-resistant ovarian cancer by repolarizing the tumor microenvironment via localized delivery of IL-12, while maintaining long-term persistence through hypoimmune modifications.

F1251

TOWARDS ENHANCING CELL REPLACEMENT THERAPIES FOR PARKINSON'S DISEASE – A NOVEL APPROACH FOR ASSESSING CLONAL EXPANSION AND GRAFT DIVERSIFICATION

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Human pluripotent stem cell-based therapies are emerging as a promising treatment for Parkinson's disease (PD), with ongoing clinical trials demonstrating the feasibility of targeted regenerative approaches. A significant and yet often overlooked challenge in cell replacement therapies for the central nervous system is the limited initial survival of transplanted cells; it has been estimated that less than 10% of the transplanted progenitors survive and form the graft. This limited initial survival contributes to variability of therapeutic outcomes, complicates dosing strategies in clinical trials, and necessitates the production of significantly larger quantities of cell product to achieve the desired therapeutic effect. Therefore, understanding which progenitor cells survive and mature into functional grafts is critical to advancing translational cell replacement therapies and will enable us to define the factors that influence the survival of transplanted progenitor cells. To address this, we have developed a molecular barcoding strategy to track the clonal expansion and survival of transplanted mesencephalic dopaminergic (mesDA) progenitor cells. These molecular barcodes are expressed after genomical integration by viral transduction into human embryonic stem cell-derived mesDA progenitor cells and subsequently transplanted into 6-hydroxydopamine (6-OHDA)-lesioned nude rats, an established preclinical model for PD. Graft survival and cellular composition were assessed by immunohistochemistry and single-nucleus RNA sequencing at early time points after transplantation. These analyses of these barcoded mesDA progenitors enabled assessing the evolving composition of the graft at early time points after transplantation and tracking the clonal expansion and modelling of surviving progenitors. The data from this study improves our understanding of cell survival and graft diversification after transplantation. It will enable the development of strategies to obtain more consistent graft composition and to increase initial cell viability. Ultimately, these advances could improve clinical trial outcomes by reducing variability and minimizing the required cell dose, bringing us closer to more reliable and effective cell replacement therapies for PD.

F1253

USING HUMAN PLURIPOTENT STEM CELLS DERIVED CARDIOVASCULAR CELLS FOR DISEASE MODELING AND TISSUE REPAIR

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Human pluripotent stem cells (hPSCs) serve as a powerful platform for disease modeling and regenerative medicine. We developed efficient protocols for differentiating cardiovascular cells and macrophages (MACs) from hPSCs under chemically defined conditions. Using hPSC-

derived cardiomyocytes (CMs), endothelial cells (ECs), and smooth muscle cells (SMCs), we constructed 3D mini-cardiac organoids (MCOs). Single-cell transcriptome analysis revealed that the 3D microenvironment enhanced CM maturation and led to the emergence of DLK1+ fibroblasts with immunomodulatory potential. Transplantation of MCOs into a rat model of myocardial infarction significantly improved cardiac function and reduced fibrosis, demonstrating their therapeutic promise. In addition, hPSC-derived ECs expressed markers of choroidal endothelial cells (CECs) and could integrate into the choriocapillaris. In a rat model of choroidal ischemia, transplanted hPSC-ECs restored choroid thickness and vasculature. Remarkably, EC transplantation improves the visual function of CI rats, highlighting their potential for treating ocular diseases such as age-related macular degeneration. We also demonstrated the potential of hPSC-derived macrophages as a powerful model for investigating immune interactions between pathogens and human tissues. hPSC-MAC responded to human pathogens, including hepatitis C virus (HCV), SARS-CoV-2, and Streptococcus pneumoniae, by activating distinct inflammatory and immune pathways. RNA sequencing identified pathogen-specific gene networks, and image-based analysis of iMACs interacting with HCV demonstrated their utility in evaluating infection and therapeutic strategies. Our study highlights the versatility of hPSC-derived cells in advancing disease modeling, uncovering molecular mechanisms, and developing regenerative therapies for tissue repair and regeneration.

F1255

GELMA AND POLYDOPAMINE NANOPARTICLES PROMOTE THE EFFICACY OF MSC SPHEROIDS ON DIABETIC WOUND THROUGH AN INTEGRATED LIVING DRESSING

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Wound dressing based on mesenchymal stem cells (MSC) is promising for the treatment of diabetic foot ulcer (DFU). However, the unfavorable microenvironment of the DFU including hyperglycemia, ischemia, hypoxia, hyper-oxidation, infection, and the resultant chronic inflammation confers challenges to the survival and efficacy of transplanted MSC. We have previously demonstrated that spheroids (Sp) formed by MSC differentiated from human embryonic stem cells (E-MSC) were more effective than dissociated E-MSCs to promote wound healing in non-diabetic mice. However, reduced efficacy was observed in diabetic mice in the present study. We hypothesize that improvement can be achieved by strengthening the E-MSC Sp efficacy with strategies to tackle the diabetic environment. To this end, we encapsulated E-MSC Sp with gelatin methacryloyl (GelMA) doped with polydopamine nanoparticles (nPDA) to generate a living dressing (named GnP-Sp). First, GelMA combined with nPDA (GnP) manifested excellent features of shape adaptation, adhesion, pore structure and degradation, and biocompatibility. E-MSC Sp combined with nPDA also demonstrated stronger antioxidant ability and induced higher M2/M1 macrophage ratio in vitro compared to E-MSC Sp alone. Upon application in a mouse DFU model, the GnP-Sp dressing repressed the local inflammation, expedited angiogenesis and collagen deposition, and accelerated wound repair. Furthermore, E-MSCs Sp outperformed dissociated MSCs in stimulating regenerative cytokines, chemokines, and angiogenesis factors associated with elevated hypoxic signaling. These findings suggest that GnP enhance the therapeutic efficacy of E-MSC Sp by improving the diabetic conditions and the new living dressing may serve as a more effective therapy for DFU than E-MSC Sp alone.

Funding Source: Macau Science and Technology Development Fund (FDCT) Key grant #0002/2021/AKP, National Science Foundation China General Grant #32270842, and National Key Research and Development grant of the Ministry of Science and Technology of China #2022YFA1105000.

F1257

ENHANCING THE ANTI-INFLAMMATORY PROPERTIES OF MSCS AND MSC-EVS BY OVEREXPRESSING IL-10

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Mesenchymal stem cells (MSCs) have low immunogenicity with immunosuppressive properties, making them a promising tool for cell-based therapy. They can exert their anti-inflammatory effects through direct cell-to-cell interaction and secretion of bioactive molecules, including cytokines such as IL-10. The primary function of IL-10 is to suppress inflammation. Thereby, the interaction between MSCs and IL-10 was mutually beneficial and could bring a bigger extent in promoting anti-inflammatory. On the other hand, the use of MSCs extracellular vesicles (EVs) rather than MSCs themselves becomes more attractive approach because there are certain concerns regarding MSCs as a clinical application. In this respect, we aim to enhance the anti-inflammatory properties of MSCs and their EVs by overexpressing IL-10. IL-10 was overexpressed in immortalized MSCs (IL-10-iMSCs) and their EV (IL-10-iMSCs-EV) was isolated. Both IL-10-iMSCs and IL-10-iMSCs-EV were characterized and tested in various in vitro assays for their anti-inflammatory properties. Furthermore, IL-10-iMSCs-EV content was analyzed by RNA sequencing to evaluate potential microRNA for anti-inflammatory role. During cell culture and EV production, inhouse formulated clinical grade serum free MSC medium were used. Our result showed that overexpression of IL-10 was observed in IL-10-iMSCs with downregulation of inflammation-related genes such as IL-6. Through the in vitro assays, IL-10-iMSCs treatment showed a significant reduction in pro-inflammatory genes expression and enhanced anti-inflammatory M2 related markers than normal iMSCs. This promising anti-inflammatory result was further enhanced by IL-10-iMSCs-EV. Furthermore, from the RNA sequencing result, we could identify serval miRNA that accounted for the anti-inflammatory role in IL-10-iMSCs-EV. This combination has shown promise in several models of inflammatory diseases that IL-10-iMSCs were able to reduce inflammation more effectively than unmodified MSCs. The use of IL-10-iMSC-derived EVs further extends the therapeutic potential. Overall, the synergy between IL-10 and MSCs offers a promising approach to develop advanced therapies for inflammatory diseases to achieve better clinical outcomes.

F1259

LONG-TERM SAFETY OF NOVEL DUAL INJECTION OF HUMAN NEURAL STEM CELLS (HNSCS) AND PEPTIDE CHEMOATTRACTANT IN THE ALS SOD1 MOUSE MODEL

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Amyotrophic Lateral Sclerosis (ALS) is an incurable lethal neurodegenerative disease caused by motoneuron (MNs) death leading to loss of skeletal muscle innervation, resulting in paralysis and respiratory failure in ALS patients. We previously published results showing human neural stem cells (hNSCs) delayed ALS onset and prolonged life by as much as 300% in the SOD1 mouse when injected at 4 cardinal life-preserving locations along the neuraxis, including cervical spinal cord (SC) that mediates respiration. Mechanism of action was in part via secretion of neuroprotective factors, inhibition of host NSCs from elaborating toxic astrocytes, and supplying trophic astrocytes and gray matter oligodendrocytes. The greater the expanse of donor hNSCs, the more MNs and longer life were preserved. We previously reported that a novel synthetic peptide (“SDV1a”) selectively engages the CXCR4 binding pocket on NSCs and can act as a chemoattractant directing transplanted hNSCs to desired CNS regions. Last year we presented findings from a novel minimally-invasive non-surgical route-of-administration (ROA) for hNSCs –cell suspension injection into the cisterna magna (CM) and SDV1a into the lumbar SC of pre-symptomatic SOD1 mice (60-70 days-old) to direct hNSCs throughout the SC from rostral-to-caudal, allowing ventral horn entry via CSF space by “crawling along” ventral roots and being attracted toward MNs. Preliminary results suggest this approach may be safe, effective and sufficiently non-traumatic that hNSCs can be re-administered when symptoms develop/condition begins to deteriorate. Initial hNSCs dosing allowed SOD1 mice to live at least 3.5 months longer than untreated controls. Re-dosing mice (with 1x106 hNSCs via CM + SDV1a intra-SC) when symptoms worsened (by 20% on rotarod test and BBB scale) extended life by at least another 4-5 months (when experiment was terminated, despite animals remaining minimally symptomatic) to perform MN counts. These preliminary findings suggest long-term safety of this novel ROA in SOD1 mice. By studying the efficacy, safety, and minimal discomfort of re-dosing SOD1 mice at symptomatic deterioration, we may suggest an atraumatic, well-tolerated approach to treat ALS patients recurrently when symptoms worsen, potentially extending their lives with manageable symptoms and improving quality-of-life.

Funding Source: California Institute for Regenerative Medicine (CIRM) and SENS Research Foundation.

F1261

CREATION OF THE FIRST GLOBAL CONTINUING EDUCATION COURSE FOR HEALTHCARE PROFESSIONALS ON STEM CELL MEDICINE

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The field of stem cell medicine is advancing rapidly, with several interventions progressing to clinical trials. Despite this progress, there is a rise of unproven stem cell treatments that presents significant challenges. This transformative era in stem cell medicine has the potential to revolutionize treatment options in the coming years, but effective integration into clinical practice requires clinicians to have authoritative, evidence-based knowledge for optimal patient care. To address this, the ISSCR andHarvard Medical School (HMS), has developed an open-access Continuing Education (CE) course, Stem Cell Medicine: From Scientific Research to Patient Care. The course was developed in three phases: 1) global consultation to identify unmet needs in stem cell education for clinicians; 2) prioritization of content and educational delivery methods and; 3) development of on-demand, online training material. The course consists of seven modules covering topics from basic stem cell biology to effective patient communication strategies. Key content includes distinguishing between approved, investigational, and unproven stem cell treatments, and the transition from preclinical research to clinical trials and applications. Examples from scientific literature are used throughout to illustrate key concepts. The final module synthesizes the content focusing on patient communication, and explores the concept of therapeutic hope and persuasive communication to support informed decision-making among patients and their families. Launched in May 2025, this open-access course offers American Medical Association (AMA) PRA Category 1 Credits™ and ANCC contact hours. It serves as an essential resource for clinicians, scientists, and healthcare providers, empowering them to navigate the evolving field of stem cell medicine. Future disease-specific courses will expand on areas where stem cell medicine has seen significant progress.

F1263

NOVEL MEMBRANE STIRRER FOR EFFICIENT AERATION AT HIGH CELL DENSITY BIOPROCESSING OF HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cell (hPSCs) derivatives hold immense potential for advanced drug screening, in vitro disease modelling and regenerative therapies. However, the envisioned routine application of these cells will require robust and economically viable production processes, compatible with industry and regulatory standards. In the biopharmaceutical industry, instrumented stirred tank bioreactors (STBR) are widely used for mammalian cell cultivation. This platform has also been successfully adapted for matrix-free suspension culture of hPSCs and recently enabled advanced high cell density bioprocessing of hPSCs by metabolic control and in silico modelling by our group, including process scalability to up to a working volume of 500 mL. The potential working scales for future cell productions are anticipated to exceed 2,000 L. However, oxygen supply using the conventionally applied headspace gassing becomes increasingly difficult at higher process scales, revealing a potential bottleneck for further process upscaling. In addition, using conventional sparger technologies to supply enough oxygen, leads to viability decrease and even cell death and thus is not applicable with iPSCs. BioThrust has recently developed a novel, scalable bioreactor featuring a first-of-its-kind membrane stirrer technology, enabling advanced and more physiological oxygen transfer into the cultivation medium. This innovation offers a promising solution to mitigate oxygen depletion and cell death associated with bubble aeration, particularly at high cell densities or larger process scales. We have optimized the membrane stirrer for our established high-density hPSC culture strategy through stepwise improvements in its design. These refinements enable reproducible aggregation at process inoculation and ensure efficient oxygen delivery throughout the entire cultivation process.

Funding Source: BioThrust GmbH.

F1265

A BASE EDITING PLATFORM OPTIMIZED FOR HEMATOPOIETIC STEM AND PROGENITOR CELLS RETAINS STEMNESS AND DIFFERENTIATION POTENTIAL

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Hematopoietic stem and progenitor cells (HSPCs) are a foundational cell type for the development of engineered therapies. Since engineered HSPCs are meant to persist throughout a patient's lifetime and are sensitive to DNA damage, it is important to utilize a technology which minimizes genotoxicity and allows engineered cells to retain functionality. Nuclease-induced double strand breaks (DSBs) trigger a DNA damage response which may lead to chromosomal aberrations, decreased viability and cell function, and ultimately reduce the efficacy and safety of engineered therapies. Base editors are not reliant on DSBs and represent an effective mitigation strategy but must be optimised for efficient delivery into HSPCs. Our proprietary Pin-pointTM platform is a flexible modular base editor typically comprising a DNA binding Cas, a DNA modifying deaminase, and a sequence-targeting guide

RNA which assembles at the target locus, and demonstrates an advanced safety profile compared to nuclease-based technologies. This makes it well suited to the development of cell and gene therapies in sensitive cell types such as HSPCs. In this study we used a Pin-point base editing system composed of Rat APOBEC1 mRNA, SpCas9 nickase mRNA and a synthetic aptamer-containing gRNA to edit CD34+ HSPCs with 80% efficiency. Flow cytometry of edited cells revealed an unaltered frequency of hematopoietic stem cells (HSCs) compared to the control and high levels of gene knock out in both the bulk and HSC population which was retained over 5 weeks. Moreover, primitive hematopoietic cells edited with the Pin-point platform retain their stemness capacity as validated in a Long-Term Culture-Initiating Cell (LTC-IC) assay. Additionally, qPCR revealed no significant DNA damage response, further supporting the gentler activity on cells. Next, we validated the technology on two separate loci known to reactivate γ-globin expression and achieved high levels of base editing that corresponded with an increase in γ-globin mRNA and protein expression as a relevant therapeutic outcome. The ability to base edit HSPCs efficiently and safely, while maintaining their stemness and differentiation potential, demonstrates the strength of the Pin-point platform as a tool for the generation of advanced cell therapies using sensitive multipotent cell types.

F1267

TOWARDS THE SCALABLE PRODUCTION OF IPSC-DERIVED NK CELLS

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As a cell type with minimal risk of causing graft-versus-host disease, natural killer (NK) cells are of interest for clinical applications in allogeneic adoptive cell therapy, particularly with chimeric antigen receptor-based therapies. In contrast to T cells, they are also able to mediate tumour cell killing via a variety of pathways, including direct ligand activation and antibody-dependent cellular cytotoxicity. The expansion of large NK cell quantities from healthy donors is still a technical challenge, with limited cell numbers available via the culture of primary cells. To address this, functional protocols to produce NK cells from induced pluripotent stem cells (iPSCs) at laboratory scale have been developed. However, these established protocols have process development challenges, including the use of unscalable plate-bound DLL4 to provide Notch signalling. Here, we outline the development of a scalable protocol for the differentiation of iPSCs to NK cells. Using DLL4-conjugated microbeads, we have developed a differentiation protocol that is amenable to scale-up studies, in particular dynamic cell culture systems that are compatible with eventual bioreactor-scale processes – the addition of DLL4 microbeads under wave-like mixing conditions provides the differentiating cells with sufficient Notch signalling required for the HSPC-to-NK cells differentiation phase. Using this protocol, we can generate approximately 10^3 NK cells from each iPSC over the course of 3.5 weeks, producing functional NK cells that can be further expanded. The process outlined here has implications for the scalable manufacture of NK cells, which will be required to produce sufficient quantities of this class of cell therapeutics for clinical applications.

Funding Source: ICP2300163; A*STAR-SCG Joint Laboratory for Manufacturing of Allogeneic

Cellular Immunotherapies.

F1269

TRANSPLANTATION OF ENCAPSULATED LIVER ORGANOIDS FOR THE TREATMENT OF LIVER FAILURE

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Alginate-encapsulated hepatocyte transplantation represents a promising universal hepatocyte cell therapy for treating liver failure. However, its clinical application has been limited by the scarcity of primary human hepatocytes (PHH). We previously generated proliferating human hepatocytes (ProliHH) through dedifferentiation of PHH and engineered them into encapsulated liver organoids (eLO), providing an unlimited cell source for transplantation. The preclinical efficacy and safety of eLOs have been demonstrated in mouse models in our previous study. Here, we report the large-scale manufacturing of eLOs under GMP conditions. Furthermore, we assessed the safety of the surgical procedure by transplanting clinical-scale eLOs under ultrasound guidance in dogs and pigs, demonstrating that the transplantation had no adverse effects on the animals. Based on these preclinical data, we conducted an investigator-initiated study to evaluate the feasibility and safety of eLO transplantation in patients with liver failure. Four patients with chronic liver failure or acute-on-chronic liver failure were successfully enrolled. Three of them had completed the six-month follow-up, demonstrating good tolerance to eLO transplantation. Notably, patient #1 and #3 showed improved liver functions, including increased albumin, reduced total bilirubin (TBIL) and ammonia levels, and improved INR. Collectively, these findings provide preliminary evidence supporting the safety and potential efficacy of eLO transplantation for liver failure.

Clinical Trial ID: NCT05727722.

F1271

ABSTRACT PREVIEW: LIPID NANOPARTICLES MEDIATED DUAL GENE KNOCK-OUT (KO) IN INDUCE PLURIPOTENT STEM CELLS (IPSCS)

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Human induced pluripotent stem cells (iPSCs) have emerged as novel autologous cell resources for studying degenerative diseases. Their ability to differentiate into clinically relevant phenotypic cells makes them invaluable for in vitro disease models. iPSC-derived neurons have

been used for in vitro neurotoxicity testing and the evaluation of neuronal transfection efficiency. In this study, we utilized iPSC-derived cortical neurons (iPSC CN) as an in vitro neuron model and demonstrated the efficiency of dual gene knock-out (KO) delivered through lipid nanoparticles (LNPs) containing CRISPR encoding for ALS target 1 and 2 (confidential targets). We hypothesise that target 1 and 2 can be KO in iPSC CN. SgRNA encoding for target 1/2 has been designed and validated by in vitro nuclease assay. LNPs were formulated and characterised using DLS. Encapsulation efficiency of nucleic acids was measured by RiboGreen™ assay. LNPs were incubated in serum to assess their ability to protect NA from degradation. Single and dual knockout efficiency (target 1 and 2) were assessed in iPSC CN using T7 endonuclease assay (T7EI), Sanger Sequencing and Inference of CRISPR Edits (ICE) analysis and western blotting. Off-target effect was assessed using T7EI. Serum-stable LNPs produced were 130-140 nm in diameter with -90% nucleic acid loading efficiency. Successful transfection with LNP-mediated single gene KO was achieved with a 23.3% and 23.7% gene KO in gene 1 and 2, respectively, leading to 40.5% and 54.3% reduction in target 1 and 2 protein expression. The dual gene KO LNPs encapsulating the combination of sgRNAs encoding target 1 and 2 achieved significant reductions in both gene and protein levels, with 29% and 42% KO of target 1 and 2 genes, respectively. This led to a reduction of 51.43% and 56.42% in target 1 and 2 protein levels. No off-target effects were generated by the LNPs, indicating that our LNPs are specific and efficient for delivering the dual gene KO CRISPR in iPSCs. LNPs achieved efficient single and dual gene knockout in the iPSC CNs, lays the foundation for predicting therapeutic effects in humans and facilitates the translation of in vitro results into potential in vivo clinical applications. The next stages of the project involve assessing functional studies of target 1 and 2 in iPSCs to evaluate their effects in ALS therapy.

Funding Source: Sanofi Innovation Awards; King's-China Scholarship Council.

F1273

GENERATION OF HUMAN IPSC-DERIVED HEMATOPOIETIC PROGENITOR CELLS AND DIFFERENTIATION TO FUNCTIONAL INK CELLS IN A FLEXIBLE AND FEEDER-FREE CULTURE SYSTEM

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Having a reliable source of high-quality hematopoietic stem and progenitor cells (HSPCs) is critical for developing various autologous and allogeneic cell and gene therapies. iPSC-derived HSPCs (iHSPCs) can be a consistent source for generating a variety of clinically relevant cell types, including Natural Killer (NK) and T cells. However, these differentiation processes typically take weeks or months, making labor- and material-intensive testing costly during development. Here we describe a fit-for-purpose feeder-free iHSPC differentiation system that uses all cell therapy grade and regulation-compliant reagents. This flexible protocol supports

cost-effective, small-scale testing in multi-well plates and can be scaled up through shake flasks. By avoiding steps that can limit culture scale up, like spin embryoid body formation and complicated handling, this protocol facilitates the translation of process development learnings to large-scale manufacturing. Using CTS™ StemPro™-34 serum-free xeno-free medium, we generated iHSPCs that were >65% CD34+CD43+ and >90% viable. To demonstrate functionality, the iHSPCs were differentiated into iNK cells in 100 mL cultures in shake flasks, followed by two weeks of expansion resulting in >10e9 CD56+ CD3- iNK cells, with >90% purity and >80% viability. NK cells have great potential as an “off the shelf” allogeneic therapeutic product, as they can target cancer cells in an antigen- and HLA-independent manner. We therefore tested the functional ability of the expanded iNK cells to mediate anti-tumor activity against the K562 erythroleukemia cell line and cancer organoids (“tumoroids”) derived from human colorectal adenocarcinoma. The iNK cells displayed significant cytotoxic activity, killing >80% of target K562 cells and adenocarcinoma tumoroids in a dose-dependent manner, without requiring enrichment prior to use. In conclusion, we demonstrate the use of a versatile differentiation workflow to generate clinically relevant cell types, including functional iHSPCs and iNK cells. Utilizing a cost-effective differentiation system during process development can aid in effectively characterizing both the therapeutic cells and production processes, to facilitate a smooth transition to scale-up or scale-out for clinical applications.

F1275

NOVEL COATED CONTACT LENSES AS THERAPEUTIC SCAFFOLDS FOR STEM CELL CULTURE AND TRANSPLANTATION

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Limbal stem cell deficiency (LSCD) is a major cause of blindness worldwide. The condition arises from the depletion or dysfunction of limbal epithelial stem cells (LESCs), a specialized stem cell population located in the limbus at the corneal edge. LSCD can arise from physical or chemical injuries, infections, or genetic causes, and its treatment poses significant challenges due to the loss of the cornea's natural immune privilege, heightening the risk of immune rejection. LESC transplantation is the most effective treatment for LSCD. However, achieving sufficient LESC transfer and precise placement in the eye are critical barriers to transplantation. We previously pioneered silicon hydrogel contact lenses (CLs) for LESC transplantation and demonstrated that CLs offer a practical substratum for adhesion, migration, and rapid expansion of LESCs. In this study, we aimed to assess the morphology, adhesiveness and proliferative capacity of LESCs on novel CSIRO's two distinct coatings, aminomalononitrile-30T (AMN-30T) and poly (ethylene glcol)-based cRGD. Human telomerase-immortalized limbal epithelial stem cells (T-LSCs) were seeded on coated and uncoated CLs for 2 weeks. The

effectiveness of CLs in maintaining the T-LSCs morphology, adhesion, and cellular connections was evaluated using light and scanning electron microscopy (SEM). AMN-30T-coated CLs exhibited notable improvement in cell adhesion and proliferation compared to both cRGD-coated and control CLs, underscoring the impact of CL composition on cellular behaviour. While cRGD-coated CLs were less effective in promoting adhesion, cells seeded across all CL types were morphologically similar, typically uniform with evident cell-to-cell contacts and the development of multiple cell layers. SEM revealed extensive microvilli on the apical surface and cell projections that were suggestive of anchorage points on the CL surface. In conclusion, this study offers preliminary insights into the potential use of novel-coated CLs as therapeutic scaffolds for cultivating LESCs. Future studies employing cell-laden CLs in a murine model of LSCD will further validate their efficacy for LESC transplantation and ocular surface disease treatment.

Funding Source: This research was funded by the Medical Research Future Fund (MRFF) Stem Cell Therapies Mission Grant (Application ID: 20222757).

F1277

CLINICAL SCALE NON-VIRAL GENE EDITING AND FEEDER-FREE PRODUCTION OF CAR-NK CELLS

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Engineered primary human natural killer (NK) cells demonstrate significant potential in immuno-oncology due to their proven safety and potent anti-tumor effects, particularly against solid tumors. Unlike primary T cells, NK cells do not rely on a matching human leukocyte antigen to function, reducing the risk of graft-versus-host diseases in allogeneic therapies. Gene delivery into NK cells is crucial for studying NK cell biology and developing NK cell-based immunotherapies. However, efficient, and safe non-viral gene delivery methods for NK cells remain limited. In this study, we describe non-viral based closed modular automated methods for CAR-NK cell engineering methods for clinical scale manufacturing. We first optimized the electroporation parameters using the Neon™ NxT Electroporation System 8-channel, emphasizing gRNA, buffers and payload concentrations to enhance gene delivery efficiency and cell viability. We isolated PBMCs using CTSTM RoteaTM, a closed counterflow centrifugation system. Isolated NK cells from PBMCs, were then expanded in feeder-free culture using CTS™ NK-Xpander™ Medium. The expended cells were washed, concentrated in genome editing buffer and subjected to cell engineering using CRISPR/Cas9 RNP and electroporation. First, we optimized conditions for small-scale cell editing using the Neon™ NxT, and successfully translated to a clinically relevant scale using the CTS™ XenonTM Electroporation System. Our results showed that electroporation system efficiently achieved knockout (60-80%) and knock-in (15-25%) of different genes including site-specific CAR knock-in at AAVS1 locus. The edited NK cells maintained their viability and phenotypic characteristics and achieved clinically relevant levels of cell expansion. Further, we performed functional cytotoxicity assays of CAR-NK cells and showed enhanced cytotoxicity against target cells compared to unmodified NK cells. In conclusion, this study presents closed, scalable, non-viral

gene delivery methods for primary NK cell engineering that enables efficient transgene expression without compromising NK cell immunophenotyping or function. The ability to efficiently modify primary NK cells will facilitate further studies on NK cell biology and enable the development of NK cell-based immunotherapies.

F1279

STABLE EXPRESSION SYSTEM DURING DIFFERENTIATION OF HUMAN IPSCS INTO MSCS

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Mesenchymal stem/stromal cells (MSCs) are adult multipotent stem cells that can differentiate into osteocytes, chondrocytes, and adipocytes in vitro, and are being applied in several clinical treatments. MSCs can be differentiated from human induced pluripotent stem cells (iPSCs), and our group has developed a protocol to generate induced MSCs (iMSCs) from iPSCs via the neural crest cell (NCC) lineage. To maximize the value of iMSCs for medical applications, we aim to generate highly functional iMSCs and, to this end, employ a method to transfect functional genes into iPSCs and establish transgene-overexpressing iMSCs. However, transgene silencing may occur during iPSC differentiation, which poses a significant challenge to our research. Here, we explore methods to avoid silencing using cHS4 insulator sequences combined with the piggyBac transposon system. We tested a polycistronic vector expressing neutrophil elastase (ELANE), EGFP, and Puromycin-resistant genes under the regulation of CAG, EF1A, or CMV promoter. Our results suggest that the cHS4 insulator is effective with the CMV promoter, evidenced by the higher puromycin resistance of cHS4 (+) cells during NCC expansion. Although there is no clear difference between EF1A promoter with and without cHS4 in terms of ELANE and EGFP expressions during NCC and iMSC inductions, gene expression of ELANE and EGFP was decreased during inductions, suggesting that EF1A promoter is not suitable for our stable expression system. CAG promoter is most suitable for our system because iMSCs from CAG promoter with and without cHS4 expressed the highest levels of ELANE mRNA and protein, and their expressions are higher in cHS4 (+) cells compared with cHS4 (-) cells. Now we are trying more complex vectors and optimizing vector design to achieve a more efficient and robust expression system in iMSCs.

Funding Source: Management Expense Grant from University.

F1281

COMPARISON OF THE IPSCS AND IPSC-DERIVED RETINAL PIGMENT EPITHELIAL CELLS IN TREATING SODIUM IODATE-INDUCED DRY-TYPE AGE-RELATED MACULAR DEGENERATION

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Age-related macular degeneration (AMD) is a leading cause of vision loss, with dry AMD being the most prevalent type, accounting for approximately 0.37% of global cases in 2020. Currently, there is no cure for dry AMD, and treatment focuses on disease prevention and slowing disease progression. The emergence of induced pluripotent stem cells (iPSC) and iPSC-derived retinal pigment epithelial (RPE) cells offers a promising avenue for treating dry AMD through organ transplantation, replacing dysfunctional RPE cells in the macula. However, the mechanisms and cellular aspects of iPSC and iPSC-RPE therapy are not well-understood, prompting an investigation into their underlying mechanisms. iPSC and iPSC-RPE cells were cultured to study their effects on ARPE19 cells (a cell model resembling dry AMD) subjected to oxidative stress damage. The study observed the mechanisms related to oxidative stress and cell apoptosis. Co-culturing with our cultured cells aimed to evaluate the therapeutic effects of stem cells on the cell model of dry AMD. A dry AMD cell model was established using sodium iodate, causing cells to lose control of apoptosis regulation, leading to cell necrosis. Apoptosis protein array analysis revealed a significant increase in gene expression of casp-3 and casp-8. However, co-culturing with stem cells showed a substantial reduction in cell necrosis, with the normalization of apoptosis pathways. This suggests that iPSC and iPSC-RPE exhibit the ability to treat cells, preventing extensive cell death in ARPE19 cells. Experimental results indicate that further modulation of cell apoptosis pathways could be a potential avenue for developing dry AMD inhibitors as a therapeutic approach.

Funding Source: NSTC 113-2314-B-A49-046.

F1283

SCALING UP THE EXPANSION OF MESENCHYMAL STEM CELLS DERIVED FROM WHARTON'S JELLY USING MICROCARRIERS IN BIOREACTORS FOR CLINICAL APPLICATIONS

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Mesenchymal stem cells (MSCs) derived from the Wharton’s jelly of the human umbilical cord (hWJ-MSC) are a promising source for cell therapy due to their ease of procurement and expansion capacity. However, scalable and efficient processes that maintain cell viability and multipotency are required for clinical applications. This study focuses on scaling up hWJ-MSC expansion from 150 mL spinner flasks to 500 mL bioreactors using Cytodex 1 microcarriers. Spinner flask cultures, starting with an inoculum of 1.6E+05 viable cells/mL, achieved a maximum density of 2E+06 viable cells/mL, yielding 300 million viable cells in 150 mL. When supplementing cultures under xeno-free conditions with hPL, a significantly lower yield was observed compared to FBS supplementation, with an 83% reduction in biomass due to suboptimal cell adhesion to the microcarriers. However, treating Cytodex 1 microcarriers with

APTES-GA improved adhesion, resulting in a 39% increase in biomass compared to uncoated microcarriers. Bioreactor conditions, including agitation speed, pH, and oxygenation, were optimized to achieve successful scale-up from spinner flasks, replicating the concentrations obtained in smaller-scale systems. Cells were characterized according to ISCT criteria by analyzing morphology, surface markers, and trilineage differentiation potential. Metabolic profiling of the bioreactor cultures was performed to evaluate glucose consumption and lactate production. This work represents a significant step toward scalable and clinically relevant systems for hWJ-MSC production in advanced cell therapy applications.

Funding Source: Supported by ANID-FONDECYT #11230208, ANID-Basal funds for CeBiB #AFB240001 and #FB0001, and the ANID-National Master's Grant #22240802.

F1285

INNOVATIVE POLYCLONAL GENE EDITING TECHNIQUES FOR GENERATING FAILSAFE®+ AND IMMUNE EVADING IPSCS: ADVANCING ENGINEERED CELL THERAPIES

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Engineered iPSC lines are critical for advancing allogeneic cell therapies by addressing immune rejection and ensuring that transplanted therapies can be safely removed from the patient if needed. However, current workflows for generating iPSCs harboring multiple edits are costly, time-intensive, and compromise genetic and epigenetic diversity. There is a pressing need for innovative approaches to reduce timelines and manufacturing costs while improving process efficiency and scalability. We leveraged our highly efficient, mRNA-based reprogramming technology to generate early-passage, genetically stable, polyclonal iPSC Seed Banks. These polyclonal banks serve as a robust starting material for engineering iPSC lines with enhanced safety and immune tolerance. To achieve this, we integrated a Ganciclovir-inducible suicide gene (HSV-TK) into the CDK1 locus, enabling selective elimination of dividing cells (FailSafe®+). Additionally, we performed targeted knockouts of the B2M and CIITA genes to suppress Human Leukocyte Antigen I and II expression (B2M/CIITA-/-), creating immune-evading iPSC lines. Using CRISPR-Cas9 and MAD7 nuclease, we conducted a multi-parameter process optimization to improve knock-in and multiplex knock-out efficiencies. These enhancements allowed us to maintain polyclonal populations through sequential edits, deferring clonal selection to the final process stages. This polyclonal editing strategy significantly reduced processing time and stressful passaging events, and enabled the cryopreservation of intermediate backups at critical stages to ensure process flexibility. Polyclonal editing allowed us to derive clinically relevant FailSafe®+, B2M/CIITA-/- iPSC lines while preserving genetic and epigenetic diversity. The optimized workflow is fully compatible with GMP manufacturing and significantly reduces timelines and costs for generating engineered iPSCs. By maintaining polyclonal populations throughout, our approach offers a scalable, cost-efficient solution for

producing safe and immune-tolerant iPSC lines for allogeneic cell therapies, accelerating their translation to clinical applications.

F1287

CELL BASED MRNA DELIVERY PROMOTES CARTILAGE IMPROVEMENT IN OSTEOARTHRITIC RAT MODEL

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Osteoarthritis (OA) is characterized by progressive cartilage defect accompanied by osteophyte formation and synovial proliferation, culminating in pain, loss of mobility and even disability. Half a billion people suffer from OA globally and the prevalence is predicted to increase due to aging, obesity and injury. The current treatments for OA are merely symptomatic, and most patients ultimately require joint replacement. Thus, new treatments are required to alleviate the symptoms as well as boost cartilage regeneration to halt or reverse disease progression. Due to its anti-inflammatory and differentiational properties, mesenchymal stem/stromal cells (MSC) have been used in OA treatment, but may not be adequate for effective regeneration. Here, we generated induced human pluripotent stem cell derived MSCs (iMSC) in a homogenous and potentially scalable manner. We transfected these iMSC with modified messenger RNA (modRNA) encoding regenerative factors for the treatment of OA and assessed their regeneration effects of this iMSC-mediated modRNA delivery system (iMSC-modRNA) on articular cartilage. A surgical rat OA model with medial meniscal tear and medial collateral ligament transection in the right knee was used in the study. Joint pain was observed in this model from 1 week after operation and increased in the following 4 weeks until it reached a stable level, with significant cartilage degeneration at 6 and 10 weeks. Following a single intra-articular injection of iMSC-modRNA at 3 weeks after operation, we monitored joint pain for the duration of the study and assessed joint histology once animals were sacrificed. Compared to the untreated group, animals received iMSC-modRNA experienced symptomatic pain relief from 1 week post treatment (1wpt) until the end of the study (7wpt). The total cartilage degeneration volume showed a 15% improvement in iMSC-modRNA treated OA rats at 3wpt and enhanced further to 40% at 7wpt, indicating of cartilage regeneration in the damaged knee. In conclusion, our iMSC-mediated regenerative modRNA delivery system highlights the synergistic effect of our innovative technologies and represents significant therapeutic potential in treatment of OA.

F1289

DEVELOPMENT OF TARGETED DELIVERY OF REGENERATIVE THERAPIES DERIVED FROM NON-MODIFIED AND MODIFIED STEM CELLS

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SmartCella is an innovative Swedish biotechnology company that combines the development and GMP manufacturing of regenerative medicines with methods for the targeted and precise delivery of different payloads (cells, genes, chemicals, and biochemicals) into tissues, organs, and tumour via our unique medical device - the Extroducer ®. Our pluripotent stem cell therapy

pipeline aims to treat: - Advanced ischemic heart failure, cardiac progenitor cells (SMART01) - Parkinsons' disease, ventral midbrain dopaminergic neuron progenitor cells (SMART02) - Osteoarthritis, iMSCs transformed with modRNA (SMART03) SMART01: GLP toxicology studies and manufacturing development for Ph1/2a trial - engraftment and maturation into cardiomyocytes with improvement in function indicated by a reduction in infarction volume, and increase in ejection volume (regeneration of cardiac tissue) SMART02: pre-clinical results - increased % of Th1+ cells which mark dopamine-producing cells in vitro - Fewer contaminating cells that indicate poor engraft ability- low Col1A1 and FN1 - Manufacturing process with high efficiency and purity of drug product SMART03: pre-clinical results - Compared to the untreated group, treated rats experienced symptomatic pain relief from 1 week post-treatment (1wpt) until the end of the study (7wpt). The total cartilage degeneration volume showed a 15% improvement at 3wpt and peaked at 40% at 7wpt, indicative of cartilage regeneration in the damaged knee.® SMART01- potential to improve cardiac function with reduced risks of current open chest surgical procedures - cardiac progenitor cells delivered as single cells endovascularly with the Extroducer SMART02- Faster improvement in rats vs current protocols/products SMART03- iMSC-mediated regenerative modRNA delivery system highlights the synergistic effect of our technology and represents significant therapeutic potential in the treatment of OA.

F1291

EARLY AND PROGRESSIVE DYSFUNCTION OF ENDOGENOUS BONE MARROW MESENCHYMAL STROMAL CELLS IN PARKINSON'S DISEASE: IMPLICATIONS FOR REGENERATIVE THERAPY

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Chronic and self-sustaining cycles of peripheral and neuroinflammation are now recognized as key drivers of dopaminergic (DA) neuron degeneration and the progression of Parkinson's disease (PD). Intriguingly, preclinical and clinical studies have consistently shown a strong association between prior exposure to chronic inflammatory conditions and an increased risk of developing PD. Systemic immune imbalances are typically regulated by immunomodulatory cells such as bone marrow-derived mesenchymal stromal cells (MSCs). While autologous MSC transplantation in PD patients has demonstrated transient motor improvements, its lack of sustained benefits underscores the need to study endogenous MSCs under disease conditions before they can be considered viable therapeutic candidates. In a chronic in vivo PD model induced by MPTP, we examined the status of endogenous MSCs during both premotor and motor stages. Our results indicate that MSC dysfunction begins early, at the premotor stage, and progressively worsens with disease advancement. Transplantation of healthy rat MSCs at the premotor stage effectively halted neurodegeneration, promoted neurogenesis, and mitigated inflammation. Additionally, we assessed the similar physiological and functional parameters in sporadic PD patient iPSC-derived MSCs (PD-iMSCs) which showed impaired proliferation, differentiation, migration, and immunomodulatory functions, alongside elevated

basal ROS levels. When transplanted into MPTP rats, PD-iMSCs failed to curb inflammation, prevent neurodegeneration, or restore motor function, unlike healthy iPSC-derived MSCs, which were effective when administered early. This study is the first to demonstrate that endogenous MSC dysfunction in PD correlates with midbrain gliosis and inflammation, emphasizing the importance of early intervention to preserve MSC function and develop effective regenerative therapies.

Funding Source: Indian Council of Medical Research (ICMR), Department of Science and Technology (DST), India Department of Biotechnology (DBT), India.

F1293

HUMAN UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELL THERAPY FOR SEVERE BRONCHOPULMONARY DYSPLASIA REFRACTORY TO CONVENTIONAL THERAPY: A PHASE I CLINICAL TRIAL

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Bronchopulmonary dysplasia (BPD) is a common and serious complication affecting very low birth weight preterm infants, characterized by impaired lung alveolarization and dysregulated vascularization. Infants with moderate-to-severe BPD face a significant risk of long-term respiratory and developmental disabilities. To date, there are no effective pharmacological treatments for BPD. Mesenchymal stem cells have been considered as a promising therapeutic option, offering potential to improve lung structure and function in BPD patients. The aim of this study is to explore a gap in BPD treatment by evaluating the safety and efficacy of intratracheal administration of human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) in preterm infants with severe BPD. This prospective trial was conducted on preterm infants, born at 23 to 29 weeks of gestation with severe BPD, diagnosed at 36 weeks postmenstrual age. These infants remained difficult to wean from invasive mechanical ventilation, despite having received conventional therapy, including surfactants and steroid. Each infant received a single intratracheal dose of hUC-MSCs (1×10^7 cells/kg). To date, three patients have been successfully enrolled in the study. No severe adverse events related to the intervention were observed at the time of this report. Following transplantation of MSCs, there was a progressive reduction in ventilator parameters, including fraction of inspiration O2 (FiO2) and mean airway

pressure. All three patients were successfully extubated at a range of 10-19 days after MSCs transplantation, with no re-intubation required prior to discharge. The chest radiographs of the three patients showed a mitigation of atelectasis, diffuse haziness and reticular opacities in lungs, 2 months after MSCs transplantation, suggesting signs of lung recovery. In summary, intratracheal administration of allogeneic hUC-MSCs appears to be a safe and feasible therapy for preterm infants with severe BPD.

Funding Source: This work was supported by the Pediatrics development Fund of School of medicine from Zhejiang University Education Foundation.

Clinical Trial ID: ClinicalTrials.gov ID: NCT06788470.

F1295

MSC-DERIVED EXTRACELLULAR VESICLES OBTAINED USING PS-AFFINITY METHOD SHOWED HIGHER ANTI-INFLAMMATORTY ACTIVITIES THAN OTHER CONVENTIONAL METHODS

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We developed a reproducible method for affinity purification of EVs by using Tim4 protein, which specifically binds phosphatidylserine displayed on the surface of EVs. Because the binding is Ca2+-dependent, intact EVs can be easily released from Tim4 by adding Ca2+ chelators. Here, we tested the profiles of the purified EVs, including biological activities of EVs, and compared with other commonly used methods for large-scale purification of EVs, such as tangential flow filtration (TFF) combined with size exclusion chromatography (SEC) or anion exchange chromatography (AEX) methods. Agarose resin immobilized with recombinant Tim4 protein was packed into a 1mL column. 200 mL of bone marrow-derived MSC culture supernatant was directly applied into the Tim4 column. After washing the column, 4 mL of EDTA solution was applied to elute the captured EVs. The resulted EVs were analyzed by using NTA, ELISA, BCA methods, and cell-based functional reporter assay. In parallel, EVs were also purified from the 200 mL of the same culture supernatant by using TFF, TFF + SEC, or TFF + AEX, and the resulted EVs were analyzed for comparison. It was confirmed that EVs can be purified with higher yield (recovery was 70 - 90%) and purity than the other methods. We also observed that the purified EVs by using the Tim4 column showed similar surface tetraspanin distributions to what observed with EVs purified with the Tim4-immobilized magnet beads-based small scale purification method, suggesting that this affinity-based method is scalable. Furthermore, the Tim4 column purified EVs showed higher anti-inflammatory effect than other conventional methods. Therefore, we propose that our affinity purification method has potential for use in manufacturing process of therapeutic EVs.

F1297

THE ROLE OF AGING IN MODULATING ADIPOSE-DERIVED MESENCHYMAL STEM CELL EXTRACELLULAR VESICLES: INSIGHTS INTO IMMUNOMODULATION AND TISSUE REPAIR

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Adipose-derived mesenchymal stem cells have gained significant attention in regenerative medicine due to their potential therapeutic applications. Providing their paracrine signaling regulating recipient cells as the extracellular vesicles (EVs) exhibit rejuvenation and immunomodulation properties. Autologous EVs derived from a person's adipose tissue hold significant therapeutic potential due to their compatibility with an individual's immune system, reducing the risk of adverse reactions. The differences between EVs derived from young and aged adipose tissues MSCs highlight the importance of understanding age-related changes in the EVs composition and function. The different age female donors for adipose tissues were obtained from 28 to 60 years old; we discussed the shift in MSCs proliferation, differentiation potential, regenerative effect, and immunomodulatory properties. Moreover, the differences in EVs characteristics, diverse cargo of bioactive molecules, and potency for skin rejuvenation and aging have addressed the importance of considering age-related factors when utilizing the autologous adipose-derived MSC and its extracellular vesicles for aesthetic dermatology and regenerative medicine.

Funding Source: 1. Thailand Science Research and Innovation Fundamental Fund Fiscal year 2025 2. Chulabhorn International College of Medicine Research Grant 3. Stemsciences Co., Ltd.

F1299

MICRORNA PROFILE INCLUDING A-TO-I EDITOME AS DIAGNOSTIC AND PHARMACODYNAMICS MARKERS FOR ALS

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Hyperexcitation due to defective A-to-I editing of GRIA2 has been reported in motor neurons of amyotrophic lateral sclerosis (ALS) patients. To clarify the miRNA profile, specifically A-to-I editing, in extracellular vesicles (EVs) derived from the body fluids of patients with ALS. Neuronal EVs were isolated from the serum and cerebrospinal fluid (CSF) of 20 patients with sporadic ALS (SALS) (time-course samples) and 10 healthy controls who participated in the ROPALS trial, and miRNA-seq analysis was performed after extracting the miRNAs in the EVs. A-to-I editing was detected in miRNAs in neuronal-derived exosomes and the A-to-I editing rate was calculated. The A-to-I editing rate of miRNAs in the neuronal-derived exosomes of SALS patients was significantly lower than that of healthy controls, but was significantly increased in CSF by ropinirole treatment. The diagnostic performance of miRNAs in neuronal-derived exosomes by A-to-I editing rate was AUC 0.690 for Blood and AUC 0.791 for CSF. The random forest classifier achieved diagnostic performance of AUC 1.00 for Blood and AUC 0.99 for CSF. Measuring miRNAs derived from EVs in the body fluids of ALS patients may contribute to disease diagnosis and evaluation of disease progression. As a diagnostic biomarker, the A-to-I editing rate by GRIA2 mRNA in CSF has been proposed. miRNAs can be diagnosed not only in CSF but also in blood samples and are detectable in all cases. miRNAs of the exosome in CSF may be useful as pharmacodynamic useful as markers in ropinirole.

Funding Source: This study was supported by the grant support from Japan Society for the Promotion of Science (JSPS) and Japan Agency for Medical Research and Development (AMED).

F1301

PARACRINE EFFECTS OF MNCQQ CELL-DERIVED CONDITIONED MEDIUM PROMOTE ANGIOGENESIS AND WOUND HEALING

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We have previously established a novel type of cells named MNCQQ cells which is from a serum free quantity and quality control culture (QQc) of peripheral blood mononuclear cells (MNCs) that increases the vasculogenic and tissue regeneration ability of MNCs. The medium derived from MNCQQ cells (QQ-Sup), has shown potential due to its rich composition in growth factors, cytokines, and exosomes, including numerous angiogenesis-related factors. Recently, emerging research on various cell-conditioned media has revealed significant therapeutic effects, prompting us to further investigate the specific therapeutic impact of QQ-Sup. This study aims to evaluate the angiogenic and regenerative capabilities of QQ-Sup on ischemic condition, assessing its potential in both in vitro and in vivo and exploring the molecular

mechanisms behind its therapeutic properties. QQ-Sup was collected from MNCQQ cultures and tested for its angiogenic potential on Human umbilical vein endothelial cells (HUVEC). The effects on HUVEC proliferation, migration, and tube formation were assessed through MTT assays, wound healing assays, and tube formation assays, respectively. Key angiogenesis-related factors in QQ-Sup were quantified using ELISA. Inhibition experiments using specific blockers validated the roles of these factors in angiogenesis. A hindlimb ischemia mouse model was also established to evaluate the therapeutic efficacy of QQ-Sup in vivo. QQ-Sup treatment significantly enhanced HUVEC proliferation (0.46 ± 0.09 vs. 0.84 ± 0.10/24h; $p < 0.01$), migration (29.21% ± 3.87% vs. 96.1% ± 3.21%/18h; $p < 0.001$), and tube formation (16.17 ± 3.16 vs. 22.29 ± 2.25/5h; $p < 0.01$). ELISA identified elevated levels of two key angiogenic factors in QQ-Sup, further confirmed by the reduced angiogenic activities upon their inhibition in functional assays. In vivo experiments showed that QQ-Sup-treated mice exhibited significantly better outcomes, with reduced ischemic damage. This study confirms that QQ-Sup can significantly promote angiogenesis and accelerate wound healing through paracrine actions of key angiogenic factors, providing a promising therapeutic option for ischemic and vascular-related disorders in regenerative medicine.

F1303

VALIDATING GMP-CONFORM CRYOPRESERVATION OF HUMAN UMBILICAL CORD TISSUE FOR DOWNSTREAM ISOLATION OF WHARTON'S JELLY MSCS

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The isolation and cryopreservation of Wharton's Jelly mesenchymal stem cells (WJ-MSC) under Good Manufacturing Practice (GMP) conditions is an important prerequisite for the future clinical implementation of WJ-MSC. Although the isolation of WJ-MSC from fresh human umbilical cord (UC) has become a standard procedure in most laboratories, the quality of WJ-MSC after cryopreservation of UC tissue remains a topic of debate. Human UCs were transported from the hospitals to the class 5 cleanroom facility, samples were cleaned of residual blood, vessels were removed, and UCs were minced into small pieces 1-2 mm in size. Finally, the minced UCs were cryopreserved in 1.5 cryo-vials, which were stored in -80°C for 24 hours. The cryo-vials were then transferred to liquid nitrogen. After three-months of cryopreservation, WJ-MSCs were isolated from UC tissue of different donors. At the three-month mark, the cord from one donor was used for isolation, while at the six and nine-month marks, the cord from two donors were used, including the cord from the three-month isolation and one additional cord. The enzymatic isolation of WJ-MSCs was performed according to current GMP guidelines. After the isolation process, the sterility test was negative. Bright field images showed the typical spindle shape of adherent cells. Flow cytometry revealed expression of MSC-specific markers CD73, CD 90 and CD105 at both passage 0 and 1 in 99% of the cells. Less than 1% of cells expressed negative MSC markers (CD34:CD45). In conclusion, cryopreservation of minced human UC tissue fragments is a feasible and safe method for

preserving UC tissue for the post-stored isolation of WJ-MSCs.

F1305

PRECLINICAL EVALUATION OF AUTOLOGOUS IPSC-DERIVED MIDBRAIN DOPAMINERGIC PROGENITORS FOR THE TREATMENT OF PARKINSON'S DISEASE, UX-DA001

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Parkinson's disease (PD) is primarily characterized by the selective loss of midbrain dopaminergic (mDA) neurons in the substantia nigra. Aiming at replacing the lost neurons, cell replacement therapy using human pluripotent stem cell-derived midbrain dopaminergic progenitors (mDAPs) is a promising therapeutic avenue for PD with recent rapid advancements in clinical trials. Here we present the preclinical data supporting the first-in-human phase 1 clinical trial of a personalized autologous cell therapy product, UX-DA001, for PD. We established clinical-grade induced pluripotent stem cells (iPSCs) by reprogramming patients' peripheral blood mononuclear cells in a GMP facility. The iPSCs from multiple patients were then differentiated into cell products with consistent cellular composition and high mDAP purity using our newly developed 3D cultivation protocol, as confirmed by scRNA-seq analysis, and free from residual iPSCs or genetic aberrations related to cancer- or neurodegenerative diseases. No adverse effects were observed in a mouse GLP-compliant safety study for toxicity, biodistribution, and tumorigenicity. Notably, we achieved high in vivo mDA neuron yields across batches derived from multiple patients, with more than 50% of the human cells in the graft positive for TH (DA neuron marker), accounting for more than 20% of the number of transplanted cells, at 6 months post-grafting. Most of these DA neurons are positive for EN1, a classical midbrain marker These in vivo outcomes demonstrate the high efficiency and robustness of our new differentiation protocol, bolstering the feasibility of personalized cell therapy for numerous patients with PD. An efficacy study confirmed that the transplanted cells mediated full dopamine level restoration in the grafted striatum (by microdialysis coupled with HPLC) and behavioral recovery in a mouse model of PD. Furthermore, the parkinsonian non-human primates receiving mDAP transplantation exhibited behavioral improvements accompanied with strong DA activity in positron emission tomography. Based on these results, the IND application of UX-DA001 has been recently cleared by the National Medical Products Administration in China for a phase 1 clinical trial for PD, and a clinical trial (NCT06778265) for treating patients with PD has been initiated.

Clinical Trial ID: NCT06778265.

F1307

IPSC-DERIVED ENDOTHELIAL CELLS PROMOTE NEOVASCULARIZATION AND LIMB SALVAGE IN PERIPHERAL ARTERY DISEASE

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Peripheral artery disease (PAD) is a common vascular disorder caused by atherosclerosis and endothelial dysfunction, leading to reduced blood flow to the limbs and increasing the risk of critical limb ischemia, gangrene, or limb necrosis. Risk factors such as diabetes, hypertension, dyslipidemia, smoking, and obesity accelerate its progression. Despite its widespread prevalence, effective therapeutic options for PAD remain limited. To address this unmet need, we investigated the therapeutic potential of induced pluripotent stem cell (iPSC)-derived endothelial cells as a novel cell-based therapy for PAD. Using a well-established hindlimb ischemia (HLI) model in immunodeficient NOD-SCID mice, we evaluated the therapeutic efficacy of iPSC-derived endothelial cells delivered via intramuscular injection at dosages ranging from 0.3 to 10 million cells, administered 24 hours post-HLI surgery. Blood flow recovery was assessed with laser Doppler imaging, and histological evidence was confirmed using immunofluorescence staining. Following HLI surgery, blood flow in the ischemic limb decreased to less than 15%, indicating severe vascular compromise. At 28 days post-treatment, iPSC-derived endothelial cells demonstrated a dose-dependent effect, with the administration of 0.3–1×10^6 cells significantly enhancing blood flow recovery. However, higher doses (10 million cells) were less effective compared to 1×10^6 cells. Limb salvage scores showed a consistent trend with blood flow results. Histological analysis revealed that iPSC-derived endothelial cells contributed to neovascularization in the ischemic limbs. This proof-of-concept study demonstrates the therapeutic potential of iPSC-derived endothelial cell therapy to restore vascular function in PAD. These findings offer a promising foundation for future translational and clinical applications, addressing a critical need in PAD treatment and improving the quality of life for affected individuals.

Funding Source: National Science and Technology Council (NSTC) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan.

F1309

FACET-BASED DNA NANOSTRUCTURES FOR ENHANCED STABILITY OF NUCLEIC ACIDS IN VIVO

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Traditional methods for targeted cellular delivery have issues related to biocompatibility. High dosage of lipofectamine or coacervate would lead to cell damage that a high delivery efficiency

is almost impossible. A biocompatible delivery agent is urgently needed to tackle this challenge. We recently developed a facet-based design where equimolar canonical DNA oligonucleotides self-assemble to form a nested cube linked by trapezoids, denoted as a tesseract inspired by the four-dimensional hypercube. Cryogenic electron microscopy (Cryo-EM) and atomic force microscopy (AFM) analysis revealed a fully formed tesseract structure with exceptional stiffness and a melting temperature of 84°C, significantly higher than other unmodified DNA nanostructures. The stability of tesseract allowed us to further study the efficacy of cellular delivery. Luciferase mRNA and antisense oligo were tethered onto the tesseract separately for the delivery in mouse and cellular model. The expression of luciferase was found to be superior to using coacervate in animal models. The antisense oligo on tesseract was found to be delivered into the MC3T3E1 for the knock down of MMP14 protein to enhance proliferation of bone tissue tackling the issue of osteopenia. These two examples illustrated the potential of using DNA tesseract as a versatile platform for the delivery of imaging or therapeutic agent in vivo with extraordinary stability and biocompatibility.

Funding Source: GRF (No. 17125920, 17125221) TBRS T12-201/19-R HMRF 09202246 HKU Seed Funding for Strategic Interdisciplinary Research (No. 102009959) HKU Seed Funding for Basic Research (No. 328279218) NSF (Grant no. CMMI 2323969).

F1311

APTAMER-EMBEDDED DNA SUPRAMOLECULAR HYDROGELS ENHANCE CELL PROTECTION, RETENTION, AND CHONDROGENIC DIFFERENTIATION TO PROMOTE CARTILAGE REPAIR

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The precise regulation of stem cell fate remains a central challenge in stem cell-based cartilage tissue engineering for post-injury joint regeneration. While stem cell transplantation is considered a promising clinical intervention for early-stage cartilage defects, its therapeutic potential is limited by suboptimal engraftment efficiency, low cell survival rates, and inconsistent differentiation outcomes. DNA nanomaterials, known for their molecular programmability and high biocompatibility, hold promise to improve the outcomes of stem cell-based cartilage repair strategies. However, three-dimensional (2D) DNA biomaterials suffer from limited structural stability and rapid enzymatic degradation, while 3D DNA nanomaterials are complex to fabricate. Herein, we developed a hybrid DNA supramolecular hydrogel that combines the design flexibility of 2D architectures with the mechanical robustness of 3D frameworks. Through molecular dynamics simulation and rolling circle amplification techniques, we synthesized multilayer DNA hydrogels with stable hierarchical organization. The introduction of aptamer structures into the DNA hydrogel network enhanced its affinity for cartilage and mesenchymal stem cells (MSCs). The 3D hydrogel network demonstrated favorable rheological properties, rapid self-healing capacity, and excellent cytocompatibility in vitro. Functional assessments revealed the hydrogel's capacity to mitigate shear-induced cell damage and enhance MSC retention at defect sites. Furthermore, 3D culture experiments demonstrated that the DNA supramolecular hydrogel created an conducive environment for MSCs, effectively supporting

their differentiation into cartilage tissue. In vivo evaluation using a rat osteochondral defect model confirmed the efficacy of aptamer-functionalized DNA hydrogels, which showed significant improvements in cartilage regeneration and functional recovery compared to scaffold-free MSC transplantation approaches. This work established a proof-of-concept for engineered DNA-based hydrogels that synergistically improved the protection, spatial retention, and chondrogenic differentiation of delivered MSCs for cartilage repair.

Funding Source: This work was supported by the National Key Research and Development Program of China (to QYG, 2019 YFA 0110600) and the CNRM (to RST and ZAL), under the Health@InnoHK program launched by the ITC, Hong Kong SAR Government.

F1313

XT-THRIVE, A NON-DMSO ALTERNATIVE TO CRYOSTOR FOR SUPERIOR CRYOPRESERVATION OF BONE MARROW DERIVED MESENCHYMAL STROMAL CELLS

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Cryopreservation is crucial for off-the-shelf cell-based therapies, stabilizing the supply of vital products and supporting centralized manufacturing. Conventional cryopreservation involves the application of DMSO which can effectively preserve most cellular products, but can impair functional recovery in sensitive cells, induce mild to severe toxic effects in patients, and even in some cases cause chromosomal and epigenetic alterations. Using DMSO-free cryoprotectants minimizes these risks, ensuring cell viability and functionality after thawing, and enable safer injectable therapies. In this study, we compared the effect of DMSO-free cryoprotectant XT-Thrive® (X-Therma Inc.) and DMSO-containing CryoStor®10 (CS10, Biolife Solutions) on bone marrow-derived (BM) MSCs expanded in serum-containing and serum-free medium. The BM-MSC cell viability after a pre-freeze incubation at room temperature showed that XT-Thrive® maintained a ~30% higher viability compared to CS10, even after extended incubation at room temperature for up to 24 h before freezing (~93% vs. ~61% viability). This was mirrored in a similar post-thaw 6 h incubation at room temperature, with a higher cell recovery and viability (~87% vs. ~63% viability). Furthermore, cells preserved in XT-Thrive® exhibited a ~2.5-fold expansion in serum-containing media when cultured in a monolayer and microcarrier format, similar to the expansion capabilities of CS10 (2.4 fold expansion). XT-Thrive® also showed improved cell expansion when the cells were cultured in microcarriers cultures in serum-free medium (XT-Thrive®: 2-fold vs CS10: 0.9-fold expansion) for 6-days. These findings highlight XT-Thrive®s ability to support superior cell recovery, viability, and expansion, making it an effective, non-toxic and resilient solution for largescale manufacturing.

Funding Source: Agency for Science Technology and Research (A*STAR).

F1315

DERIVATION OF ENTERIC NEURAL PROGENITORS FROM HUMAN INDUCED PLURIPOTENT STEM CELL AND EVALUATION OF THERAPEUTIC POTENTIAL AS A SOURCE FOR CELL-BASED THERAPY OF HIRSCHSPRUNG'S DISEASE

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Dysfunction of enteric nervous system (ENS) leads to severe motility disorder of gastrointestinal tract including rare diseases such as Hirschsprung's disease (HSCR). To date, no cell-based treatment has yet been developed to fully restore its function. Here, we introduce our novel culture condition that enables efficient induction and expansion of enteric neural progenitors (ENPs) from human induced pluripotent stem cells (hiPSCs) via neural crest cell (NCC) lineage. Induced ENPs (iENPs) co-expressed key progenitor genes SOX10 and PHOX2B and showed high differentiation potential into subtypes of enteric neurons and glial cells in vitro. The single cell RNA sequencing revealed that iENPs followed trajectory of ENS development and their similarity of iENP-derived enteric neurons to those from human fetal colon. Expanded iENPs could be engrafted into intestinal muscle layer of immune deficient mice and showed differentiation into neural and glial lineage. Potential of iENPs to recover motility function in the HSCR gut environment was demonstrated using two different HSCR-relevant models. We developed immune deficient HSCR disease model mice (B6RGs-Retwt/S811F) which showed reduced gut motility due to aganglionosis at the distal end of the colon. Four months after transplantation of iENPs into aganglionic region of the B6RGs-Retwt/S811F mice, statistically significant recovery of electrical field stimulation-induced gut contraction was detected by organ bath assay. Also, efficacy of iENP transplantation was confirmed in the human intestinal gut organoid-based model derived from hiPSCs harboring HSCR-related point mutation. These data suggest that iENPs are a desirable source for cell-based therapy of HSCR.

F1317

A SIMPLE AND FLEXIBLE CELL FACTOR ASSAY PANEL FOR CLINICAL STUDY SAMPLE ANALYSIS

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Inflammatory factors and cytokines are an important indicator of the immune response towards new cell therapies and other biologics. Based on its original inflammatory triggers, the diversity of inflammatory factors can vary widely. As such, clinical study samples are focused on

quantifying the level of cell factors (such as cytokines) after administration of novel therapeutics. However, samples obtained from clinical trials are subject to several limitations such as sample type, amount, and volume. As such, only a select number of factors can be quantified at a single time. Through traditional methods, this greatly limits the number of studies that can be performed on a single sample due to incompatibilities with the sample matrix, sample volume consumed per test, and total number of samples to be tested. Another challenge is also the resources available at clinical study centers across the world. Whereas top-of-the-line flow cytometers may not be readily available, utilizing cost-effective instruments can reduce the entry barrier and information gathered. To overcome these challenges, a simple, multiplexed flow cytometry-based assay platform was developed to meet the stringent conditions to operate in clinical studies. This platform requires only two fluorescent activation channels for operation: the APC channel dedicated towards separating each marker by its fluorescent intensity and the PE channel used to quantify the concentration. Unlike traditional multiplexed assays that require multiple fluorescent channels, this platform unlocks the upper boundary of quantifying over 10 factors simultaneously within a single 15 µL sample injection and a limit of detection of 1 pg/mL. By minimizing the instrument requirements, sample volume, and enabling a flexible, 10+ multiplexing panel capability, clinical study samples across the globe can obtain a wider variety of information from each patient sample and improve clinical research.

F1319

EVALUTATING CLOAKED-FAIL/SAFE CELLS AS A UNIVERSAL ALLOGENIC SOURCE FOR TRANSPLANTATION IN HUMANIZED MOUSE MODELS

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Cell transplantation faces significant hurdles: finding an abundant source of healthy cells, the risk of allograft transplantation rejection, and tumorigenicity of in vitro expanded cells. This study introduces a universal allogeneic donor ES cell line, Cloaked-FailSafe™ (CFS) cells, designed to tackle these issues. The cell line can be used to differentiate cells into various cell types and expanded in vitro using any established protocol. CFS cells are engineered with ‘cloaking’ offensive and defensive mechanisms to evade immune rejection. Furthermore, these cells contain a drug-inducible "kill-switch" to eliminate uncontrolled proliferating cells via the application of the drug ganciclovir. However, prior to clinical application, these cells need to be tested against human immune cells. Earlier, we tested the CFS cells and control H1 parent

cells with human NK and T cell lines in addition to human PBMC to show suppression of immune responses by CFS cells. However, recognizing that in vitro testing using immune cells cannot fully replicate in vivo conditions, we proceeded to assess the engraftment or rejection of the CFS cells following their transplantation in a humanised mouse model. We subcutaneously injected 10^6 cells into humanised mice and compared these to the 'uncloaked' H1 control cells and assessed this over time using bioluminescent imaging. We showed that the CFS cells increased in number with time and formed visible lumps by 6 weeks at the injection site. On the other hand, the H1 cells reduced in number and resulted in either no or very small lumps, likely scar tissue, at the site of injection. For the mice injected with CFS cells, when the lumps reached 400-500mm^3 in size, ganciclovir was injected daily for between 1-2 weeks, resulting in a size reduction of the lumps and then tissue stabilization. In conclusion, the CFS cells show acceptance of transplanted cells in an allogeneic humanized mouse model, and their growth can be effectively regulated with a drug. This makes them a promising and safe universal cell source for in vitro differentiation into therapeutic cells aimed at addressing a wide range of degenerative diseases.

Funding Source: The work in this study was supported by the RGC European Union - Hong Kong Research and Innovation Cooperation Co-funding Mechanism (E-HKU703/18).

F1321

IMPROVED VASCULARIZATION OF 3D BIOFABRICATED HUMAN STEM CELL-DERIVED KIDNEY TISSUE

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Chronic kidney disease is a global health crisis, with end-stage renal disease (ESRD) patients requiring long-term dialysis or kidney transplantation. Dialysis has an exceptionally low 5-year survival of under 40%, restores only partial renal function, and is associated with both high healthcare costs and low quality of life for patients. Kidney transplantation remains the only cure for ESRD, but is severely hindered by the organ shortage, highlighting a clinical imperative for alternative therapeutic strategies. Recent advances in pluripotent stem cell-derived kidney organoids, exhibiting multicellular complexity and aspects of renal structure and function, support their use as an innovative source of functional nephrons for organ repair/replacement strategies. Translation will require the ability to reliably scale organoid production, pattern tissue geometry, establish functional vasculature, and promote developmental maturation in vitro. We have developed an innovative approach to bioengineering kidney by integrating stem cell biology, 3D biofabrication, primary human microvessels, and whole tissue perfusion to generate vascularized, anatomically patterned human kidney tissues. Here we show that exposure of biofabricated kidney tissues to flow/shear stress promotes nephron maturation in vitro, characterized by improved cellular organization of developing glomeruli, with expansion of Bowman's space, as well as increased renal tubule lumenization, polarization, and transporter expression. Additionally, we demonstrate that incorporation of human microvessels, in combination with perfusion culture, leads to high density tissue vascularization, with evidence of glomerular wrapping by endothelial cells, capillary invasion, and formation of vascular structures with patent lumens. These findings mark a significant advancement in the field of renal tissue engineering, demonstrating the feasibility of scaling kidney organoids into

vascularized, patterned tissues with functional potential.

Funding Source: This work is supported by Wellcome Leap funding as part of the HOPE Program.

F1323

THE THERAPEUTIC EFFECTS OF ADIPOSE STEM CELL EXTRACELLULAR VESICLES ON LYMPHEDEMA

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In the past decade, cell therapy has provided researchers with new inspirations in the treatment of secondary lymphedema. Adipose derived stem cell (ADSC) therapy and their derivatives are emerging as a promising treatment. This study aims at exploring the therapeutic effects of ADSC extracellular vesicles (EV) and its potential as an assistive therapy for vascularized lymph node transfer (VLNT) on a mouse lymphedema model. The mouse lymphedema model was established with three separate procedures, radiation 1 week before and 2 days after surgery (a dose of 20 Gy in two fractions), and surgery of resecting popliteal and inguinal lymph nodes. Mice are divided into 4 groups: therapeutic groups (PBS/EV injection on the 7th day postoperatively) and assistive therapy groups (VLNT immediately following the surgery and PBS/EV injection on the 7th day postoperatively). Circumferential measurement of hindlimb, weekly micro-CT imaging for volumetric measurement and the limb excessive volume % is used. The recovery of lymph flow is monitored monthly using near-infrared fluoroscopy. At 1 month after injection, histological staining, immunohistochemical staining, western blotting and qPCR analysis were conducted to observe any lymph vessel regeneration, local inflammation or fibrosis. Both EV group and EV+VLNT group indicated significant decrease in limb circumference and limb excessive volume % compared to PBS group and PBS+VLNT group respectively. ICG fluorescence results showed earlier lymph flow recovery in EV-treated groups. The numbers of lymphatic vessels by LYVE-1 immunohistochemistry, and VEGF-C expressing cells were significantly increased in EV group compared to PBS group ($p < 0.05$). The IHC results of type I collagen and pro-inflammatory factors indicated that the inflammatory level and fibrosis were significantly lowered by EV treatment. Lymphangiogenesis gene expression was upregulated in EV group compared to PBS group. The prescription of ADSC-EV alleviates the symptoms of lymphedema and increases the number of lymphatic vessels. It also helps accelerate the recovery of lymph flow after VLNT. This study offers a novel, promising approach in lymphedema management.

F1325

DEVELOPING A FLEXIBLE CELL PROFILING PLATFORM TO QUALIFY RAW CELLULAR MATERIALS AND FINAL THERAPEUTICS

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Flow cytometry cell profiling is a laboratory technique that relies on multiparameter flow cytometry to characterize immune cell heterogeneity. Despite its common use as a diagnostic tool in monitoring immunodeficiencies, cell profiling also plays a role in ex vivo cell manufacturing, especially for stem-cell derived therapeutics. In particular, the precise identification and characterization of specific cell populations provides important information regarding the composition of the raw cellular material. In turn, this knowledge can help control the quality of the raw material to the required standards for manufacturing and greatly increase the quality and consistency of the final therapeutic. This is especially crucial in autologous therapies where the patient variability can significantly compromise quality and therapeutic efficacy. With the advent of standard of GMP guidances, drug manufacturers are striving to deliver personalized medicine quickly. Thus, the importance of rapid and high-throughput tests in evaluating patient cell type variation is paramount. A rapid, flexible cell profiling platform was developed using fluorescence-conjugated antibodies alongside gating logic and cell analysis. The entire method was designed to be run in less than 30 minutes with clearly distinguishable cell subpopulations and validated using varying sample types CAR-T, PBMCs, and more. We successfully utilized this platform to perform cell profiling of a pool of healthy donor samples, elucidating the relative abundance of CD3+ T lymphocytes, T helper cells, T cytotoxic cells, B lymphocytes and NK lymphocytes. However, this platform has the flexibility to be adapted for different cell profiling requirements and is rapid enough to be performed throughout the manufacturing process. With the availability of a cell profiling platform, this tool highlights the utility of multiparameter flow cytometry for ex vivo cell manufacturing of autologous and allogeneic cell therapies.

F1327

TRANSPLANTATION OF HYALINE-CARTILAGE-LIKE TISSUES INDUCED FROM HUMAN PLURIPOTENT STEM CELL-DERIVED LIMB BUD-MESENCHYMAL CELLS TO A MINI PIG MODEL WITH OSTEOCHONDORAL DEFECTS

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Cartilage tissue is known to have poor self-repair capacity and lacks effective treatments for damage. Although the advancement of regenerative medicine, challenges remain in the quality of repaired tissue and biological integration at the transplant site. We have successfully developed a technique for expandable limb-bud mesenchymal-like cells (ExpLBM) while maintaining their chondrogenic differentiation potential. Furthermore, we have confirmed the engraftment of cartilage-like tissues derived from ExpLBM into immunodeficient mice and rats. In this study, we aimed to evaluate the clinical applicability of this approach using an osteochondral defect Göttingen mini-pig model. We assessed the effects of immunosuppressive agents and produced various shapes of ExpLBM-derived hyaline cartilage-like tissue, followed by xenotransplantation into large animals. Tacrolimus was administered to eight mini-pigs, and blood concentrations were measured accordingly. ExpLBM cells were induced to undergo chondrogenic differentiation using a 96-well culture plate or a self-aggregation technique, producing spherical or sheet-like ExpLBM-derived hyaline cartilage-like tissues. These tissues were transplanted into 3 mm diameter osteochondral defect on the medial femoral condyles of both knees. Two weeks after transplantation, tissue sections were prepared for Safranin O staining and immunohistochemistry (human Vimentin / Aggrecan / COL2), followed by histological evaluation. The blood concentration of tacrolimus remained stable and consistently exceeded the minimum therapeutic concentration at trough levels. Additionally, engraftment of ExpLBM-derived hyaline cartilage-like tissues, exhibiting tissue morphology similar to normal cartilage, was confirmed at all transplantation sites. Given that successful engraftment was observed in the load-bearing site of the mini-pig knee joint cartilage. ExpLBM-derived hyaline cartilage-like tissues are considered to be potentially useful for transplantation therapy in knee joint cartilage defect site.

F1329

APTAMER-MEDIATED TARGETING OF MMP14 AS A POTENTIAL THERAPEUTIC FOR LOW BONE MASS

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Osteopenia, a concern in aging and congenital disorders (1), involves Mmp14, a matrix metalloproteinase crucial for the transition from chondrocytes to osteoblasts. Ablating Mmp14 in mice increased trabecular bone, enhancing PTH impact on osteoblastogenesis (2). Inhibiting MMP14 may be a potential therapeutic strategy for osteopenia. Antisense oligonucleotides (ASO), a common gene knockdown therapy, modify gene expression and mRNA splicing in genetic disorders (3). Stabilization strategies, like self-assembled DNA nanostructures, aid intracellular delivery. Combining aptamers with DNA nanostructures addresses several limitations, allowing specific targeting of molecules, surfaces, and cells (4). Herein, this study is aiming to develop a self-assembled DNA nanostructure as a carrier of gene silencing therapeutics targeting osteoblasts for bone and cartilage. We have identified a single-stranded DNA (ssDNA) aptamer, Apt15 targeting osteoblasts through cell-SELEX and high-throughput

sequencing, utilizing the MC3T3-E1 cell line as the target and the ATDC-5 chondrocyte cell line as the control. It demonstrated nanomolar-range dissociation constants (Kd values), displaying robust binding affinity and selectivity under physiological conditions. Directly incorporated Apt15 to DNA nanostructures significantly improved its cellular delivery efficiency and also facilitate target delivery with less off-target effect. This study presents a promising approach for delivering gene silencing therapeutic agents specifically to osteoblasts, and it broadens the potential use of DNA nanotechnology in precise drug delivery.

Funding Source: This work was partially HMRF fund, grant number 09202246.

F1331

CIRCULAR RNA-MEDIATED PARTIAL REPROGRAMMING AS A NOVEL THERAPEUTIC STRATEGY FOR IDIOPATHIC PULMONARY FIBROSIS VIA REVERSING PATHOLOGICAL CELL STATES OF ALVEOLAR EPITHELIAL CELLS AND LUNG FIBROBLASTS

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Idiopathic pulmonary fibrosis (IPF) is a progressive and fatal lung disease characterized by excessive deposition of extracellular matrix and the transformation of alveolar epithelial cells and fibroblasts into pathogenic cell states. In this study, we explored the therapeutic potential of partial reprogramming (PR) using lipid nanoparticles (LNPs) encapsulating circular RNAs (circRNAs) encoding OCT4, SOX2, and KLF4 (OSK) to treat IPF. Partial reprogramming is an innovative approach that aims to revert cells to a more youthful and regenerative state without inducing pluripotency. This technique involves the transient expression of reprogramming factors, such as OCT4, SOX2 and KLF4, which can induce cellular rejuvenation. The utilization of circRNAs for expressing PR factors offers several advantages, including their stability and low immunogenicity, making them an efficient and safe gene delivery vector for therapeutic applications. Our results demonstrated that PR treatment effectively reversed TGFβ-induced epithelial-to-mesenchymal transition (EMT) phenotypes in alveolar epithelial cells, as evidenced by the upregulation of epithelial markers such as E-cadherin and the downregulation of mesenchymal markers such as N-cadherin and vimentin. RNA-seq analysis showed that this reversal was mediated through the TGFβ, WNT, and PI3K signaling pathways. Besides EMT markers, PR treatment also upregulated the AT2 cell markers such as SFTPB, SFTPC and SFTPD, indicating recovering cells to a more regenerative state. Additionally, PR also reversed the fibroblast-to-myofibroblast transition (FMT) in lung fibroblasts, with downregulation in FMT markers such as collagen type I (COL1A1) and α-smooth muscle actin (α-SMA). Moreover, the effects of PR treatment in reversing pathological cell states of alveolar epithelial cells and lung fibroblasts outperformed those of pirfenidone (PFD) treatment. To further validate these findings, we established an IPF model using lung organ-on-a-chip technology, which confirmed the therapeutic effects of PR treatment. This study provides a novel therapeutic strategy for IPF by leveraging the regenerative capacity of cells through partial reprogramming using LNP-encapsulating circRNAs, offering a promising avenue for future clinical interventions.

F1333

COMPOSITE FUNCTIONAL CARDIAC TISSUE SHEETS WITH HIPSC-CMS AND HADSCS FOR TREATING MYOCARDIAL INFARCTION

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Human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have gained significant attention in the treatment of ischemic heart disease. However, challenges such as immune rejection and low retention rates still hinder their therapeutic potential. Human adipose-derived mesenchymal stromal cells (hADSCs) have been shown to modulate immune responses, promote angiogenesis, and improve the maturation of hiPSC-CMs. In this study, we co-cultured hiPSC-CMs and hADSCs on a biodegradable poly (D, L-lactic-co-glycolic acid) (PLGA) fiber scaffold for several days to generate a composite 231.00 ± 15.14 μm thick 3D cardiac tissue sheet. As anticipated, the resulting tissue exhibited improved structure, alignment, extracellular matrix (ECM) condition, contractility, and paracrine activity compared to hiPSC-CMs cultured alone on the PLGA fibers. Additionally, the composite tissue sheet significantly enhanced cell engraftment and survival following transplantation. In a rat myocardial infarction model, this composite tissue not only improved cardiac function but also reduced ventricular remodeling, fibrosis, and promoted angiogenesis. These findings suggest that this composite 3D cardiac tissue sheet could represent a promising therapeutic approach for clinical applications.

F1335

ATTEMPTS TO DIRECT CONVERSION OF T CELLS AND ESCS/IPSCS INTO RETINAL PROGENITOR-LIKE CELLS BY SMALL MOLECULE COCKTAILS

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Blindness are consider as a terrifying disease in the world, although some can be treat by surgery or drug deliver. Photoreceptor degeneration diseases, account for 1/4 of the untreatable blindness. These diseases include macular degeneration (AMD), diabetic retinopathy (DR), and retinitis pigmentosa (RP), affects millions of patients world-wide, remain untreatable due to the lack of effective therapies or surgical interventions. Fetus derived retinal progenitor cells (RPCs) hold promise in the clinical trials and animal experiments. However, the clinical application is now limited by issues such as immune rejection, ethical concerns, and limited availability. T cells, widely used in cancer therapy, which not only can easier and safer to harvest compared to other cells, can be massively activated and expanded in vitro, but also foundation for autologous personalized medicine. ESCs/iPSCs has the capacity to self renew, unlimited proliferation in vitro, and differentiate into all cell types in the human body. Here we

aim to develop small molecules to covert T cells and ESCs(Embryonic Stem Cells)/iPSCs(Induced Pluripotent Stem Cells) into retinal progenitor-like cells. Direct conversion by chemical cocktail avoid the insertion mutagenesis of virus integration. Demonstrating a non-genetic, small-molecule-based strategy for direct cell fate conversion. Remarkably, after five days of treatment with our small molecule cocktail, T cells exhibited neuronal-like morphology and expressed key RPC markers. Similarly, ESCs/iPSCs displayed early-phase neuronal differentiation and upregulated RPC-associated genes. These findings suggest that small-molecule-induced reprogramming can provide an alternative source of RPCs, potentially overcoming the limitations of current stem cell therapies. In conclusion, this study not only presents a novel, efficient strategy to generate RPCs direct from T cells and ESCs/iPSCs, offering a potential pathway for future treatments of blinding diseases caused by photoreceptor degeneration, but also brings hope to those suffering from vision loss.

F1337

ENHANCING THE SUSTAINED RELEASE OF GROWTH FACTORS IN PRP PROCEDURES USING A FIBRIN FIBER

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PRP (Platelet-Rich Plasma) refers to a procedure that involves collecting a patient's blood, followed by centrifugation to obtain plasma with a platelet concentration 4-5 times higher than that of normal blood. The biological advantages of PRP include its autologous origin, which reduces the risk of immune reactions or side effects, an increased release of growth factors due to concentrated platelets, and superior biocompatibility compared to synthetic materials by mimicking natural healing processes. However, PRP has limitations: most growth factors are released within 10 minutes to 24 hours after injection, resulting in a short duration of effect; the use of anticoagulants (e.g., EDTA) can cause pH mismatches leading to growth factor loss; and efficacy varies depending on the patient's platelet condition. These drawbacks highlight the need to optimize PRP's therapeutic potential. Additionally, previous studies suggest that PRF (Platelet-Rich Fibrin) extends growth factor release through a fibrin matrix but may lack sufficient initial concentration.To address this, this study aimed to select an appropriate anticoagulant, adjust its concentration and centrifuge speed, extract fibrin fibers from PRF, and combine them with PRP's growth factors to enhance the duration and concentration of growth factor activity. The goal was to develop a method that achieves an optimal concentration for a relatively simple injectable procedure. Due to experimental constraints, direct experimentation was not feasible; instead, results were predicted by comparing and analyzing existing literature and validated through empirical patient cases. Theoretical considerations were discussed with various researchers and clinicians to refine the approach.This study confirmed that the proposed method could be broadly applied to burn treatment, scar therapy, and skin aesthetics.

Poster Session 3 (EVEN)
5:00 PM – 6:00 PM

TRACK: CLINICAL APPLICATIONS (CA)

F1212

COMPARATIVE STUDY OF SERUM-FREE CULTURE MEDIA ON THE GROWTH AND FUNCTION OF UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS

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MSCs (Mesenchymal Stem Cells) have garnered significant attention for their potential in regenerative medicine and cell therapy. The selection of cell culture medium is crucial, as MSCs are exceptionally sensitive to their cultivation environment. Traditionally, fetal bovine serum (FBS) has been used to supplement MSCs cultures. However, due to undefined and heterogeneous nature of animal-derived components in FBS, there have been efforts to develop serum-free culture media for MSC cultivation. Numerous commercially available serum-free media claim to support or enhance the growth and function of MSCs. Therefore, choosing the appropriate culture medium is vital for maintaining MSC quality and quantity. In our study, we utilized umbilical cord-derived MSCs (UC-MSCs) to compare and evaluate the functional changes between different serum-free culture media. We assessed parameters such as cell proliferation, MSCs' surface markers, cytokine secretion, trilineage differentiation, immunosuppressive capability, and angiogenic potential. Additionally, we conducted RNA sequencing to analyse the changes in gene expression. Our findings suggest that Rohto's culture medium significantly enhances the growth and function of MSCs. We anticipate that Rohto's media will maximize the quality and quantity of MSCs, thereby making a significant contribution to regenerative medicine research.

F1214

INVESTIGATING GENOME EDITING AS A POTENTIAL THERAPEUTIC FOR LATE-ONSET RETINAL DEGENERATION

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Late-Onset Retinal Degeneration (L-ORD) is a rare, autosomal dominant disease caused by mutations in the C1QTNF5 gene, leading to vision loss through RPE dysfunction. As a late onset disease, there is a window of opportunity to treat patients prior to vision loss. Here, we investigate a dual cutting Non-Homologous End Joining (NHEJ) CRISPR/Cas9 approach, to specifically excise and switch off the mutated allele as a potential preventive measure for vision loss. We used 3rd Generation Sequencing (Oxford Nanopore long-read sequencing) in a L-ORD patient cohort to reveal shared, mutant allele-specific Single Nucleotide Polymorphisms (SNPs) that could be targeted with CRISPR. We also generated induced Pluripotent Stem Cells (iPSC) from a L-ORD patient skin biopsy using episomal vectors. iPSCs generated were then nucleofected with spCas9 and combinations of two guides, and editing efficiency was examined by Sanger sequencing, ICE analysis and Snapgene. Dual edited clones were differentiated into iPSC-derived RPE and C1QTNF5 expression and localisation was studied. Our results

revealed the cohort of L-ORD patients had 24 shared SNPs, of which 3 created novel spCAS9 SiPAM sites (NGG) unique to the mutant allele. Using RNPs targeting these mutant-specific SiPAM sites, we successfully excised ~7.9kb within the C1QTNF5 gene. Isolation of clones demonstrated that the excision was specific to the mutated C1QTNF5 mutated allele. C1QTNF5-/+ iPSC successfully differentiated into RPE, and C1QTNF5 and collagen IV deposition in the basal lamina seen in the mutant iPSC-RPE cells was lost. Our results show that the identification of allele-specific SiPAM sites on the mutated C1QTNF5 gene can be used for dual-guided CRISPR-Cas9 targeting and excising of the dominant C1QTNF5 allele, without affecting the WT allele. Most importantly, editing corrects the dominant negative phenotype of L-ORD cells. With no treatments currently available for L-ORD, this therapeutic approach could be used to inactivate the faulty gene, preventing loss of vision in L-ORD patients. Future work will further characterise the functional implications of knocking out the mutant allele. We will also test our editing approach directly in iPSC-derived RPE using a range of delivery methods.

F1216

GENERATION OF A CARDIAC-SPECIFIC RECOMBINANT PROTEIN TOOLBOX FOR DIRECT CARDIAC REPROGRAMMING AND OTHER BIOMEDICAL APPLICATIONS

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The ever-increasing burden of cardiovascular disorders in today's world necessitates the need to find a suitable regenerative therapy for the generation of functional cardiomyocytes (CMs). Existing methods for the same involve the use of viral vectors, which eventually integrate within the genome of resulting myocytes, rendering them unfit for cell therapy. The quest for a minimal yet efficient cardiac reprogramming cocktail has thus motivated us to investigate the efficacy of using transcription factors (TFs) in the form of recombinant proteins to generate integration-free functional CMs by reprogramming human fibroblasts. The domain of cardiac reprogramming using recombinant proteins offers multiple advantages over the use of integrative methods and is yet to be fully explored to unravel its immense potential. To achieve this, we have generated a recombinant protein toolbox consisting of a unique combination of six cardiac-specific TFs, which can potentially replace their viral counterparts in the direct cardiac reprogramming paradigm. Firstly, the TFs have been expressed and purified under native conditions from a bacterial system with maximum purity. We have then validated the transduction ability and the functionality of the purified proteins in mammalian cells using various assays. Following this, each purified cardiac-specific recombinant protein was applied to fibroblasts, substituting its respective lentiviral vector in the reprogramming factor combination. This substitution successfully induced the upregulation of cardiac-specific markers, facilitating the generation of CMs from fibroblasts. Simultaneously, induced pluripotent stem cells (iPSCs) were differentiated into beating CMs, further enriched via lactate enrichment to give a homogenous population of cells. Overall, this unique toolbox of recombinant fusion proteins will thus serve as a safe alternative for the prospective derivation of integration-free functional CMs, thereby bringing cell therapy closer to clinic. Further, the knowledge gained about the crucial TFs and signalling pathways would broaden our horizon for a better understanding of the cardiovascular

milieu in general.

F1218

HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED OVARIAN IMPLANTS TO PREVENT THE ADVERSE EFFECTS OF MENOPAUSE

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Hormone deficiencies from menopause significantly affect women's health with widespread effects ranging from osteoporosis to vasomotor symptoms. The current therapeutic options are limited, with treatments to manage individual symptoms and hormone replacement therapy (HRT), which has been associated with severe side effects, being the primary courses of action. As such, innovative therapeutic solutions to address this problem are urgently needed. We are developing a bioengineered ovarian implant to restore hormone balance by leveraging human induced pluripotent stem cell (hiPSC)-derived ovarian support cells (OSCs). The OSCs have been optimized for high steroidogenic activity through comprehensive screening, including cell viability screening, secretome analysis, phenotypic profiling, and molecular characterization. To support estrogen production, we established androgen delivery systems using engineered theca cells and controlled-release androstenedione microbeads. The implant design incorporates an advanced alginate encapsulation strategy, ensuring structural integrity, long-term cell viability, efficient hormone diffusion, and non-immunogenicity. These advancements demonstrate the potential of this implant as a functional and durable option for hormone restoration therapy. The next step in the development of this implant will involve the generation of a prototype with preclinical assessment and validation, which is currently underway.

Funding Source: This work was supported by an award from the ARPA-H Sprint for Women's Health initiative, dedicated to advancing innovative solutions in women's health.

F1220

ADVANCING ALLOGENEIC CAR-T CELL THERAPY: OVERCOMING CHALLENGES IN AUTOLOGOUS GRAFTING AND LENTIVIRAL TRANSDUCTION THROUGH IPSC AND CRISPR TECHNOLOGIES FOR T-CELL MALIGNANCIES

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Recently discovered adoptive cell therapies such as CAR-T cells have shown great success in treating hematological malignancies, although several factors prevent such therapies from reaching their full potential. Parallelly, advances in stem cell differentiation and engineering

strategies hold the potential to overcome the current limitations of CAR-T cells in especially challenging scenarios such as T cell-derived malignancies. This project addresses key challenges in CAR-T therapy development for T cell malignancies such as difficult T cell isolation, fratricide, and poor phenotypes. We will achieve this by overcoming autologous grafting limitations by using iPSCs to create allogeneic CAR-T cells and optimizing CAR-T cell production by introducing strategic gene modifications on the stem cell stage resulting in an improved cell product. Our results show that individual KOs of potential T cell targets CD1a, CD2, CD3, CD5, CD7, CD30, and CD37 in T cells do not result in collateral loss of other markers and maintain proper activation upon stimulation. Moreover, lentiviraly-transduced CD3/7-targeting CAR-T cells with CD3/7 KO exhibit a favorable phenotype and excellent cytotoxic potential, as confirmed by flow cytometry and Incucyte assays. While CRISPR-based CAR delivery into T cells has been successful, further optimization is ongoing to improve efficiency. Lentiviral delivery of the CAR and clone selection in iPSCs have also been successful, yielding pure cultures of CAR-expressing stem cells ready for differentiation into CAR-T cells. Additionally, iPSCs from the CiRA Center (Kyoto), lacking human leukocyte antigen (HLA), are engineered to carry the CAR, target KO, and additional deletions (e.g., PD-1), ready to start the allogeneic T cell differentiation protocol. This work lays the foundation for allogeneic CAR-T cell products with enhanced safety for T cell malignancies. At the same time, iPSC technology promises to overcome autologous grafting challenges, addressing clinical and economic obstacles in CAR-T therapy.

Funding Source: DigitSTEM Initiative.

F1222

FEASIBILITY OF USING STEM-CELL DERIVED HUMAN TROPHOBLASTS FOR MASS PROPAGATION OF LIVE ATTENUATED VACCINE STRAINS OF SARS-COV-2 AND OTHER VIRUSES

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Existing vaccines for SARS-CoV-2 are less satisfactory in providing mucosal immunity to block viral transmission. An ideal vaccine should elicit long-lasting and robust protection in the mucosa, which is crucial to prevent reinfection. We have previously constructed a dually inactivated SARS-CoV-2 named d16i3a, which is severely attenuated in vitro and in vivo. The 2'-O methyltransferase activity of NSP16 in d16i3a has been inactivated and the ORF3a accessory gene has also been disrupted. The robustness of d16i3a in inducing mucosal and cross-variant protection in animal models indicates its potential to be used as a booster vaccine against SARS-CoV-2. In addition, we have also substituted the ancestral viral spike with that derived from the Omicron XBB.1.16 variant so that optimal protection against emerging variants can be achieved. Since it is severely attenuated, mass production of d16i3a requires special cell lines that have been optimized for viral propagation. Our previous work has shown that human early syncytiotrophoblasts derived from trophoblast stem cells are highly susceptible to SARS-CoV-2 infection. However, the susceptibility varies among derivatives of the trophoblast stem cells and its differentiation stages. In this study we optimized the derivation of SARS-CoV-

2-susceptible human early syncytiotrophoblasts from trophoblast stem cells. The cells were further engineered to enhance virion production of recombinant SARS-CoV-2 viruses. Their potential for mass production of other viruses including influenza A virus was also assessed. Our findings suggest that stem cell-derived human trophoblasts might be developed as a tool for mass production of live attenuated vaccine strains of SARS-CoV-2 and other emerging viral pathogens.

Funding Source: Hong Kong Health and Medical Research Fund grant COVID190114 Hong Kong Research Grant Council T11-709/21-N Innovation and Technology Commission grant Mainland-Hong Kong Joint Funding Scheme MHP/128/22.

F1224

POST-MARKET STUDY OF THE CLINICAL EFFICACY OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED OVARIAN SUPPORT CELLS FOR INFERTILITY TREATMENT

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Despite the popularity of in vitro fertilization (IVF), several barriers to accessibility remain, including cost, time, and the amount of hormone injection required. In vitro maturation (IVM), in which the patient undergoes an abbreviated stimulation regimen, reduces the burden associated with IVF. However, the current standard of care treatment for IVM has limited efficacy with low rates of oocyte maturation, embryo formation, and pregnancy. Recently, we demonstrated the clinical development of Fertilo, which is a novel ex vivo applied cell therapy product designed to support the IVM of oocytes via co-culture with current Good Manufacturing Practice (cGMP) compliant human induced pluripotent stem cell (hiPSC)-derived ovarian support cells (OSCs). To evaluate the clinical efficacy of Fertilo, a two phase study was conducted. In the first phase, 50 patients underwent 5 different minimal stimulation regimens prior to IVM with Fertilo to identify the optimal regimen. In the second phase, 20 patients underwent the preferred minimal stimulation regimen and retrieved oocytes were subjected to IVM in either Fertilo or MediCult IVM control conditions. In phase I, stimulation with CC and rFSH + trigger led to the most favorable outcomes, with an 11% euploid HQ blastocyst formation rate per COC and a 33% ongoing pregnancy rate per cycle. In phase II, Fertilo significantly improved the euploid HQ blastocyst formation rate (p=0.0135) and the transferable blastocyst formation rate (p=0.0040) compared to MediCult IVM. Of note, embryo transfers are ongoing in both phases and no adverse events have been reported for any patients. In phase I of this study, the first live birth of a healthy singleton female following a Fertilo treatment cycle occurred at 38.5 weeks without complication, providing evidence of the efficacy and safety of Fertilo and the first ever report of a live birth from a hiPSC-derived cell therapy application in IVF. These results demonstrate that the hiPSC-derived cell therapy Fertilo applied with a minimal stimulation regimen including CC and rFSH is an efficacious and safe option to improve IVM outcomes and broadens the population of individuals suitable to undergo IVF.

Clinical Trial ID: ISRCTN36032472.

F1226

PSEUDO-COST-EFFECTIVENESS ANALYSIS OF AUTOLOGOUS ADIPOSE-DERIVED MESENCHYMAL STEM CELL THERAPY FOR K/L 3 AND 4 PATIENTS WITH KNEE OSTEOARTHRITIS COMPARED TO TOTAL KNEE ARTHROPLASTY

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Knee osteoarthritis (KOA) is characterized by progressive cartilage degeneration, often driven by aging and mechanical stress, and results in pain and functional impairment. Although adipose-derived mesenchymal stem cells (ASCs) have emerged as a potential KOA treatment, they are costly and exhibit cellular heterogeneity that can affect efficacy. Moreover, there is insufficient evidence to determine whether ASCs are superior to existing therapies. This study developed a novel patient-level economic model, incorporating individual patient attributes, to examine whether ASCs are more cost-effective than total knee arthroplasty (TKA), the gold standard for severe KOA, within a relatively short timeframe. Knee Injury and Osteoarthritis Outcome Score, Visual Analogue Scale and Timed Up and Go Test were used as outcome measures. This study conducted scenario analyses under different reimbursement structures, demand estimation and preferences, and cost assumptions to account for the uncertainty in future health care policies. In a scenario where ASCs and TKA operate under the same reimbursement system, occupy equal portions of the market, and apply different markup rates, ASCs prove to be cost-effectively dominant over short periods (1, 3, and 6 months). In a scenario where the reimbursement system for ASCs differs significantly from that for TKA—yet both hold equal market shares and apply different markup rates—TKA is cost-effectively dominant, whereas ASCs are dominated over the same short-term intervals. In the most realistic scenario, where reimbursement systems, market shares, and markup rates all differ between ASCs and TKA, insufficient long-term evidence remains to determine whether ASCs are more cost-effective than TKA. Results from additional scenarios—such as those involving demand estimation, patient preferences, and cost assumptions—will be presented in full at the conference. In summary, this study is the first to develop a novel, individual-level model for cell therapy from an economic perspective. It aims to contribute to optimal treatment for severe KOA patients while emphasizing that patient cooperation in data collection is essential for verifying long-term cost-effectiveness.

Funding Source: This work was supported by JST SPRING, Grant Number JPMJSP2123.

F1228

DIRECTED DIFFERENTIATION OF MRNA-REPROGRAMMED IPSCS VIA BIOREACTORS GENERATES CLINICALLY RELEVANT YIELDS OF NEURAL STEM AND PROGENITOR CELLS THAT CAN BE ENGINEERED TO SECRETE THERAPEUTIC PROTEINS

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Neural stem and progenitor cells (NSPCs) hold potential as a potent cell therapy because of their unique ability to provide trophic support, modulate neuroinflammation, and further differentiate into neurons and glia to restore structure and function to damaged areas of the CNS. Allogeneic brain-derived NSPCs engineered with viral vectors have been explored as a therapeutic modality; however, this approach faces major challenges that hinder its clinical translation, including limited source material and risk of viral integration. Here, we demonstrate a scalable process for differentiation of mRNA-reprogrammed induced pluripotent stem cells (iPSCs) to iNSPCs. Differentiation in a 0.1L bioreactor generated 1.9 x 10^6 iNSPCs with a percent yield >2-fold higher than a static differentiation performed in parallel. Bioreactor-derived iNSPCs were passaged in static and expanded approximately 17-fold over nine days, implying a theoretical yield of 3.2 x 10^7 cells from a 0.1L bioreactor (or 3.2 x 10^8 cells per liter), exceeding the number of cells administered in recent or ongoing clinical trials for ALS and retinitis pigmentosa, which ranges from 0.3 – 10.5 x 10^6 NSPCs per patient. Key iNSPC markers SOX1, nestin, and PAX6 were upregulated in both bioreactor- and static-derived iNSPCs; however, bioreactor-derived iNSPCs displayed approximately 4.6-fold higher expression of PAX6 and 2-fold lower expression of SOX10 (a negative iNSPC marker) than static-derived iNSPCs. After one additional passage and cryopreservation, bioreactor-derived iNSPCs were positive for SOX1 (90%) and nestin (95%), and negative for pluripotency markers TRA-1-60 and TRA-1-81 (< 1%). Additionally, we demonstrated that iNSPCs could be non-virally engineered to overexpress neurotrophic factors. Electroporation with mRNA encoding glial cell line-derived neurotrophic factor (GDNF) or brain-derived neurotrophic factor (BDNF) resulted in >1ng/mL of secreted protein, compared to undetectable GDNF levels and >25-fold lower BDNF levels in untransfected iNSPCs. Our results suggest that mRNA-reprogrammed iPSCs can be effectively differentiated using a scalable process to generate engineered iNSPCs, which could accelerate the clinical translation of cell therapies for neurodegenerative and neuroinflammatory diseases.

F1230

HOLOCORE: A PAN-EUROPEAN PHASE 4 CLINICAL TRIAL ON THE EFFICACY AND SAFETY OF HOLOCLAR FOR LIMBAL STEM CELL DEFICIENCY

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In 2015, the European Medicines Agency (EMA) granted conditional market authorization for Holoclar, a treatment based on ex vivo expanded autologous human corneal epithelial cells aimed at restoring the corneal epithelium in patients with limbal stem cell deficiency (LSCD) from ocular burns. Subsequently, a large-scale pan-European trial was conducted to fulfil EMA requirements to confirm efficacy and safety in patients with moderate to severe LSCD and obtain the full market authorization in the EU. This study presents the design and key findings of HOLOCORE and HOLOCORE FU trials, multinational, multicentre, prospective, open-label, uncontrolled phase 4 clinical trials. HOLOCORE enrolled 80 patients (13 female, 67 male), aged 2 to 84 years, who met the specific inclusion criteria for this treatment. Following the withdrawal of some participants, 69 patients were considered suitable for evaluation. HOLOCORE FU produced the results of 6-year follow-up. The primary parameter assessed was the treatment's efficacy, defined as the proportion of patients with fewer than two quadrants of superficial corneal neo-vascularization, no central corneal involvement by neo-vascularisation, and the absence of epithelial defects at day 360 following Holoclar□ treatment. Efficacy was estimated at 77% by site investigators (based on real clinical assessments) and 50.9% by independent assessors (based solely on 2D images of the ocular surface). The adverse event analysis revealed that 63.8% were unrelated to Holoclar, while only 7.2% were directly associated with treatment failure with no severe adverse events. LSCD symptoms significantly reduced, from 87% at baseline to 55.7% after 12 months, accompanied by improvements in corneal and conjunctival sensitivity and an overall enhancement in quality of life. Most importantly, Best Corrected Visual Acuity improved from 32.7% to 57.8% following transplantation, with 100% of patients without stromal scarring showing visual improvement after treatment. Based on these results, Holoclar received full market authorization in the EU and the UK in 2024. The treatment demonstrated comparable efficacy and safety to previous trials, solidifying its role in managing moderate to severe LSCD and establishing a model for developing other advanced therapy medical products.

Funding Source: Holostem terapie avanzate Srl; Chiesi farmaceutici Spa.

Clinical Trial ID: EudraCT: 2015-001344-11; 2014-002845-23.

F1232

ENHANCED SKIN CELL REJUVENATION BY INITIATION PHASE PARTIAL REPROGRAMMING USING AI-DESIGNED CIRCRNA-EXPRESSED PHASE SEPARATION-DEFICIENT TRANSCRIPTIONAL FACTORS

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Partial reprogramming (PR) is an emerging therapeutic approach to rejuvenate cells via epigenetic remodeling. However, methods to precisely constrain PR from altering cell identity in clinical applications remain elusive. To address this challenge, we established initiation phase partial reprogramming (IPR) involving two key techniques to retain cellular reprogramming in the initiation phase, thus extending the time window for rejuvenating cells without losing their identities. Firstly, we built an AI-assisted protein design pipeline to generate a liquid-liquid phase separation (LLPS)-deficient OCT4 which loses the ability to promoting cellular reprogramming towards late stages while continuously exerting rejuvenation effects. Secondly, we built an AI-assisted circular mRNA (circRNA) design pipeline to generate two multi-gene circRNA vectors, one co-expresses the wild-type OCT4, SOX2, and KLF4 (OSK), and the other co-expresses the LLPS-deficient OCT4, SOX2, and KLF4 (O'SK). The two vectors were capsulated with LNP and tested to reprogram human skin fibroblasts derived from aged donors. DdPCR examination of the embryonic stem cell-related gene (ESRG) showed that OSK induced ESRG expression quickly in the cells, neither did O'SK, indicating that O'SK is safer than OSK. The epigenetic ages were reduced 10 years by OSK and 14 years by O'SK. The SA-β-Gal-positive cells were decreased 80% by OSK and 92% by O'SK. Single-cell RNA-seq and ATAC-seq data revealed a novel set of transcription factors (TFs) related to cell survival and proliferation, rather than the pluripotency-related TFs, elevated in rejuvenated cells, indicating that the rejuvenation and dedifferentiation processes were controlled by different molecular mechanisms. Furthermore, the two circRNA vectors were examined in UV-radiated human skin tissues. The histological analysis showed 72%, 138%, 191%, and 64% recoveries in elastin, collagen I, III, and IV in the O'SK-treated group, and merely 45%, 75%, 65%, and 36% recoveries in the OSK-treated group, respectively. The rejuvenation effects of O'SK were much better than those of OSK. Collectively, these results demonstrate an augmented cell rejuvenation effect through IPR induced by the LLPS-deficient OCT4, thus offering a novel therapeutic strategy for reversing age-related diseases.

F1234

INSIGHTS INTO THE CLINICAL DEVELOPMENT OF REGENERATIVE MEDICAL PRODUCTS BY REGULATORY FRAMEWORK

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Regenerative medicine has emerged as a transformative solution for addressing unmet needs in ophthalmology, leveraging the body's innate ability to repair and replace pathologically affected tissues. Pharmaceuticals and Medical Devices Agency (PMDA, Japan) have approved five notable regenerative medical products: Vyznova (allogeneic corneal endothelial cells for bullous keratopathy), Luxturna (gene therapy using adeno-associated virus for biallelic RPE65 mutation-associated inherited retinal dystrophy), and Nepic, Ocural, and Sakracy (autologous or oral mucosa-derived epithelial cell sheets for limbal stem cell deficiency). We analyze the latest points of review, dosage and administration, clinical data package, overview of Phase 3 clinical trials, efficacy and safety evaluation, and primary endpoints. In the development of new products, it is necessary to demonstrate the risk-benefit assessment based on the efficacy and safety of appropriate studies while capturing the features of the product and based on the basic information on the target disease. In particular, in the development of new products, it is necessary to implement the study design and safety measures based on the characteristics of the target disease and the formulation, and it is important to be able to market a useful product with the cooperation of the relevant stakeholders. The presentation will give the current status and future perspective of regenerative medical products in Ophthalmology from experiences of the PMDA.

F1236

OPTIMIZED CONDITIONS FOR STEM CELL-DERIVED EXTRACELLULAR VESICLES IN BIOREACTOR CULTIVATION

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Stem cells and extracellular vesicles (EVs) are emerging as promising tools for regenerative therapies, with wide-ranging applications in biomedicine, drug development, and disease modeling. However, the scalability of traditional 2D culture systems remains a significant challenge in meeting the demand for large quantities of cells and extracellular vesicles required for therapeutic applications. In this study, we optimized a scalable system for mass production of cell secretions, focusing on generating large amounts of EVs using a floating culture system. A scaffold-free protocol was developed in vertical-wheel bioreactor, enhancing the proliferation and EVs secretion of placental stem cells. EVs mass-cultured in the bioreactor using the optimized protocol demonstrated superior therapeutic efficacy compared to those produced under 2D culture conditions. These EVs promoted cell proliferation, enhanced cell motility, and facilitated angiogenesis in a co-culture environment, underscoring the potential of this approach for advancing regenerative therapies. The mass culture system established in this study opens

the way to efficient cell secretome production, suggesting potential commercial applications in regenerative medicine and biopharmaceutical production.

Funding Source: This work was supported by Korea IPET through High Value-added Food Technology Development program, funded by MAFRA (321025-05).

F1238

STEM CELL THERAPY FOR CHRONIC LUMBAR DISC DISEASE: PHASE 2 CLINICAL SAFETY AND FEASIBILITY DATA OF INTRADISCAL INJECTIONS OF HYPOXIC CULTURED MESENCHYMAL STEM CELLS

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Lumbar disc disease (LDD) is a leading cause of disability with no effective therapy. Orthobiologics have emerged as a strategy for regenerative therapy. Hypoxic culturing of MSCs results in increased therapeutic activity of MSCs post-transplant into the nutrient-poor, low oxygen microenvironment of the disc. The use of this biologic to treat LDD is a promising strategy, due to their hypoxic engineering and known, immuno-modulatory/anti-inflammatory properties. We report early safety/feasibility data in subjects (n=10) dosed in an ongoing Phase 2 trial. The study is double-blind randomized, controlled, and multicenter to determine the safety/efficacy of a single injection of hypoxic cultured MSCs combined with platelet lysate to treat LDD with 12 month safety/efficacy endpoints. 99 subjects will be randomized 2:1 to treatment or control. Data collected at baseline, week 2, week 12, week 26, and week 52. Pain and function scales used; Visual Analog Scale (VAS), Oswestry Disability Index (ODI). Primary endpoint is safety and secondary a clinical response (30% decrease in pain and a 30% increase in function at Week 52). 10 subjects were dosed with 40 x 106 hMSCs or control. At 52 weeks there were no AEs/SAEs related to cell dose. At 12 weeks average baseline ODI was 44 vs 12 week ODI of 43 (2.27% change), at 26 week average baseline ODI was 48 vs 26 week ODI of 24 (50% change), at 52 week average baseline ODI was 43 vs 52 week ODI score of 12 (75% change). For VAS, subjects at 12 weeks had an average baseline VAS score of 63.7 vs 12 week VAS score of 36.4 (42.8% change), at 26 week average baseline VAS score was 69.6 vs 26 week VAS score of 35.5 (48.9% change), at 52 week average baseline VAS score was 71.5 vs 52 week VAS score of 17 (76.2% change). Studies suggest that the harsh microenvironment of the disc could impact cell viability resulting in non-efficacy or worsening symptoms. At 52 weeks in our trial AEs/SAEs reported were not related to cell dose and although blinded, early clinical data at 12 week, 26 week and 52 weeks demonstrated a positive trend with no worsening of pain and function. Results demonstrate that hMSCs may be used for treating LDD. Early data from the present study suggest that a cell dose of 40x106 of hMSCs are suitable for intra-discal injection, not limited by dose limiting toxicity and may be an effective therapy for treating LDD.

Clinical Trial ID: NCT04042844.

F1240

GENERATION OF STEM CELL DERIVED ISLETS WITH ENHANCED PURITY AND FUNCTIONALITY FOR TREATMENT OF TYPE-1 DIABETES

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Type 1 diabetes (T1D) is an autoimmune disease where immune cells destroy the insulin producing pancreatic beta cells. Pancreas or islet transplantation is a promising treatment and provide a better glucose homeostasis in T1D patients. However, limited availability of donors, poor yield of islets and immune rejection are the major obstacles with primary islet or pancreas transplantation. Yet stem cell derived islets (SC-islets) provide an alternative to overcome challenges associated with primary islet transplantation. Although, several protocols have been developed by different labs, the composition and purity of these SC-islets remains a challenge. SC-islets, from most of the protocols, contains approximately 20% to 50% unwanted or off-target cells (ductal, exocrine and enterochromaffin cells) apart from the intended alpha and beta cells. Preclinical studies have shown that such unwanted cells are associated with unwanted cell growth following transplantation. Therefore, eliminating these unwanted cells is important for the safety of clinical testing. Furthermore, published SC-islets have only show in vitro functionality with approximately 2-5-fold increased insulin secretion following high glucose challenge, which is significantly lower than high quality pancreatic islets. In this study we have used multiomic CITE-Seq and flow cytometry-based approaches to identify novel cell surface markers which can be utilized for enrichment of stem cell derived alpha and beta cells. We found a specific cell surface marker which is detected on stem cell derived beta and alpha cells, but not in the unwanted cells. We have utilized this antibody to enrich the alpha and beta cells while efficiently eliminate unwanted cells. This resulted in SC-islets with 98% purity (beta and alpha cells), devoid of exocrine, ductal or enterochromaffin cells. Unexpectedly, we also found that elimination of the unwanted cells strikingly improved functionality of the islets, achieving more than 10-fold increased insulin release following glucose stimulation. This suggest that the unwanted cells are not only a potential risk but also exhibit a negative impact on the SC-islet function. To summarize, we have developed a method to generate pure and highly functional SC-islets for treatment of T1D.

Funding Source: Knut and Alice Wallenberg Foundation, Vinnova, StratRegen at Karolinska Institutet.

F1242

SCALABLE HEMATOPOIETIC STEM CELL EXPANSION IN STIRRED-TANK BIOREACTORS FOR ADVANCING CELL IMMUNOTHERAPIES

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Hematopoietic stem cells (HSCs) are pivotal for off-the-shelf allogeneic immune cell therapies, including natural killer (NK) cells. While HSC expansion molecules like IBR403 enable CD34+ cell growth, conventional 2D cell culture methods are inefficient, costly, labor-intensive, and lack scalability, limiting large-scale clinical and commercial production. To overcome these challenges, we developed a robust, scalable, and high-throughput platform using automated stirred-tank bioreactors to expand cord blood derived HSCs (CBUs) and differentiate them into NK cells. Using a Design of Experiments (DoE) approach with MODDE® software, we optimized culture conditions by comparing GMP-grade media with RUO media, supplemented with IBR403 and key cytokines. 72 bioreactor conditions were tested evaluating perfusion rates (0.5-2 VVD) and cytokine concentrations (0-100 ng/mL) across multiple donors over 28 days. CD34+ cell expansion and differentiation were performed in the Ambr® 15 bioreactor system, with functionality and purity assessed for clinical relevance. HSCs cultured at 2 VVD perfusion rate produced an average of 5×10^7 CD34+ cells per mL of input cord blood in GMP-grade media, compared to 1.4×10^6 in RUO media. The DoE identified an optimal formulation that reduced costs by 50% while achieving >6×10^7 CD34+ cells/mL of input cord blood. A high (2 VVD) perfusion rate was critical for maximizing CD34+ yields per mL of input CBU. Variations in CO2 (5% vs. 10%) and dissolved oxygen setpoints (15% vs. 30%) had no impact on CD34+ expansion. NK cells differentiated in bioreactors under optimal conditions showed >95% CD56+ expression (55-98% on Day 28) and >75% cytotoxicity against A549 cells at a 1:1 effector-to-target ratio, with donor variability impacting HSC expansion and NK differentiation. This study successfully optimized CBU-derived HSCs expansion and NK cell differentiation in GMP-grade media using the Ambr® 15 bioreactor system. By refining key process parameters, we achieved high-yield, cost-effective CD34+ cell expansion and scalable NK cell production. These findings establish a robust platform that transforms cord blood into a valuable resource for advancing cell therapies.

F1244

INTER- AND INTRA-DONOR VARIABILITY OF IPSC GENERATED FOM HUMAN SKIN PUNCH BIOPSIES VIA AN ACCELERATED AND VALIDATED PIPELINE USING NON-INTEGRATIVE RNA-BASED REPROGRAMMING

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Human induced pluripotent stem cells with their potential to differentiate into countless different cell types hold great potential for allogenic as well as autologous cellular therapy. However, many traditional techniques used for generation of iPSCs entail the risk for genomic integration and therefore limit their use in a clinical setting. In addition, the established protocols are often lengthy and require the subsequent screening for unwanted vector integration. Transient mRNA based reprogramming eliminates this risk and is one of the fastest and most efficient

reprogramming technologies available today. We hereby present an accelerated and robust protocol to generate cryopreserved and quality controlled stocks of monoclonal iPSC lines from human skin biopsies within 6-7 weeks. Fibroblasts were isolated from abdominal skin via combined mechanical and enzymatic dissociation. The cells were expanded for only one passage before starting the reprogramming routine, eliminating the need for extensive upstream expansion of fibroblast and thus minimizing the risk for cultivation induced aberrations. For reprogramming, cells were transfected for five consecutive days with an mRNA cocktail containing the transcription factors OCT3/4, SOX2, KLF4, MYC, NANOG and LIN28A. During a short growth phase iPSC colonies formed with high efficiency and could be further expanded into stable monoclonal cell lines. We derived and characterized 36 iPSC lines from five healthy donors (age 50-62). All tested lines showed characteristic stem cell morphology, typical pluripotency-associated marker expression and successfully differentiated into the three germ layers endoderm, ectoderm and mesoderm. While phenotypically all iPSC lines revealed low intra- and inter-donor variability, the verification of genetic integrity (digital PCR / SNP-based CNV analysis) and the assessment of their oncogenic risk profile (whole exome sequencing) was more selective, highlighting the need for comprehensive quality control of newly generated iPSC lines. In summary, the described protocol allows for efficient and reliable integration-free generation of high quality monoclonal iPSC lines from human skin punch biopsies within only 6-7 weeks and will therefore facilitate the generation of iPSCs from individual donors.

F1246

VERSATILE HIGH-SPEED MICROFLUIDIC CELL SORTING OF HUMAN PLURIPOTENT STEM CELLS AND THEIR DERIVATIVES

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iPSCs with their potential to differentiate into numerous different cell types hold great potential for cellular therapy. Innovative therapeutic approaches often entail the application of clearly defined cells types or subsets that need to be isolated and enriched from heterogenous cell populations. Conventional flow sorters were predominantly designed for research applications. Open systems and harsh sorting conditions therefore often prevent the translation of protocols into the clinic. We hereby show how a gentle microchip-based sorting technology can enable enrichment of PSCs and their derivatives in the safety of a sterile cartridge. Human fibroblasts were reprogrammed via repeated mRNA transfections and resulted in a mixture of successfully generated iPSCs and non- or partly reprogrammed undefined cells. This heterogenous cell culture was stained with Tra-1-60-PE and sorted immediately after reprogramming. The iPSCs

could be successfully enriched from purities of 4-41% to 85-99 %. Furthermore transgenic iPSC constitutively expressing GFP to a differing degree could be effectively enriched from 13-51% to a population of 72-98%. To obtain monoclonal transgenic iPSCs the sorted fraction was applied to a single-cell dispenser and seeded in 96-well plates. The cells showed high plating efficiency after the combination of sorting and subsequent single-cell dispension while maintaining typical iPSC morphology. Next, various iPSC-derived cell types were enriched after differentiation. Retinal pigmented epithelial cells could be enriched from a purity of 51-69% to 93-98% and showed a strongly pigmented phenotype after sorting. In other approaches cardiomyocytes were enriched from 56% to 96% and CD144-positive endothelial cells could be enriched from 48-49% to 90-96%. In all applications a high viability of over 85% was maintained after sorting. In summary, this gentle, sterile sorting technology allows for efficient, reliable enrichment of PSCs and their derivatives and therefore facilitates working with clearly defined cell populations in a wide variety of PSC applications.

F1248

SAFETY AND EXPLORATORY EFFICACY OF HUMAN ALLOGENEIC WHARTONS' JELLY-DERIVED MESENCHYMAL STEM CELLS IN PATIENTS WITH CMT1A: THE PHASE 1 TRIAL

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Charcot-Marie-Tooth disease type 1A (CMT1A) is an inherited peripheral neuropathy resulting from a duplication mutation in the PMP22 gene and is one of the most prevalent orphan diseases. Symptoms include sensory loss, muscle atrophy, and gait disturbances, affecting an estimated 1.5 million people globally, with no cure available. This study is a Phase 1 clinical trial to assess the safety, tolerability, and preliminary efficacy of Wharton's jelly-derived mesenchymal stem cells (EN001) in patients with CMT1A. A total of nine patients with CMT1A participated in this study. The participants received EN001 intravenously, with three participants assigned to the low-dose group (5 × 10^5 cells/kg) and six to the high-dose group (2.5 × 10^6 cells/kg). The safety and tolerability assessments were conducted 16 weeks post-administration. Exploratory efficacies were evaluated during a 48-week follow-up period. Exploratory efficacy endpoints included CMTNSv2, CMTNSv2-R, CMTES, CMTES-R, ONLS, 10 MWT, NCS, and MRI. There were a total of four adverse events, but mild (Grade 1) and transient during 16 weeks. Those events were determined to be unrelated or unlikely to the investigational products (EN001). There were no additional adverse events related to EN001 from Week 16 to Week 48. Significant improvements were found in CMTNSv2, CMTNSv2-R, CMTES, and CMTES-R in the high-dose groups at Week 16. The disease severity classification of CMTNSv2 was changed from moderate to mild in the high-dose group. All subjects maintained improvements at Week 48, in particular, CMTES and CMTES-R in the high-dose groups showed significant improvements in disease severity classifications from moderate to mild. FDS scores decreased significantly at Week 48 compared to the baseline. Specifically,

two participants in the high-dose group scored zero, which is normal, at Week 16 and remained until Week 48. Intravenous administration of EN001 in patients with CMT1A confirmed safety and tolerability. Significant improvements in motor function, particularly in the high-dose group, were observed for 48 weeks after administration. Long-term follow-up is planned for five years. These results suggest that EN001 could be an effective treatment option for CMT1A.

Funding Source: This research was supported by the Ministry of Science and ICT, and the Ministry of Health and Welfare (RS-2023-00215098, HX23C1756); the National Research Foundation (2022M3E5E9016662); and the Samsung Medical Center (SMO1240041).

Clinical Trial ID: NCT05333406.

F1250

SCALABLE MSC EXPANSION WITH CELLFIBER® TECHNOLOGY AS A COST-EFFECTIVE MANUFACTURING PLATFORM

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Mesenchymal stem cells (MSCs) possess potential for differentiating into diverse cell types, offering therapeutic benefits in tissue repair and immune modulation. Despite their pluripotency and accessibility, the process of scaling-up of MSCs is laborious and time-consuming. Due to the necessity of handling a great number of vessels to provide sufficient surface area for growth, maintaining stable quality and biological activity becomes challenging. Beyond standard static adherent cultures in multilayer vessels, novel systems have been developed for large-scale manufacturing, most of which, particularly in the context of cell harvesting, efficiency and workflow complexities are still being refined. CellFiber® is an encapsulation technology that utilizes medical-grade alginate to form a protective tubular structure providing a stable environment. This approach maximizes surface area for high-density cell culture at various scales. This study focuses on cultivating MSCs using CellFiber technology in T75 flasks, gas permeable bags (300mL and 1000mL) and a cellbag bioreactor (2L), offering larger surface areas for adherent culture compared to traditional non-fiber cultures in the same vessels (160 cm2 with fiber versus the conventional 75cm2 in a T75 flask). Cell yields were 5.74 x 10^7 in the 1000mL gas permeable bag and 1.6 x 10^8 in the 2L cellbag bioreactor, demonstrating the feasibility of fiber culture through normal marker expression. The adjustable fiber parameters offer flexibility, enhancing the footprint efficiency and increasing the “space-effectiveness” (surface area-to-culture volume ratio). Manufacturing assumptions and cost attributes are separately outlined for further investigation of the Cost of Goods (CoGs) associated with the adoption of this innovative technology. CellFiber® technology offers advantages in maximizing surface area within a manageable culture batch, while enabling non-enzymatic cell harvest from hydrogel fiber, thereby reducing the risk of cell loss. Preliminary results demonstrate CellFiber® benefits large-scale manufacturing with a functionally-closed workflow. This approach could serve as a compelling, cost-efficient solution to address the current challenges in MSC manufacturing processes.

Funding Source: AMED CiCLE.

F1252

BIODISTRIBUTION ASSESSMENT OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CELL THERAPY PRODUCT

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Human induced pluripotent stem cells (hiPSCs) hold immense potential for regenerative medicine, particularly in treating ischemic cardiovascular and neurodegenerative diseases. Despite their promise, the clinical translation of hiPSCs and/or hiPSC-derived cell therapy (CT) products has been hindered by challenges associated with the inherent characteristics of hiPSCs such as the tumorigenic potential. Indeed, the presence of a small amount of unexcluded hiPSCs may pose safety risks particularly if they are retained within unintended parts of the body. To address the challenges for CT products before human application, biodistribution studies serve as a critical component of preclinical safety and toxicity evaluations. Quantitative methods based on PCR techniques have been developed and widely applied for biodistribution assessment, offering high specificity, sensitivity, and absolute quantification. Yet, due to the unique diversity and complexity of every biological therapy product's characteristic, procedural considerations may vary, and regulatory guidance may not clearly suggest detailed, standardized criteria for all. As a result, direct implementation of a simple, universal approach is limited. In this study, we compile and summarize key guidance documents issued by regulatory authorities worldwide. Next, we propose a fit-for-purpose framework for establishing GLP-compliant biodistribution evaluation process for hiPSC-derived CT products. This framework consists of four essential steps: (1) study design of biodistribution assessment; (2) qPCR assay development; (3) assay validation; and (4) data analysis and interpretation. We further demonstrate the application of this framework conducting a biodistribution study of hiPSC-derived endothelial cells (hiPSC-ECs) in BALB/c nude mice, confirming its feasibility and suitability. We found that intramuscularly injected hiPSC-ECs were undetectable in 13 organs, excluding the injection site, after 12 hours post-injection across 86 animals distributed into 11 experimental groups. By providing a structured and practical approach to biodistribution assessment, our fit-for-purpose framework offers a clear path forward for advancing the development of safe, effective, and standardized hiPSC-derived therapies.

Funding Source: This work was supported by grants from the National Research Foundation of Korea funded by the Korean government (RS-2024-00509295), and Korea National Institute of Health research project (2023ER130202 and 2023ER130102).

F1254

AN INTEGRATED APTAMER-BASED PLATFORM FOR EFFICIENT EXOSOME ISOLATION, MOLECULAR ANALYSIS, AND REAL-TIME DETECTION

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Extracellular vesicles (EVs) are critical mediators of intercellular communication and play key roles in both physiological and pathological processes. In this study, we employed the Exo-SELEX technique to screen and identify aptamers specifically targeting exosomes, thereby achieving efficient exosome isolation and detection. By immobilizing the selected aptamers on magnetic beads, we developed a novel exosome capture method. Compared with traditional approaches, this aptamer-based strategy is gentler, faster, and requires smaller sample volumes, significantly improving the efficiency and convenience of exosome isolation. To demonstrate the bioanalytical utility of this method, we analyzed the miRNA expression profiles within the captured exosomes, providing new insights into the molecular mechanisms underlying disease progression, particularly in cancer. In addition, to better investigate the molecular characteristics of the exosome surface, we introduced the DNAzyme Proximity Biotinylation technique, in which aptamers specifically bind to their targets, while DNAzyme catalytic activity generates biotin labels near the binding site, enabling precise localization of key surface molecules on exosomes. Furthermore, to expand the application of this platform, we integrated aptamers with fiber optic technology by utilizing tilted fiber Bragg gratings (TFBG) combined with a gold-coated optical fiber surface to exploit surface plasmon resonance (SPR) signals. This integration enabled the development of a highly sensitive biosensor for rapid, real-time exosome detection. In conclusion, this study utilized aptamer technology to achieve efficient exosome isolation, coupled with miRNA analysis to elucidate the biological significance of molecular cargo within exosomes, and employed DNAzyme technology for precise identification of key surface molecules on exosomes. Finally, the fiber optic sensor facilitated real-time, high-sensitivity detection of exosomes. This integrated platform not only provides an innovative tool for exosome research but also demonstrates its broad potential in disease diagnostics, biomarker discovery, and molecular mechanism exploration.

F1256

IPSC-BASED THERAPY FOR PARKINSON'S DISEASE

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We pursue a cell replacement therapy for Parkinson's disease (PD) using induced pluripotent stem cells (iPSCs). To this goal, we have developed a method for 1) scalable dopaminergic (DA) neuron induction on human laminin fragments and 2) sorting DA progenitor cells using a floor plate marker, CORIN. The grafted CORIN+ cells survived well, functioned as midbrain DA neurons in the PD model rats and monkeys, and showed a minimal risk of tumor formation. Based on these results, we started a clinical trial to treat PD patients at Kyoto University

Hospital in 2018. The trial evaluated the safety and efficacy of transplanting human iPSC-derived DA progenitors into PD patients' putamen. Using a stereotaxic surgical technique, we implanted approximately 5 or 10 million cells into the bilateral putamen of the patients. The target was seven patients, and we finished the two-year observation of all patients in 2023. In this presentation, I will summarize this project.

Funding Source: This study was supported by a grant from the Research Project for Practical Application of Regenerative Medicine of the Japan Agency for Medical Research and Development (AMED) [23bk0104126h0003] to J.T.

Clinical Trial ID: jRCT2090220384 and UMIN000033564.

F1258

CHARACTERISTICS OF MESENCHYMAL STEM CELL THERAPEUTICS CULTURED IN 3D USING GELATIN HYDROGEL SCAFFOLDS AND THEIR EFFICACY IN REFRACTORY INTESTINAL INFLAMMATION

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In inflammatory bowel disease (IBD), refractory erosions and multiple ulcers form in the gastrointestinal tract. Surgical intervention is often performed for complications such as strictures associated with refractory ulcers and complex fistulas. However, these procedures are not curative. Challenges include short bowel syndrome caused by repeated intestinal resections, reduced quality of life (QOL) due to long-term drainage tubes placement, and the potential development of malignancies. Moreover, current immunomodulatory agents are administered systemically, raising concerns about side effects from prolonged use. Therefore, there is an urgent need to develop novel therapies that provide localized, high-efficacy treatment for severe lesions. Mesenchymal stem cells (MSCs) secrete a variety of humoral factors and extracellular vesicles, demonstrating immunomodulatory and tissue repair capabilities. Studies have shown that 3D culturing of MSCs suppresses replicative senescence, prolongs cell viability, and enhances functional properties such as anti-inflammatory effects. However, MSC characteristics vary depending on the culture method. Gelatin hydrogel, which mimics the extracellular matrix in vivo, promotes the proliferation and intercellular interactions of co-cultured cells. It also facilitates the diffusion of nutrients and oxygen essential for cell survival and exhibits excellent biocompatibility. In this study, we developed 3D-cultured MSCs using gelatin hydrogel particles as scaffolds (Gel-MSCs) and evaluated their potential as mucosal-localized therapeutics. We also investigated their characteristics using umbilical cord-derived MSCs and adipose tissue-derived MSCs. Compared to 2D-cultured MSCs (2D-MSCs), Gel-MSCs exhibited enhanced expression of immunomodulatory and tissue repair-related factors, along with increased secretion of prostaglandin E2 (PGE2), which promotes intestinal epithelial regeneration and the regulates intestinal inflammation. Additionally, local administration of Gel-MSCs to severe ulcers in a TNBS-induced colitis rat model enhanced epithelial regeneration and improved MSC retention in mucosal tissues. This report discusses the mechanisms underlying the therapeutic efficacy of Gel-MSCs.

Funding Source: Grants-in-Aid for Scientific Research in Japan SHIBUYA CORPORATION.

F1260

"IT'S REALLY AN ADVENTURE": A QUALITATIVE STUDY EXPLORING EXPERIENCES OF PROFESSIONALS INVOLVED IN WORKING WITH ADVANCED THERAPEUTICS IN THE NETHERLANDS

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To date, few clinical studies have been conducted in Australia involving novel cell and gene therapies. Even fewer products have been approved for use. However, as an increasing number are being evaluated across the globe, there is an increasing need and interest to provide Australian patients with access to these products, often referred to as “advanced therapies”. Given the complexity of implementing these therapies, understanding barriers and enablers to implementation in a comparable healthcare system is key to successfully translating these new technologies into clinical practice. In this study we focused on the experiences of professionals in the Netherlands based at a single tertiary hospital who were involved in delivering approved cell or gene therapies, or who were conducting or starting up trials of gene or cell therapies. Particular attention was paid to a current international clinical trial of a genetically modified-cell therapy developed by researchers based at the same tertiary hospital in The Netherlands. Semi-structured interviews were conducted with 11 professionals and explored their experiences including what they found facilitated the process or made it more challenging. Participants were from a range of specialities and roles within the hospital. Therefore it represented a variety of facets of involvement in the implementation process. Inductive Content Analysis was used to identify barriers and enablers. Categories of barriers included the complexity of the regulatory system (particularly for those who were doing this for the first time), and the logistical challenges of implementing such a procedure within an existing hospital structure. Categories of enablers included strong clinic-lab relationships, an institutional investment in appropriate facilities and knowledgeable personnel, and the operational similarity of the novel procedure with existing therapeutic options. This study provides insights into proactive steps that can be taken towards being prepared to offer cell and gene therapies. This is a timely reminder for Australia that attention should be paid to questions of implementation and access to advanced therapies, and provides useful steps that healthcare institutions need to consider as they ready themselves and their workforce to deliver these products.

Funding Source: The Novo Nordisk Foundation Centre for Stem Cell Medicine, reNEW, Novo Nordisk Foundation grant number NNF21CC0073729 The Henry and Rachael Ackman Travelling Scholarship 2024 (UTR6.170).

F1262

MODULATION OF SERCA2A OF INTRA-MYOCYTIC CALCIUM TRAFFICKING IN PATIENTS WITH HEART FAILURE WITH REDUCED EJECTION FRACTION (MUSIC-HFREF) AND STEM CELL MODELS OF HEART FAILURE

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Patients with Heart Failure with reduced Ejection Fraction (HFrEF) continue to experience high morbidity and mortality. A critical characteristic of failing hearts is abnormal intracellular Ca2+ handling, which is due to a decrease in expression and function of the cardiac sarcoplasmic reticulum calcium ATPase pump (SERCA2a). Delivery of an adeno-associated type 1 vector carrying SERCA2a (AAV1.SERCA2a) improved contractile function in a dose dependent manner in many animal studies, and also in dose optimization studies using human stem cell-based engineered mini-heart tissue models of heart failure (HF). In patients, low doses were safe but led to marginal outcomes due to poor myocardial transduction. Through a local intracoronary delivery, we have titrated to a dosage high enough for efficacy without causing immunogenicity or adverse events, and when normalized by cardiomyocyte number, the estimated dose per cell is consistent with the optimal range obtained with the mini-heart models in vitro. Compared to earlier trials, our ongoing phase 1/2a clinical trial for the treatment of HFrEF (MUSIC-HFrEF) delivers higher doses of AAV1.SERCA2a (3E13 viral genome(vg)/patient and 4.5 E13 vg/patient) through intracoronary infusion in both ischemic and non-ischemic patients with left ventricular ejection fraction (LVEF) of 35% or less. To date six patients have received a dose of 3E13 vg/patient of AAV1.SERCA2a and two patients received 4.5E13 vg/patient, and the follow-up period has been 1 to 26 months. There have been no gene therapy or procedure–related serious adverse events at both doses post-injection. Four of the six patients in the 3E13vg/patient dose have shown improvements in NYHA HF classification at 6 and 12 months. Clinically meaningful improvements have been observed in LVEF and 6-minute walk test (6MWT), along with decreases in HF biomarkers NT-Pro-BNP and troponin I. Based on the early clinical efficacy at a dose of 3E13vg/patient of AAV1.SERCA2a, the enrollment of patients at a higher dose of 4.5E13 vg/patient is continuing. These encouraging results of high-dose AAV1.SERCA2a in patients with HFrEF (ischemic and non-ischemic) not observed previously may offer alternative treatments to patients with severe heart failure where a large medical unmet need remains.

Clinical Trial ID: US FDA, NCT04703842.

F1264

ENHANCED GENETIC STABILITY OF HUMAN PLURIPOTENT STEM CELL CULTURES WHEN SINGLE-CELL PASSAGING USING ETESR™

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Genetic instability in human pluripotent stem cells (hPSCs) is a well-documented challenge. Recurrent cytogenetic abnormalities arise during culture and confer a selective advantage to genetically variant cells through mechanisms including resistance to cell death, increased cell proliferation and reduced differentiation capacity. We previously showed that routine single-cell passaging of hPSCs can result in a high incidence of de novo genetic abnormalities, and that eTeSR™, a novel hPSC maintenance medium optimized for single-cell passaging, can significantly reduce the appearance of these recurrent abnormalities. To further demonstrate the genomic stability of routinely single-cell passaged hPSCs in eTeSR™, 135 clonal hPSC sublines (derived from H1, H9, and SCTi003-A hPSCs) were independently expanded for 20 weeks in eTeSR™ while 136 clonal sublines were independently expanded as single-cells in two other commercially-available hPSC media. Single nucleotide polymorphism microarray analysis revealed that, after 20 weeks, 70% of clonal sublines maintained in control media acquired at least one de novo abnormality compared to only 25% eTeSR™-maintained sublines. This can be attributed to fewer small (< 10,000 kb) structural variants detected in cells maintained in eTeSR™ (3%) compared to control media (69%). Notably, 51% of control sublines displayed a gain in chromosome 20q11, a well-characterized, recurrent copy number variant which conveys a selective advantage and is often undetectable by G-band karyotyping. Conversely, the 20q11 abnormality was not detected in any of the eTeSR™ samples. To investigate the reduced incidence of 20q11 observed in eTeSR™-maintained sublines, we performed a competitive mixing assay in which hPSCs with a gain of 20q11.21 were mixed with fluorescently labelled wild-type cells (1:20 ratio) and cultured for 5 passages. We observed a 4-fold reduction in the takeover rate of 20q11.21 when cells were cultured in eTeSR™ (14 ± 8%) compared to 62 ± 7% in an alternative commercial single-cell workflow (mean ± STDEV; n = 4). This study underscores the role of innovative media formulations in mitigating culture-acquired genetic aberrations in hPSCs, addressing a critical challenge in the field.

F1266

A SINGLE-STEP PROCESS FOR NON-VIRAL ENGINEERING HYPOIMMUNOGENIC PLURIPOTENT STEM CELLS WITH THE PIN-POINTTM BASE EDITING PLATFORM

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Pluripotent stem cells (PSCs) hold great promise for manufacturing advanced cell therapies. Off-the-shelf allogeneic products derived from PSCs, engineered to be compatible with large patient cohorts, can broaden access to these therapies. However, their sensitivity to DNA damage presents challenges for complex genome editing. Base editors offer a solution due to their reduced genotoxicity compared to nuclease-based technologies. We developed the Pin-point platform, which enables the modular assembly of base editors composed of DNA binding Cas and DNA modifying deaminase components associated via an aptamer encoded in the sequence-targeting guide RNA (gRNA). Owing to the aptamer-dependent recruitment of the deaminase component to target DNA sequences, the Pin-point platform uniquely allows multi-purposing of a single Cas nickase component for simultaneous multiplexed base editing and targeted transgene knock-in. To demonstrate the platform's utility for engineering allogeneic PSCs, transient delivery of mRNAs encoding a Pin-point base editor (Rat APOBEC1 and SpCas9 nickase) with synthetic aptamer-encoding gRNAs were used to generated clonal hypoimmunogenic iPSC lines with various genotypes, including large cargo transgene integration, using an automated clone tracking and picking workflow. These hypoimmunogenic iPSC lines, created via multiplexed base editing and simultaneous base editing with targeted transgene integration, retained pluripotency, exhibited expected human leukocyte antigen (HLA) phenotypes, and evaded both innate and adaptive immune responses when differentiated into therapeutic cell products. The Pin-point platform thus represents a safe and efficient solution for multiple genome engineering operations via a novel single-step process compatible with downstream automation, dramatically streamlining the development of allogeneic iPSC-derived cell therapies. Pin-point™ Technology Platform: The Pin-point™ base editing platform technology is available for clinical or diagnostic study and commercialization under a commercial license from Revvity.

F1268

FABRICATION OF ECM-MIMICKING HYDROGEL FOR ADIPOSE TISSUE ENGINEERING

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Adipose tissue engineering is an emerging field that repairs tissue defects from trauma or tumor resection and serves as filling materials in plastic surgery. Currently, in vitro 3D culture of adipose tissue overcomes the limitations of 2D models and complex animal studies, closely mimicking the physiological environment of real fat and applying on research of adipose-related diseases. The extracellular matrix (ECM) is crucial for adipose tissue, providing structural support and facilitating essential functions like cell adhesion, migration, and differentiation. However, existing studies often neglect the specific biochemical composition and mechanical properties of the extracellular matrix (ECM) that are essential for effective adipogenesis. By utilizing combinations of Collagen I (Col I), Collagen VI (Col VI), Fibronectin (FN), and Glycosaminoglycans (GAGs), we aim to fabricate dynamic ECM niches with controllable compositions of ECM components. Adipose-derived stem cells (ADSCs) sourced from autologous adipose tissue possess multipotent differentiation potential and secrete various growth factors. To evaluate scaffold functionality, we utilized ADSCs and induced adipogenic

differentiation. Our findings indicated that the Col-GAG, Col VI, and combined culture groups effectively promoted adipogenesis. This study will demonstrate that our ECM-mimicking scaffolds can support adipogenesis and provide a foundation for future tissue regeneration research. By advancing the design of hydrogels that mimic native ECM, this work aims to significantly contribute to adipose tissue engineering and foster innovative solutions for tissue repair in clinical settings.

Funding Source: This work was supported by the Health@InnoHK program of the Innovation and Technology Commission of the Hong Kong SAR Government.

F1270

CELL THERAPY SCALE-UP: BIOPROCESS DEVELOPMENT FOR THE PRODUCTION OF HEMATOPOIETIC CELLS

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Blood transfusion is currently the most common cell therapy applied worldwide to treat various medical purposes [1]. One of the purposes is the treatment of severe anemias, as these diseases can only be treated with blood transfusions and stem cell transplantations. An attractive therapy that could overcome the risks involved with donor-derived transfusion products is in vitroproduced red blood cells (RBCs) or the corresponding stem cells, namely hematopoietic stem cells (HSCs) [2,3]. In vitro RBCs can be produced from induced pluripotent stem cells (iPSCs), which are pluripotent cells created by reprogramming human somatic cells. This reprogramming technique started a new era in regenerative medicine due to their self-renewing properties and multilineage differentiation potential to produce patient-specific progenitor or functional effector cells [4, 5]. However, hematopoietic cells and RBCs from iPSC are mostly produced via multiple differentiation steps using static adherent protocols which hinders scalability towards clinically relevant volumes. A dynamic shake flask cultivation was developed to produce hematopoietic stem/progenitor cells (HSPCs), which can further be differentiated into functional RBCs. The dynamic cultivation led to a ~16,000 fold increase in cell number compared to static cultivation. This project aims to transfer the developed protocol to a suspended stirred-tank bioreactor to produce HSPCs. Employing bioreactors will improve reproducibility and facilitate upscaling towards the desired volumes, as mini-transfusions (1011 RBC, required in phase I trial) could be generated with 3 to 4 threelitre bioreactors. The main challenges to be optimized are specific nutrient and oxygen requirements for each differentiation step, as well as shear stress effects.

F1272

OPTIMIZING PLURIPOTENT STEM CELL SPHEROID GROWTH IN LITER SCALE BIOREACTORS THROUGH THE USE OF CONSTANT MEDIUM PERFUSION

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In order to efficiently meet clinical manufacturing needs requiring high quantities of pluripotent stem cells (PSCs) for downstream differentiation, the development and optimization of PSC culture methods in large volume bioreactors is critical. We have previously shown how PSCs can be expanded as spheroids in 3L stirred-tank reactors (STRs). These types of bioreactors use impellers that continuously mix the medium but also generate shear stress that may negatively affect PSC growth. Additionally, current protocols that rely on gravity sedimentation of PSC spheroids and manual aspiration of spent medium are impractical beyond a 3L culture scale. Notably, STRs can be combined with perfusion systems to constantly supply fresh medium and mitigate the need to manually exchange spent medium. Here, we describe our efforts to optimize PSC spheroid growth in liter scale bioreactor cultures by balancing stir speeds, shear stress effects, and medium exchange rates using constant perfusion of StemScale PSC Suspension Medium. Cells were initially seeded into STRs at low stir speeds (i.e., low RPM) to promote spheroid formation. The RPM was then gradually increased to prevent spheroid aggregation and maintain culture homogeneity. Shear was detrimental to spheroid growth at high speeds, though this could be minimized with shear protectants (e.g., Pluronic). Our results indicated that the addition of 0.1 – 0.2% Pluronic enabled spheroids to grow larger and more uniform in size, resulting in greater cell yields. We further evaluated spheroid growth by using tangential flow depth filtration (TFDF) and alternating tangential flow (ATF) perfusion systems to perform medium exchanges. We determined that both systems were able to support spheroid expansion with similar efficiencies. Taken together, with these optimized parameters we observed yields of up to 25 billion PSCs (12x106 cells/mL, equaling ~80-fold expansion) capable of maintaining pluripotency (>95% OCT4+/NANOG+ cells) after 10 days in culture. Overall, these results demonstrate that PSC culture in perfused STRs is an effective means to improve PSC expansion workflows.

F1274

APPLICATION OF INDUCED PLURIPOTENT STEM CELL-DERIVED MITOCHONDRIA TRANSFER FOR IN VITRO OSTEOGENESIS

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Mitochondria play an important role in cell metabolism and survival, producing chemical energy through oxidative phosphorylation. When mitochondria are damaged, normal cells transfer their mitochondria to dysfunctional cells, promoting their revitalization. For this reason, its potential application in the treatment of damaged tissues has been widely investigated. Mitochondria derived from induced pluripotent stem cells (iPSCs) are expected to exhibit superior therapeutic effects due to their pluripotent nature. The mitochondria of iPSCs are distinguished by their low metabolic rate and reduced reactive oxygen species (ROS) production while generating sufficient ATP. Therefore, we aim to develop iPSC-derived mitochondria therapy for osteoporosis (OP), where ROS play a critical role in pathogenesis, and first confirmed its effect on in vitro osteogenesis. This study focuses on isolating mitochondria from iPSCs and delivering them directly to cells. We first isolated mitochondria from iPSCs using a commercial

mitochondria isolation kit and validated their presence and purity using Western blot and TEM imaging. The quality of isolated donor mitochondria is important for mitochondria transfer therapy efficiency. Therefore, we checked the mitochondrial membrane potential and ATP levels of the isolated donor mitochondria to assess the quality of them. Next, we observed internalized donor mitochondria in the recipient cell after transfer by confocal microscopy, and we also determined senescence of the recipient iPSC after mitochondria transfer. After checking mitochondrial quality and mitochondria transfer validity, we transferred the isolated mitochondria to iPSCs prior to osteogenic differentiation and observed changes in their differentiation capacity. This proof-of-concept study demonstrates the potential of iPSC-derived mitochondria transfer to be applied to in vitro bone formation, providing a basis for the development of mitochondrial transfer therapy for OP using iPSCs.

Funding Source: This work was supported by the National Research Foundation of Korea (NRF) and ministries including Science and ICT, Trade, Industry and Energy, Health and Welfare, and Food and Drug Safety (RS-2023-KH142779, RS-2019-NR040058).

F1276

INJECTABLE MICROGEL PLATFORM FOR CELL-BASED REGENERATION IN INTERVERTEBRAL DISC DEGENERATION

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Intervertebral disc (IVD) degeneration is a major cause of low back pain, with limited treatment success. Stem cell-based therapy offers a promising treatment option. Direct cell delivery faces challenges like cell leakage and low cell survival due to stressing microenvironment in the IVD. To address this challenge, we developed an injectable photo-cross-linkable methacrylated fibrinogen-based (FibMA) microgel system to encapsulate and precondition the cells, improving their stability. Human nucleus pulposus-derived progenitor cells were used as the target cells in this study. Cell-encapsulated microgels fabrication process was optimized for FibMA concentration, UV crosslinking intensity, and exposure duration. No DNA damage occurred in cells with this protocol. With an optimized process we have prepared and compared two different sizes of microgels. Microgels with 120µm and 450µm diameters after 7 days in culture exhibited cell viability of about 250% and about 100%, respectively and ECM gene expression after 5 weeks of culture. The larger microgels (450µm) facilitate greater cell cytoskeleton formation and spreading throughout the microgel compared to the smaller ones (120µm) and immunostaining confirms the secretion of COL2 and ACAN matrix proteins within the microgels. Furthermore, we assessed the safety and efficacy of the cell-loaded microgel using the rat lumbar disc needle puncture model. Disc regeneration analysis was done by CT imaging and histopathological analysis. Additionally, biobehavioral studies, including cold sensitivity and von Frey tests, were used to test if the treatment not only facilitates disc regeneration but also

alleviates pain. To investigate the cell fate of the human encapsulated cells in the rat disc single cell RNA sequencing was used. This study demonstrates that the optimized FibMA microgel system supports high-quality cell encapsulation, and stability, and promotes tissue regeneration in vivo. The advanced injectable microgels and stem cell therapies offer promising prospects for clinical applications to treat discogenic low back pain.

Funding Source: This study was supported by NIH R34NS126032, CIRM DISC2-14049 and CIRM scholarship (to NM) EDUC4-12751.

F1278

STRUCTURAL CHARACTERIZATION OF THE 20Q11.21 REARRANGEMENT RECURRENTLY OBSERVED IN HUMAN PLURIPOTENT STEM CELLS

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There are currently over 115 clinical trials worldwide using human pluripotent stem cells (hPSC)-derived cells. Culture conditions exert a selective pressure that can give an advantage to cells accumulating genomic alterations. Therefore, ensuring their genomic integrity is an important prerequisite. Seventeen years ago, using a-CGH and SNP array technologies, we identified, in hPSCs, a recurrent chromosomal defect (2.5 to 4.6 Mb) located on chromosome 20 at position q11.21. Cells carrying this amplification possess some features of neoplastic progression. 20q11.21 duplication is found in many cancers and is an important event in tumor progression. This anomaly is the most frequently observed worldwide in hPSCs. It is estimated that it represents more than 20% of the recurrent anomalies listed. However, this duplication is not well characterized. Only few studies have looked at the position of the extra copy of chromosome 20q11.21. Using fluorescence in situ hybridization we determined in 5 hPSC lines that the extra copy was inserted either at 1p36.3 region or as a tandem or inverted repeat at 20q11.21. Only one study analyzed the local genomic architecture and breakpoints of two samples using long-read next generation sequencing and found that in both cases, the duplications were arranged in a head-to-tail orientation. Optical genome mapping (OGM) has only rarely been used to monitor genomic integrity of hPSCs and their derivatives. Our study aims to evaluate in which way OGM could become a more sensitive and resolutive tool to qualify clinical-grade lines and might contribute to a better understanding of the mechanisms responsible for genomic instability. To this end, we analyzed 6 samples in which a 20q11.21 duplication has been previously identified with SNP arrays. Beyond the field of hPSC use in biotherapy, this study could also contribute to a better understanding of early events that play a role in tumor progression.

Funding Source: This study was supported by the Agence Nationale de la Recherche under the “Investissements d’avenir” program [ANR-10-IAHU-01].

F1280

WHAT REALLY ARE THE TROPHIC REQUIREMENTS OF MATURING HUMAN PLURIPOTENT STEM CELL-DERIVED NEURONS?

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Differentiation of human pluripotent stem cells into defined cellular populations have vast research, industry and clinical applications ranging from modelling development and disease through to advancing drug development and cell-based therapies. Necessary for their use is the establishment of defined protocols that emerge from a culmination of developmental knowledge, yet these protocols rarely perform systematic assessment of all the media components and their resultant necessity, potential detriment and not to mention cost implications, particularly during maturation, post regional specification. Assessing 16 combinatorial conditions, here we examined the 4 trophic proteins commonly included in neuronal maturation media - Brain Derived Neurotrophic Factor (BDNF), Glial-cell derived neurotrophic factor (GDNF), dibutyryl cyclic Adenosine Monophosphate (dcAMP) and transforming growth factor- ß3 (TGF-ß3). Cultures were analysed for survival, retained specification and functionality as well as the impact of trophic factor ablation on subsequent graft survival and function in a rat model of Parkinson's disease (PD). Omission of growth factors had no impact on cell survival or dopamine neuron proportions, with cells able to regulate expression of pro-survival transgene PITX3 in response to altered culture conditions. Furthermore, transplantation of neural progenitors cultured in the absence of these trophic cue had no detrimental impact on graft outcomes in PD rats. Parallel assessment in maturing cortical and motor neuron differentiations similarly suggest that these trophic cues are dispensable within cultures, with this knowledge providing a significant cost benefit for human stem cell-based research including clinical and industry translation.

F1282

NOVEL POROUS MICROSCAFFOLD ENABLES MACROPHAGE DIFFERENTIATION AND PROLIFERATION FROM HPSCS FOR THERAPEUTIC APPLICATIONS

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Macrophages play crucial roles in a wide range of physiological and pathological processes, including immune response and tissue homeostasis. In this study, we present a novel approach using a three-dimensional porous microscaffold (PMS) to generate a large population of

induced macrophages (iMacs) from human pluripotent stem cells (hPSCs). Interestingly, PMS facilitated the formation of vascular-like hematopoietic structures resembling the early aorta-gonad-mesonephros (AGM) region during embryogenesis. Subsequently, a population of macrophages undergoing robust proliferation emerged in PMS. Multi-omics analysis identified diverse paracrine signals within the 3D microenvironment that stimulated macrophage proliferation and activated key genes associated with proliferation and self-renewal. Functional experiments demonstrated that iMacs generated in 3D PMS could serve as off-the-shelf therapeutics and effectively alleviate symptoms of drug-resistant Streptococcus pneumoniae infections, underscoring their potential for innovative cell-based therapies.

Funding Source: This work was supported by the National Key Research and Development Program of China Grants 2022YFA1103103 and 2023YFA1800302 and the National Natural Science Foundation of China (NSFC) Grants 32270784 and 31970819 to Jie Na.

F1284

CHARACTERISTICS OF ENDOMETRIAL STEM CELLS AND EXTRACELLULAR VESICLES ACCORDING TO THE ESTRUS CYCLE

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The endometrium is the lining of the uterus that develops and regresses in response to changes in female hormone secretion during the reproductive cycle, playing a critical role in embryo implantation and successful pregnancy. Similar to many mammalian species, the canine endometrium undergoes regular cyclical changes, including growth, differentiation, remodeling, destruction, and repair. This study compared the characterization and gene expression of endometrial stem cells (EnSCs) and EnSC-derived extracellular vesicles (EVs) during the estrous cycle (diestrus and estrus). Genetically identical cloned dogs were used to allow a precise comparison of mesenchymal stem cell (MSC) activation, characteristics, and EVs secretion during different stages of the estrous cycle. The estrous cycle stages were confirmed through vaginal smears and estrogen level measurements, and uterine tissues were collected during neutering surgeries. Cell counts were compared between tissues collected from estrous and diestrus endometrium of the same weight, and cell morphology was observed. Each isolated particle was characterized by analyzing the expression of three lineage differentiation markers and MSC-specific markers to compare their properties. Additionally, EVs derived from estrous and quiescent EnSCs were extracted and their properties were compared. As a result, we identified differences in the efficiency of isolating cells from endometrial tissue at different stages of the estrous cycle, changes in cell morphology, and distinct miRNA expression profiles in EVs derived from EnSCs during the estrus and diestrus stages. These findings provide a deeper understanding of the interplay between stem cells, their secretions, and hormonal cycles, with applications in fertility treatment, uterine repair, and regenerative medicine.

Funding Source: This research was supported by a grant of the Korea Health Technology Research and Development Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health and Welfare, Republic of Korea (grant number: HI22C1754).

F1286

STREAMLINING CELLULAR REPROGRAMMING: A CLINICAL-GRADE WORKFLOW FOR MANUFACTURING ALLOGENEIC AND AUTOLOGOUS IPSCS

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Induced Pluripotent Stem Cells (iPSCs) are a transformative and ethical alternative to Embryonic Stem Cells for regenerative medicine, disease modeling, and drug discovery. Their ability to be derived from somatic cells and differentiated into various lineages makes them a versatile platform for developing stem-cell-based therapies. Most reprogramming methods are low efficiency and require early clonal isolation to generate iPSC, resulting in high passage number and limited genetic diversity that negatively impact efficiency, safety and robustness. These limitations increase production costs and timelines, hindering the scalability of iPSCs for clinical use. We developed a novel, efficient, footprint-free mRNA-based reprogramming workflow to address these gaps. Here we demonstrate that this technology safely reprograms fibroblasts and mesenchymal stromal cells with efficiencies as high as 20%, enabling bulk, polyclonal reprogramming and reducing reliance or eliminating the need for early clone selection. Clone selection is deferred to gene editing or Master Cell Bank (MCB) stages, reducing culture bottlenecks and genome stress, minimizing population doublings and passage numbers, while preserving genetic and epigenetic diversity. Optimization of this workflow across six compliant cell lines highlights its robustness and scalability. Results demonstrate a stable polyclonal population suitable for downstream gene editing and processing, reducing timelines and manufacturing costs. Enhanced in-process and release testing ensure manufacturing of iPSCs with safety and quality for both allogeneic and autologous applications. This platform represents a major advancement in iPSC manufacturing, overcoming critical barriers in cost, safety, and efficiency. Its broad applicability in cell therapy, disease modeling, and drug screening positions it as a pivotal technology for accelerating iPSC-based research and autologous and allogeneic clinical therapies.

F1290

VERIFICATION OF TISSUE REGENERATION EFFECTS IN A HIND LIMB ISCHEMIA AND SKIN ULCER MODEL MOUSE USING NOVEL CELL THERAPY WITH EX VIVO CULTURED PERIPHERAL BLOOD MONONUCLEAR CELLS

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We conducted clinical research on cell transplantation therapy using ex vivo expanded human peripheral blood mononuclear cells (MNCQQ) for intractable limb ulcer diseases, confirming safety and efficacy as a vascular regeneration therapy. We have now established a new pharmaceutical-grade cell type, RE01, which overcomes patient variability, allows simpler cultivation, and shows higher angiogenic and wound healing effects than MNCQQ cells. Our non-clinical research with RE01 cells verified therapeutic effects in mouse models of limb ischemia and skin ulcers. In the limb ischemia model, we ligated the femoral artery, administered cells intramuscularly, and observed blood flow changes and limb necrosis over time. For the skin ulcer model, we created ulcers on mice backs, administered cells subcutaneously, and measured ulcer reduction rates. Results revealed significantly enhanced vascular and tissue regeneration in ischemic tissue. RE01 cells represent an innovative peripheral blood cell therapy. We are conducting an investigator-initiated clinical trial for patients with Buerger's disease and collagen disease-derived lower limb ischemic ulcers, aiming to provide a minimally invasive, safe, and effective vascular regeneration therapy. RE01 cells offer a more reliable and potentially more effective treatment option, particularly beneficial for conditions traditionally difficult to treat. Positive preclinical results provide a strong foundation for ongoing clinical trials. If results translate to human patients, RE01 cell therapy could offer new hope for chronic limb ischemia and non-healing ulcers. Potential regulatory approval of RE01 cells as a regenerative medicine product could significantly impact vascular medicine, providing a standardized autologous cellular therapy option. This could improve accessibility and consistency of treatment worldwide. Future research will focus on investigating RE01 cells' therapeutic mechanisms, potentially leading to therapy optimizations and new applications in regenerative medicine. The development and clinical testing of RE01 cells represent a significant advancement in regenerative medicine for vascular diseases, with the potential to revolutionize treatment of limb ischemia and chronic wounds.

F1292

ENHANCING T CELL IMMUNOTHERAPY FOR SOLID TUMORS USING A NOVEL FUNCTIONAL SORTING APPROACH WITH A MICROFLUIDIC DEVICE

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Immunotherapy for cancer, especially solid tumors, has made great strides, but challenges remain, particularly in T-cell infiltration and tumor-specific killing. Improvement of T cell-mediated immunity is essential to enhance therapeutic efficacy. In this study, we developed a unique functional sorting approach using microfluidic devices with the aim of developing next-generation immuno-cell therapies with enhanced tumor infiltration and continuous killing capacity. This method allowed sorting immune cells according to their ability to migrate against target cells. In fact, when the sorted cell populations were evaluated in in vitro cytotoxicity

analysis, it was possible to enrich the populations with significantly enhanced killing activity. In the future, this study aims to use these cell populations as tools to identify and optimize the genes and pathways that define or generate these populations, thereby enhancing the functionality of T cells in solid tumor immunotherapy. This research will contribute to the advancement of cellular therapies and provide new opportunities for more effective treatment of patients with solid tumors.

Funding Source: Japan Science and Technology Agency (JST) Scholarship.

F1294

ENHANCING TISSUE REGENERATION USING PLACENTAL STEM CELL-DERIVED EXTRACELLULAR VESICLES INTEGRATED WITH THERMOGEL

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Cell-derived extracellular vesicles (EVs) are mediators of intercellular communication capable of delivering biologically active molecules. EVs inherit specific properties from their parent cells, which define their composition, biological activity, and therapeutic potential. Stem cell-derived EVs are particularly enriched in molecules related to tissue regeneration, making them highly valuable for therapeutic applications. Placental mesenchymal stem cells (MSCs) can be obtained easily and noninvasively after childbirth, offering unique therapeutic advantages such as low immunogenicity, promotion of tissue repair, reduction of inflammation, and enhancement of angiogenesis. In this study, we investigated the therapeutic potential of a thermogel complex (EVs-thermogel) composed of placental MSC-derived EVs, designed to overcome the limitations of stem cell delivery and retain therapeutic agents at wound sites for extended periods. Placental MSC-derived EVs exhibit superior capabilities in cell proliferation and immuno-modulation, and when blended with a thermossensitivity sol-gel matrix, their therapeutic efficiency is enhanced. The temperature-dependent sol-gel transition was achieved through hexanoylation of glycol chitosan (HGC). The placental MSC were characterized through analysis of cell proliferation, differentiation, and the expression of stem cell markers. The EVs-thermogel, formulated by the physical amalgamation of HGC and EVs, was optimized to evaluate its physical properties. This EVs-thermogel demonstrated no cytotoxic effects and promoted cell proliferation in co-culture environments. Its regenerative capacity was validated through positive outcomes in in vitro scratch assays and angiogenesis evaluations. It was confirmed biocompatibility through blood wettability properites. These results confirmed that EVs-thermogel is effective in tissue regeneration and holds significant promise for commercialization as a therapeutic treatment.

Funding Source: This work was supported by KOITA (Korea Industrial Technology Association) grant funded by MSIT (Korea Ministry of Environment). [Research and Development CENTER Capability Enhancement Project, grant number: 1711199718].

F1296

HETEROGENEITY OF MESENCHYMAL STEM CELL-DERIVED EXTRACELLULAR VESICLES: INFLUENCE OF CELL ORIGIN AND CULTURE CONDITIONS ON MOLECULAR CARGO AND SKIN REGENERATIVE POTENTIAL

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Mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) display significant heterogeneity, influenced by their cellular origin and culture conditions. This study investigates the diversity of MSC-EVs derived from adipose tissue, umbilical cord, amniotic fluid, and corneal sources, analyzing their intravesical cargo and molecular profiles. Additionally, we examine the impact of hypoxia and normoxia culture conditions on EV composition and functionality. Using advanced molecular characterization techniques, we identify distinct signatures across MSC subtypes, highlighting protein, lipid, and RNA content variations. Functional assays in in vitro and ex vivo skin models further evaluate the regenerative potential of these EVs. Our findings underscore the critical role of MSC source and environmental factors in shaping EV therapeutic efficacy, offering insights for optimizing MSC-EV-based skin regeneration therapies.

Funding Source: Thailand Science Research and Innovation Fundamental Fund Fiscal Year 2025 Bioflirt Labs. Co., Ltd.

F1298

THERAPEUTIC POTENTIAL OF EX VIVO CULTURED MONONUCLEAR CELLS FOR HAIR REGENERATION: PRECLINICAL AND CLINICAL EVALUATION IN ALOPECIA TREATMENT

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The ex vivo cultured mononuclear cells (MNC-QQ cells), developed in our laboratory, are a highly functional cell population derived from small amount of peripheral blood after approximately one week of culture. These cells exhibit exceptional angiogenic, antifibrotic, and anti-inflammatory properties. In a phase 1/2 clinical study on patients with refractory limb ulcers, MNC-QQ cells demonstrated a certain degree of efficacy and safety. Alopecia, characterized by a shortened anagen phase of the hair cycle and an increased number of hair follicles remaining in the telogen phase, is linked to reduced blood flow around hair follicles. To examine whether the angiogenic properties of MNC-QQ cells are effective in treating alopecia, preclinical and clinical studies were conducted. In preclinical experiment using mice, dermal papilla cells, and keratinocytes MNC-QQ cells' efficacy in promoting hair regeneration was evaluated. In an ulcer mouse model, MNC-QQ cell-treated groups exhibited a significant increase in blood vessel numbers within scar tissue, along with evidence of hair follicle regeneration. Moreover, results from in vitro experiments indicated that MNC-QQ cells directly promoted the elongation of hair follicles. In collaboration with Tokyo Skin Clinic and Plastic Surgery, Houju Medical Corporation, and Juntendo University's Department of Regenerative Therapy, a phase 1/2 clinical trial was

conducted on six patients with alopecia. MNC-QQ cells were manufactured from the patients' peripheral blood and subcutaneously injected into the scalp, with safety and efficacy evaluated over a six-month period. No adverse events were reported in any patients, and improvements in scalp findings and quality of life were observed. These findings suggest that MNC-QQ cells promote hair regeneration not only by improving blood flow around hair follicles but also through direct action on the follicles themselves, demonstrating their potential as a treatment for alopecia. Further research is underway to elucidate the underlying mechanisms of these effects.

Funding Source: This work was supported in part by a Grant-in-Aid for Special Research in Subsidies for ordinary expenses of private schools from The Promotion and Mutual Aid Corporation for Private Schools of Japan.

Clinical Trial ID: jRCTb030230286.

F1300

ENHANCEMENT OF ERYTHROCYTE DIFFERENTIATION VIA THE PRECISE INTRODUCTION OF A NATURALLY OCCURRING VARIANT IN EPOR USING CRISPR/CAS9 GENOME EDITING

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Biomanufacturing with induced pluripotent stem (iPS) cells for industrial or therapeutic applications faces significant limitations, including scalability, cost-efficiency, and consistency of the differentiated cell product. Naturally occurring variants in humans are often studied for their impact on disease, yet some variants confer traits seen as beneficial in vitro. For example, C-terminal truncating variants in the erythropoietin receptor (EpoR) increase sensitivity to erythropoietin (Epo), resulting in uncontrolled expansion of red blood cells, a condition known as congenital erythropoiesis (CE). Genetic engineering of the EPOR gene in iPS cells represents an approach to overcome biomanufacturing challenges and meet global demands for blood and blood cell products. In this work, we engineered human iPS cells with a 7-bp deletion (NM_000121.4: c.1299_1305del) resulting in truncated EpoR reported in CE. Our strategy employs CRISPR/Cas9 genome editing coupled with DNA repair via endogenous microhomology-mediated end-joining to achieve precise editing without the need for an exogenous DNA template. In two iPS cell lines commonly used for research, the 7-bp deletion variant accounted for 90.0% of all indels where the target site was modified at the frequency of 86.5%. Our approach was reproducible in an additional eight iPS cell lines established from healthy donors, with an average of 90.8% of edited alleles carrying the 7-bp deletion variant. During differentiation of EPOR-edited iPS cells into erythrocytes, iPS cells homozygous for the truncated EPOR allele demonstrated a selective advantage in proliferation, with a remarkable 93-fold increase in the production of erythroid cells compared to the wildtype allele. Moreover, cell surface markers of the erythrocyte lineage CD71 and CD253a were presented earlier in iPS cells with truncated EPOR, indicating accelerated differentiation. Finally, a survey of single

nucleotide polymorphisms (SNPs) in population genomic data suggests that our guide RNA (gRNA) targets a highly conserved region of EPOR across various ethnicities. Taken together, these data suggest that our editing approach is broadly applicable to the general human population and may be integrated into biomanufacturing processes to treat hematological disorders.

Funding Source: iPS Cell Research Fund.

F1302

ADVANCING AMNIOTIC STEM CELL THERAPEUTICS THROUGH OPTIMIZED 3D CULTURE SYSTEMS

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Amniotic membrane-derived mesenchymal stem cells (AM-MSCs) hold great promise for regenerative medicine due to their pluripotency, immunomodulatory abilities, and non-invasive isolation, which eliminates ethical concerns. However, traditional 2D culture methods fail to replicate the in vivo microenvironment, limiting their therapeutic capabilities. This study focuses on developing optimized 3D culture systems to enhance the stemness and chondrogenic differentiation potential of AM-MSCs. Utilizing specialized 3D culture platforms (LabSpheroTM, LabToLab, Republic of Korea), uniform spheroids were generated to foster cell aggregation and improve cell-to-cell interactions, critical for chondrogenesis. The findings revealed significant upregulation of stemness-related markers, such as OCT4 and SOX2, alongside increased chondrogenic differentiation, indicated by elevated expression of SOX9, COL2A1, and aggrecan. Additionally, 3D-cultured AM-MSCs demonstrated enhanced viability, better mimicked the natural extracellular matrix (ECM), and exhibited superior paracrine signaling and regenerative potential. These results suggest that 3D culture systems can significantly enhance the therapeutic efficacy of AM-MSCs, offering a promising approach for cartilage regeneration and the treatment of orthopedic conditions like osteoarthritis.

Funding Source: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (RS-2024-00405287).

F1304

INDUCED RETINAL PROGENITOR CELLS FOR TREATING PHOTORECEPTOR DEGENERATION DISEASES

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Retinitis pigmentosa (RP), diabetic retinopathy (DR), age-related macular degeneration (AMD), and Stargardt's disease are serious eye diseases which lead to to irreversible photoreceptor loss and incurable blindness. RP affects over 2 million people worldwide, with less than 5% of cases which has Rpe65 mutation, benefitting from gene therapy. DR impacts 93 million individuals globally and can be slowed by treatments such as anti-VEGFs and laser therapies, though late-stage DR remains incurable. AMD affects nearly 200 million elderly people worldwide, leading to blindness in its late stages. Stargardt's disease, the most common inherited juvenile macular degeneration, affects 1 in 8,000 to 10,000 people, with no available treatments to slow its progression. In recent regenerative therapy efforts, ReNeuron and jCyte have conducted phase 2 clinical trials using retinal progenitor cells (RPCs) derived from fetal retina to treat RP patients. The transplanted cells showed efficacy in rescuing patients' vision for at least one year. However, ReNeuron halted further trials due to surgical complications, while jCyte plans to proceed with a US pivotal trial, emphasizing that visual function restoration depends on patients' central visual field at baseline. The use of human retinal progenitor cells (hRPCs) derived from fetal tissue is limited by availability and ethical concerns. Additionally, differentiating human embryonic stem cells (hESCs) or human induced pluripotent stem cells (hiPSCs) into RPCs is time-consuming, labor-intensive, and yields low efficiency. To address the unmet medical need for photoreceptor degeneration, this study aims to generate retinal progenitor-like cells through chemical-based direct reprogramming without genome manipulation of exogenous transcription factors. Our data indicate that these chemical-induced retinal progenitor-like cells can be efficiently and safely produced in just 5 days and potentially aid in the recovery of visual function in blind animal models. More than 90% of the conversion cells show electrophysiology activity by calcium image. These encouraging results suggest that chemical-induced retinal progenitor-like cells have high clinical potential. We are progressing through preclinical tests and preparing to start clinical trials.

F1306

GENETICALLY ENHANCED MESENCHYMAL STEM CELLS SIGNIFICANTLY IMPROVED SURVIVAL OF SEPSIS IN ANIMALS AND APPEARED WELL TOLERATED IN A PHASE 1 SAFETY TRIAL OF SEPTIC SHOCK PATIENTS

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Sepsis is a disease of organ dysfunction, caused by a dysregulated host immune response to infection. Despite >1000 clinical trials, no new product has been approved. It is a global health priority as sepsis is a leading cause of morbidity and mortality in these critically ill patients. Our novel approach is based on genetically engineering immunomodulatory mesenchymal stem/stromal cells (MSCs) to express vascular protective angiopoietin-1 (ANGPT1) and the Gram-negative bacteria-targeting protein acyloxyacyl hydrolase (AOAH), which were tested to show proof of concept efficacy in improving mice survival in two sepsis models (p=0.002 in E. coli peritonitis, p=0.01 in polymicrobial sepsis, compared to vehicle). A first-in-human, dose-escalation phase 1 safety trial (AMETHYST: Advanced Mesenchymal Enhanced cell THerapY for SepTic, NCT04961658) was designed to determine the safety and maximum feasible tolerated dose (MFTD) in adult septic shock patients. A cryopreserved, allogeneic, genetically enhanced MSC therapy product (GEM00220) was manufactured at a GMP facility, with an established stability of 24 months in liquid nitrogen. Products were thawed at the bedside of intensive care units (ICU) and given at 4 ascending dose levels: A - 15 million cells, B - 60 million cells, C - 150 million cells, and D - 300 million cells given as two doses of 150 million cells, separated by 24 hours. Safety and tolerability were also assessed by monitoring adverse events up to 28 days, with day 28, day 90, and 1-year survival assessment. Eleven participants (3 each in cohort A-C; and 2 in cohort D) were enrolled with a median age of 58 years old (range: 28 to 81); median SOFA score of 15 (range 5 to 18) with a median predicted mortality risk of 80% (range: < 10% to >90%). In patients treated with GEM00220, twenty-eight-day mortality was 36% in all cohorts, with no death in cohorts C and D. Infusion for all doses was well tolerated. No stopping rule criteria were met for any dose cohort. In conclusion, infusing cryopreserved GEM00220, up to 300 million cells, into patients with septic shock seemed safe and feasible. This easy-to-use MSC therapy, engineered to express ANGPT1 and AOAH, represents a novel approach to the treatment of sepsis, independent of microbial identity or antibiotic resistance.

Clinical Trial ID: NCT04961658.

F1308

EXPLORING THE THERAPEUTIC POTENTIAL OF NETRIN-1 PRIMED MSC-DERIVED SECRETOME IN WOUND HEALING

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Wound healing is a multifaceted biological process that necessitates synchronized cellular activities, involving cell proliferation, migration, and the remodeling of the extracellular matrix. Mesenchymal stromal cells (MSCs) have received significant attention due to their regenerative capabilities, primarily attributed to their secretome, which consists of bioactive molecules and extracellular vesicles. Recent studies indicate that priming MSCs with external factors can

enhance their therapeutic efficacy. This study investigates the effects of Netrin-1 (N1) priming on the regenerative potential of MSC-derived secretome, focusing on key cellular functions essential for wound healing. MSCs were isolated from Wharton's Jelly of human umbilical cords, characterized, and subjected to priming with recombinant N1 protein for 48 hours. Further the conditioned media from these cultures were collected and assessed for their wound healing potential. Functional assays demonstrated that the secretome from N1-primed MSCs exhibited significantly improved wound healing capabilities compared to that of unprimed MSCs. Specifically, the primed secretome displayed enhanced anti-inflammatory properties by facilitating macrophage polarization, promoting fibroblast migration and proliferation, and significantly increasing tube formation by HUVECs, indicating superior vascularization and regenerative abilities. These findings indicate that priming of MSCs with N1 results in a notable increase in the secretion of factors associated with cell survival, anti-inflammation, and angiogenesis in the conditioned media. Thus, the N1-primed MSC secretome emerges as a potent therapeutic agent for wound healing, vascularization, and regeneration. Future research will focus on developing and evaluating an appropriate delivery system for the secretome to further enhance its therapeutic potential and ensure targeted delivery in preclinical models for wound healing. This integrated approach opens significant avenues for advancing therapeutic modalities in regenerative medicine.

Funding Source: Manipal Academy of Higher Education.

F1312

DEVELOPMENT OF XENO-FREE, FAST-DISSOLVING MICROCARRIERS FOR SCALABLE STEM CELL THERAPY APPLICATIONS

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Scaling up anchorage-dependent cell cultures is essential for clinical and industrial applications, such as regenerative medicine, cell therapy, and biomanufacturing. However, current microcarriers often lead to suboptimal cell yield, reduced viability, and high costs. Traditional microcarriers also require complex separation steps, increasing mechanical damage and contamination risks. Dissolvable microcarriers provide a promising solution by enabling gentle cell harvesting, minimizing mechanical stress and preserving cell quality. As demand for scalable, high-yield cell culture systems grows, dissolvable microcarriers address key challenges in large-scale production and clinical manufacturing. This study developed xeno-free, customizable, fast-dissolving P2 microcarriers for cell therapy. C2C12 myoblast cells were cultured in a 5 mL system without shaking, seeded at 48,000 cells/mL. Cell attachment was assessed after 24 hours by counting unattached cells in the supernatant. After 4 days, cells were harvested using DPBS washes and a 10-minute TrypLE enzymatic treatment, followed by counting with a haemocytometer. P2 microcarriers achieved 92.4% cell attachment within 24 hours, similar to X1 (96.1%) and Cytodex 3 (97.4%). After 4 days, P2 microcarriers dissolved in 3 minutes, compared to 10 minutes for X1, while Cytodex 3 was non-dissolvable. Cell yields

were 842,500 cells/mL for P2, 1,117,500 cells/mL for X1, and 551,250 cells/mL for Cytodex 3, corresponding to 17.6-, 23.3-, and 11.5-fold increases, respectively. Preliminary data indicated that differences in stiffness between P2 and X1 microcarriers influenced exosome expression levels. Xeno-free, fast-dissolving P2 microcarriers offer high yield, efficient harvesting, and customizable properties for scalable anchorage-dependent cell culture. These advantages position P2 microcarriers as a promising option for stem cell therapy, ensuring gentle handling and consistent cell quality essential for clinical-grade production.

F1314

PROGENITOR REPROGRAMMING FOR CELLULAR REJUVENATION BY DEFINED FACTORS

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Aging leads to tissue dysfunction and death, driven by cellular degeneration. Rejuvenation strategies using reprogramming factors like OCT4, SOX2, KLF4, and c-MYC (OSKM) can reset aging hallmarks by generating induced pluripotent stem cells (iPSC). Later, the transient expression of OSKM was reported to reset some aging hallmarks, extend the life span, restore youthful DNA methylation patterns, and reverse vision loss in mice. However, uncontrolled OSKM expression poses risks of unintended iPSC induction and loss of original cellular identity. To address this, here we developed a novel alternative cellular reprogramming method using c-MYC, BMI1, and Bcl-xL (MBX) to revert iPS-derived lineage-committed cells to their progenitor state, termed “progenitor reprogramming,” without generating iPSCs. Mesenchymal stem cells (MSCs) lose their proliferative and differentiation potential with age, reducing the expression of

stemness markers like CD90. During long-term cultures of iPSC-derived MSCs (iPS-MSCs), we observed declining proliferation potential accompanied by gradual loss of cell surface expression of CD90. We confirmed MB or MBX (MB(X)) overexpression (O/E) reversed these trends by reprogramming CD90- differentiated cells back to CD90+ MSCs and enhancing stable proliferation for several months. In contrast, either the continuous or transient OSK O/E impaired cell growth and produced unknown lineage cells and iPSC-like cells. Furthermore, transcriptomics revealed that compared to control and OSK groups, MB-induced progenitor reprogramming upregulated the expression of cell cycle-related genes and pathways while decreasing markers of cellular senescence and factors associated with senescence-associated secretory phenotype (SASP). Finally, we confirmed progenitor reprogramming in iPSC-derived megakaryocytes, myeloid cells, vascular endothelial cells, and vascular smooth muscle cells. In summary, MB(X) represents a distinct set of cellular reprogramming factors that restore differentiated cells to their progenitor state without the risk of unintended cell generation. Thus, MB(X)-driven reprogramming holds significant potential for developing innovative therapeutic strategies with promising applications in regenerative medicine, disease modeling, and drug discovery.

Funding Source: Japan Agency for Medical Research and Development under (JP23bm0404072), Tokyo Biochemical Research Foundation, the JSPS KAKENHI (23K18284), and the JST Fusion Oriented Research for Disruptive Science and Technology (JPMJFR234Q).

F1316

SCALABLE STEM CELL-DERIVED NATURAL KILLER CELL DIFFERENTIATION IN AN IN VITRO FEEDER-FREE SYSTEM

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Cell therapies in immuno-oncology has been a critical field in recent years, with natural killer (NK) cells being a promising and potent solution due to its pivotal role in the anti-inflammatory response and tumor surveillance. NK cells are well-suited for treating solid tumors, where they can penetrate the tumor matrix environment, activate fellow immune cells, and lyse cancer cells. Despite its potential, there is only a sparse following of NK-based cell therapies in clinical trials. This can be attributed to the challenges in sourcing and/or manufacturing high-quality NK cells. Representing 10 to 15% of the circulating lymphocytes in blood, NK cells are notoriously hard to obtain in sufficient numbers. NK cell lines were revealed to lack known IgG and IgM receptors which has resulted in NK-based cell therapies to use alternative sources such as pluripotent stem cells (PSCs). However, stem cell differentiation methods are extremely susceptible to variability which can easily alter indicators such as expansion rate, cell number, purity, and more. Furthermore, when considering the process from a clinical perspective, traditional culturing methods utilizing feeder cells are unfeasible due to safety and contaminant issues they pose as a secondary biological substance. This study was designed to develop a serum-free, scalable, in vitro feeder-free system using PSCs for NK cell manufacturing. By optimizing and utilizing GMP-grade reagents throughout the entire manufacturing process, an

96.33% proportion of CD3- CD56+ NK cells were achieved in 25 days. At each stage, quality control assays were performed to validate and optimize the method before evaluating final NK cells on their biological function and cytotoxicity. As a serum and feeder-free system, this proposed method can be easily upscaled and outlines the framework towards the development of a closed, automated and GMP-compliant scalable expansion system for NK cell-based immunotherapy products.

F1318

A TRANSCRIPTIONAL ATLAS OF THE PUBERTAL HUMAN GROWTH PLATE CLARIFIES THE MOLECULAR MECHANISM FOR GROWTH HORMONE-INDUCED GROWTH

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The cartilaginous growth plate is a critical organ responsible for longitudinal bone growth. It remains open throughout life in mice but closes in humans after puberty. Growth hormone (GH) is a widely used therapy for children with growth retardation and open growth plates. However, it remains unclear whether GH directly targets human growth plates. Furthermore, while cartilage stem cells have recently been identified in mouse growth plates, their presence and GH responsiveness in human growth plates are unknown. To address these gaps, we characterized the cellular and molecular organization of early pubertal human growth plates using unique tissue samples obtained during growth-restricting surgeries. Our analysis identified two distinct populations of stem cells differing in cycling activity, molecular profiles, and regulatory factors. Quiescent stem cells were localized within a niche characterized by low Wnt and TGFβ signaling. To investigate the direct effects of GH, we developed a human growth plate explant culture system. GH directly stimulated explant growth and promoted stem cell proliferation by activating the JAK/STAT, TGFβ, and ERK pathways while inhibiting the AKT pathway. Notably, activation of the TGFβ pathway occurred in an autocrine manner. These findings provide critical new insights into human longitudinal growth and the mechanisms of GH action, with potential implications for optimizing treatments for growth disorders.

Funding Source: The Swedish Research Council the Novo Nordisk Foundation the Swedish state under the ALF agreement between the Swedish government and county councils.

F1320

REVERSIBLE IMMORTALIZATION FACILITATES THE LARGE-SCALE PRODUCTION OF IL10-ENRICHED EXOSOMES FOR OSTEOARTHRITIS THERAPY

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Osteoarthritis (OA) treatment requires effective inflammation mitigation. Interleukin-10 (IL10), an anti-inflammatory protein, shows therapeutic potential but faces challenges in direct application due to delivery inefficiency. Mesenchymal stem cell (MSC)-derived extracellular vesicles (EVs) present a promising delivery platform but are limited by replicative senescence, low exosome yield, and inconsistent therapeutic outcomes. Here, we achieved reversible immortalization of human umbilical MSCs using lentivirally delivered excisable hTERT and SV40T transgenes, extending proliferation capacity while preserving stem cell properties. To produce IL10-enriched MSC exosomes, we employed two strategies: (1) engineering an N-terminal peptide sequence using the XPack lentivector to target IL10 to the exosome membrane and (2) utilizing CRISPR/dCas9 activation to enhance endogenous IL10 transcription. Combining these approaches enabled the large-scale production of bioengineered MSC_IL10_EVs, which were tested in an in vitro OA model for inflammation reduction. In vitro, MSC_IL10_EVs facilitated the polarization of CD86+ M1 macrophages into MERTK+CD206+ M2 macrophages and reduced levels of TNF-α and IL-1β, effects reversible by IL10 receptor inhibition. In vivo, MSC_IL10_EVs improved gait abnormalities in an OA rat model. These findings demonstrate an efficient platform for producing therapeutic exosomes, enhancing OA treatment efficacy through MSC-based IL10 delivery systems.

F1322

AUTOMATED IPSC CULTURE AND DIFFERENTIATION USING THE MYTOS IDEM PLATFORM: ADVANCING SCALABLE REGENERATIVE MEDICINE

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Pluripotent stem cells (PSCs) are revolutionizing regenerative medicine, yet their transition to commercialization is hindered by labor-intensive protocols and complex scaling processes. Protocols are typically developed manually in a flask-based format, and scaled by increasing operators or using 3D bioreactors for commercial production. The Mytos iDEM platform addresses these challenges by automating iPSC culture and differentiation in a closed, flask-based system, seamlessly translating manual protocols to scalable, GMP-compliant production leading to faster route to scale. In this study, we demonstrate the iDEM's versatility by automating two differentiations: hematopoietic progenitor cells (HPCs) and dopaminergic neurons (DAs). HPCs were generated using Stemcell Technologies' StemdiffTM Hematopoietic kit, achieving consistent yields and marker expression (9.5x106 HPCs per T175 with the expression levels of CD34+ >95%, CD45+ >60%, SSEA4+ < 5% and TRA-181+< 5%, n=2). DAs were produced using a previously published protocol, yielding over 95% viable cells with comparable quality to manual differentiation assessed by flow cytometry (Tuj1+>95%,

FOXA2+>95%, MAP2+>80 and TH+>65%, n=3). Additionally, on-going proof-of-concept studies differentiating iPSCs into RPE cells will highlight the platform's advantages for extended protocols. The iDEM platform simplifies scale-up by reducing variability, minimizing manual intervention, and ensuring culture integrity in a closed system. By enabling robust, scalable differentiation workflows, it accelerates the path to commercialization, providing regenerative medicine developers with a powerful tool to bring cell-based therapies to the clinic faster.

F1324

REGIONAL SPECIFIC HUMAN SPINAL INTERNEURON FOR RESTORING SPECIFIC FUNCTION IN SPINAL CORD INJURY

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Spinal cord injury (SCI) leads to locomotor deficits and neuropathic pain (NP). Ventral spinal V3 glutamate neurons are integral components of the central pattern generator (CPG) that generate the rhythm and pattern of locomotion, whereas the loss of dorsal spinal dI4 GABA neurons contributes to the NP. Here, human pluripotent stem cells (hPSCs) were differentiated into V3 and dI4 neurons in vitro. Six months after transplantation into the lesion cavity of the contused rat T10 spinal cord, human V3 and dI4 neurons extended numerous axons mainly into the ventral and dorsal horn of rat lumbar spinal cord, respectively. Human dI4 neurons form inhibitory synapses with rat sensory interneurons and nociceptive projection neurons and form presynaptic inhibition synapses with rat primary sensory afferents, whereas human V3 neurons form excitatory synapses with rat CPG neurons including V3, V2a, and V1 neurons and motor neurons. Human dI4 neurons are innervated by rat supraspinal descending pain modulation centers, including the raphe magus, dorsal raphe and rostral ventral medulla, whereas human V3 neurons are innervated by rat supraspinal motor command centers, including the corticospinal tract, reticulospinal tract and vestibulospinal tract. Functionally, NP in rats including mechanical allodynia and thermal hyperalgesia were alleviated by human dI4 neurons but not V3 neurons, whereas locomotion in rats was restored by human V3 neurons but not dI4 neurons. Importantly, recovered locomotion and alleviated NP were lost after DREADD-induced inactivation of human V3 and dI4 neurons. Finally, human dI4 and V3 neurons survive long-term, mature and integrate into the injured rhesus monkey spinal cord. These results suggest that the transplantation of hPSC-derived spinal V3 and dI4 neurons can restore locomotion and alleviate NP after SCI and highlight the importance of the regional specificity of transplanted neurons for the treatment of neurological disorders.

Funding Source: Ministry of Science and Technology of China [STI2030-Major Projects 2022ZD0204700], National Natural Science Foundation of China [82301572].

F1326

EXPLORING THE POTENTIAL OF EPSC-DERIVED NK CELLS IN A HUMAN 3D EMBRYOID BODY MODEL FOR IMMUNOTHERAPY APPLICATIONS

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Human expanded potential stem cells (EPSCs) hold promise for generating diverse cell types due to their self-renewal and differentiation capabilities, which are essential for regenerative medicine and tissue engineering. The ability of EPSCs to differentiate into both extraembryonic and embryonic lineages makes them an attractive tool for studying human development and disease. In this study, we employed a human 3D embryoid body model derived from EPSCs to investigate the generation and functionality of natural killer (NK) cells for immunotherapeutic applications. Through step-wise differentiation, our transgene-free system enabled the robust production of NK cells from EPSCs, demonstrating their potential for targeted cytotoxicity and immunomodulation. These findings highlight the potential of EPSCs as a novel source of NK cells for immunotherapy, which could lead to the development of more effective and personalized treatments for diseases.

F1328

SKIN PRECURSOR CELL DIFFERENTIATION FROM INDUCED PLUROPOTENT STEM CELLS AND APPLICATION OF SKIN PRECURSOR CELL-CONDITIONED MEDIUM IN SKIN BURNS: A PROMISING CANDIDATE FOR SKIN REGENERATION

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Skin, which consists of epidermis, dermis, and subcutaneous fat, is the largest organ in the human body and protects the body from the external environment. The skin is exposed to potential damage, and burns are one of the leading causes of skin damage. According to World Health Organization (WHO) report, approximately 180,000 people die from burns worldwide every year and millions of patients suffer from physical and mental illnesses that last a lifetime. Severe burns are characterized by the destruction of the skin structure and the disappearance of progenitor cell populations, which are essential for regenerating and restoring structure and function. Recent studies suggest that skin precursor cells (SPCs) play a crucial role in regenerating damaged tissue by differentiating into keratinocytes and other essential cell types. SPCs contribute to re-epithelialization, modulate inflammatory responses, and enhance extracellular matrix remodeling. Understanding the mechanisms by which SPCs facilitate burn wound healing could lead to novel cell-based treatments to improve burn wound treatment. In

this study, induced pluripotent stem cells (iPSCs) were differentiated into SPCs, and their morphology and expression of specific gene markers were confirmed through qPCR and immunofluorescence analysis. The regenerative effects of skin precursor cell-conditioned media (SPC-CM) were subsequently evaluated. Fibroblasts and keratinocytes were treated with SPC-CM and confirmed expression specific gene markers by qPCR and immunofluorescence analysis. The wound healing assay confirmed how much wound closure accelerated in SPC-CM treated fibroblasts and keratinocytes. Since this acceleration ability can identify the potential to promote tissue repair, Burns were induced in the 3D skin model and the skin regeneration effect was evaluated by processing the SPC-CM. The effectiveness of SPC-CM was demonstrated through the expression of inflammation-specific markers and changes the TEER values of 3D skin burns model. These results indicate that SPC-CM can improve fibroblast-mediated matrix remodeling and keratinocyte adhesion, highlighting its potential as a promising candidate for the development of therapeutic agents and cosmetic materials aimed at skin repair and regeneration.

Funding Source: This work was supported by the National Research Foundation of Korea (NRF) and ministries including Science and ICT, Trade, Industry and Energy, Health and Welfare, and Food and Drug Safety (RS-2023-KH142779, RS-2024-00512348).

F1330

GENOME-ENGINEERED PORCINE EXPANDED POTENTIAL STEM CELLS AS A NOVEL CELLULAR SOURCE FOR XENOTRANSPLANTATION

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Xenotransplantation is promising in overcoming organ shortages. Currently, somatic cells are used for genetic modifications to generate the donor pig, yet they face technical challenges like limited gene editing capacity and subsequent phenotyping. Here, we report using porcine expanded potential stem cells (EPSCs) as a unique cellular source in xenotransplantation research. We simultaneously knocked out hyperacute rejection related genes GGTA1, CMAH and B4GALNT2, and inactivated GHR to reduce organ overgrowth. Subsequently we engineered a recombinase-mediated cassette exchange (RMCE) construct at the porcine ROSA26 locus for introducing human immune modulating genes. Then we readily targeted human CD47 cDNA via this cassette exchange method. The engineered EPSCs remained pluripotent and genetically stable. By differentiating them into highly immunogenic endothelial cells and directly immunophenotyping them, we demonstrated that the edited cells exhibited substantially lower complement-mediated lysis, decreased antibody binding, and reduced macrophage phagocytosis compared to the unedited cells. The efficient and robust gene editing in porcine EPSCs and the subsequent cellular immunophenotyping of the differentiated cells can potentially streamline the xenotransplantation study and facilitate generation of improved donor animals.

Funding Source: National Key Research and Development Program of China (nos.

2022YFA1105401); Health@InnoHK, Innovation Technology Commission; HKSAR, Hong Kong Research Council (Germany/Hong Kong travel grant G-HKU704/21).

F1332

CHIMERIC ANTIGEN RECEPTOR T CELLS FOR SELECTIVE TARGETING OF CANCER-EXPRESSING PODOCALYXIN

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Chimeric Antigen Receptor (CAR) T-cell therapy has achieved remarkable success in treating CD19-positive B-cell malignancies but faces significant challenges in addressing solid tumors such as antigen specificity and safety concerns regarding off-target effects. Podocalyxin (PODXL), a transmembrane protein linked to tumor progression and poor prognosis in various cancers, represents a promising but difficult therapeutic target because of its expression in normal tissues. To address these challenges, we employed CasMab technology to develop CAR-T cells utilizing the cancer-specific monoclonal antibody PcMab-6, which selectively recognizes cancer-specific modifications on PODXL-expressing cancer cells. Compared to control CAR-T cells derived from a non-cancer-specific antibody (PcMab-47), PcMab-6 CAR-T cells demonstrated significant antitumor activity in vitro with reduced off-target effects. Furthermore, the humanization of PcMab-6 scFv enhanced the persistence and therapeutic efficacy of CAR-T cells, resulting in extended antitumor effects in vivo. These advancements highlight the potential of humanized PODXL-targeted CAR-T cells to provide safer and more effective treatments for solid tumors, paving the way for clinical application.

F1334

IL-24-IMSC: A NOVEL THERAPEUTIC STRATEGY TO REVERSE THE IMMUNOSUPPRESSIVE TUMOUR MICROENVIRONMENT IN NON-SMALL CELL LUNG CANCER

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Lung cancer remains the leading cause of cancer-related mortality worldwide, with non-small cell lung cancer (NSCLC) accounting for approximately 85% of all cases. Although immune checkpoint inhibitor (ICI) therapies have significantly improved survival outcomes in advanced NSCLC, up to 85% of patients develop treatment resistance. The immunosuppressive tumour microenvironment (TME) is believed to play a significant role in the development of ICI

resistance. Genetically engineered mesenchymal stem cell (MSC) therapy represents a promising approach for cancer treatment due to its low immunogenicity, tumoritropic nature, cost effectiveness and potential availability as an “off-the-shelf” therapy. In this study, we present IL-24-iMSC, a novel therapeutic in which MSCs are genetically modified to secrete interleukin 24 (IL-24), an immunomodulatory cytokine with anti-cancer properties. Using CRISPR/Cas9, the IL-24 gene was inserted into induced mesenchymal stem cells (iMSCs) at the B2M locus, with expression driven by the EF1α promoter. Flow cytometry revealed high expression of MSC surface markers (CD73, CD90, CD105) and absence of hematopoietic (CD34, CD45) and HLA-DR markers, confirming the MSC phenotype. Enzyme-linked immunosorbent assay (ELISA) demonstrated sustained IL-24 secretion from IL-24-iMSCs, while control iMSCs showed no IL-24 production. Tumour tropism of IL-24-iMSC was validated in xenograft models, where infiltration of stem cells into tumours was observed. Future investigations will focus on evaluating the immunomodulatory effects of IL-24-iMSC in orthotopic models using immunocompetent mice. This study aims to provide preclinical evidence supporting the development of IL-24-iMSC as a therapeutic strategy for NSCLC. Furthermore, it will offer critical insights into the potential of genetically engineered stem cell therapy to enhance immunotherapy outcomes in lung cancer patients.

F1336

HUMAN NEURAL STEM CELL-DERIVED EXTRACELLULAR VESICLES ALLEVIATES BREAST CANCER CHEMOTHERAPY-INDUCED COGNITIVE IMPAIRMENTS

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Breast cancer is one of the most common cancers affecting women throughout the world. Breast cancer chemotherapies have been shown to cause cancer-related cognitive impairments (CRCI), negatively affecting the quality of life (QOL) for ~70% of 4 million survivors in the U.S. Often referred to as chemobrain, CRCI encompasses decreased attention, disrupted processing speeds, executive function, memory consolidation, and recall long-term post-therapy. Previously, we showed the regenerative potential of extracellular vesicles (EVs) derived from human neural stem cells (hNSCs) in reversing CRCI in brain cancer mouse models receiving cranial radiotherapy. The current study focuses on a breast cancer mouse model to assess the effectiveness of a GMP-grade hNSC (UCI-191)-derived EV to reverse chemobrain following clinically relevant adjuvant chemotherapy. Nano-sized EVs can cross the blood-brain barrier and contain bioactive cargo such as lipids, proteins, nucleic acids, and mitochondrial components. WT (C57) female mice were induced with breast cancer using murine Py230 cells. Then, mice received adjuvant chemotherapy (Adriamycin, ADR, doxorubicin, 2mg/kg; and cyclophosphamide, CYP, 50mg/kg), administered an hour apart, once weekly for four weeks. This treatment eliminated breast cancer growth. After the administration of chemotherapy, mice received IV injections (retro-orbital vein, RO) treatment of hNSC-EV once weekly for four weeks and one month later, administered learning and memory, executive function, and memory consolidation cognitive function tests. Animal brains were evaluated for

neuroinflammation, gliosis, and synaptic integrity. Mice treated with ADR-CYP showed significantly reduced learning and memory, increased anxiety, decreased memory consolidation, and impaired executive function compared to ADR-CYP +EV-treated mice. Immunofluorescence and 3D algorithm-based in silico volumetric analyses revealed significant improvements in synaptic integrity and reductions in astroglial and microglial activation in the EV-treated mice. These findings demonstrate the regenerative and neuroprotective impact of GMP-grade stem cell-derived EVs in ameliorating breast cancer chemobrain that has the potential to improve QOL for millions of breast cancer survivors.

Funding Source: NIH R01 CA262213 and R01 CA276212 to M. M. A. and California Institute for Regenerative Medicine (CRIM) award: DISC-12400 to M. M. A.

UPDATES NOT INCLUDED IN ORIGINAL PUBLICATION (NOT IN ORDER):

GSI Track

Poster session 1 (ODD)

Poster number: W1359

FUTURE PERSON RESEARCH WITH HUMAN EMBRYOS AND EMBRYO-LIKE MODELS: AN ALTERNATIVE ETHICAL FRAMEWORK

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Improved embryo culturing techniques and new stem cell-based embryo models (SCEM) are challenging legal and regulatory frameworks for the ethical conduct of this research, internationally. These developments have prompted calls to reconsider limits that currently restrict human embryo research where it is legal. Questions have also arisen over whether SCEM that closely resemble early embryos should be regulated as embryos or under separate ethical and regulatory frameworks. While several proposals have been made in response to these questions, they are philosophically unsound and add unnecessary complexity to ethics review processes. This paper proposes an alternative framework for categorizing research that ought to be subject to the highest levels of ethics review from that which need not. This framework is not contingent on any biological or developmental markers, but instead draws upon philosophically and morally significant distinctions between ‘future person’ and ‘non-future person’ research.

DMDD

Poster Session 2 (ODD)

T1025

DEVELOPMENT OF A DELIVERY STRATEGY FOR NEURAL MICROTISSUES IN PARKINSON'S DISEASE CELL THERAPY

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With the rapid expansion of the field of 3D cultures and organoids, interest in their therapeutic use is growing. However, these innovative formats pose unique challenges for clinical translation as their physical and biological properties substantially differ from those of conventional single-cell-based products. Their handling imposes new constraints, such as much faster sedimentation, which must be addressed from the fill-and-finish stage to the final delivery procedure in the target region to ensure accurate dosing and precise cell placement. In this study, we present the development of a strategy to maintain homogeneity throughout the entire downstream process for the delivery of 3D neural microtissues as a cell therapy for Parkinson's disease. This includes the development of a custom-made delivery solution. Various delivery methods were compared using in vitro tests. The final selected strategy was validated through in-use testing and led to successful engraftment in a non-human primate, with the presence of dopaminergic (DA) neurons observed 1 month after transplantation. The development approach described here holds potential for broader applications in other diseases and supports using next-generation cell therapies employing 3D formats.