INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

STEM CELLS IN TISSUE MAINTENANCE, REPAIR, AND AGING

An ISSCR International Symposium

11–13 DECEMBER 2024
SINGAPORE

IN PARTNERSHIP WITH

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PROGRAM BOOK

ABOUT THE ISSCR

INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

The International Society for Stem Cell Research
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isscr.org

The International Society for Stem Cell Research (ISSCR) is a 501c(3) nonprofit organization with a mission to promote excellence in stem cell science and applications to human health. Our vision is a world where stem cell science is encouraged, ethics are prioritized, and discovery improves understanding and advances human health.

The ISSCR represents nearly 5,000 scientists, students, educators, ethicists, and business leaders from more than 80 countries. Each ISSCR member makes a personal commitment to uphold the ISSCR Guidelines for Stem Cell Research and Clinical Translation, an international benchmark for ethics, rigor, and transparency in all areas of practice.

Our Board of Directors and Committees represent leaders across research, academia, and industry who are committed to advancing the Society's mission.

Our work is made possible through generous support from our members and allied organizations towards strategic initiatives that support the mission:

- **Regulatory Affairs:** The ISSCR helps members navigate the regulatory landscape while assisting regulators by making scientifically informed recommendations for the development of stem cell therapies.
- **Policy:** The ISSCR advocates globally to support research funding, enforce ethical guidelines, and guard against unproven therapies.
- **Education:** The ISSCR provides resources and programs for the general public, educators, physicians, policy makers, and regulators. Aboutstemcells.org and ISSCR's patient handbook provide scientifically vetted resources for patients seeking unbiased and trusted information.
- **Standards and Guidelines:** The ISSCR sets international guidance for ethical and rigorous research, adopted by public and private organizations, regulatory bodies, funders, and publications. These references strengthen the pipeline of research and therapies, ultimately to benefit the patient.
- **International Conferences:** The ISSCR hosts a portfolio of international and digital meetings designed for knowledge sharing and collaboration to further the field. Discover upcoming programs, including the ISSCR 2025 Annual Meeting.
- **Publishing:** The ISSCR publishes *Stem Cell Reports*, an open access journal communicating basic discoveries in stem cell research alongside translational and clinical studies.

ABOUT THE SSCS

STEM CELL SOCIETY SINGAPORE

Stem Cell Society Singapore
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Singapore 138648
secretariat@stemcell.org.sg
stemcell.org.sg

The Stem Cell Society Singapore (SCSS) is a non-profit organisation dedicated to advancing stem cell research and education in Singapore. Established in 2008, it serves as a platform for scientists, clinicians, and industry professionals to collaborate, share knowledge, and promote the development of regenerative medicine. SCSS organises conferences, workshops, seminars & webinars, and public outreach programs to raise awareness about stem cell therapies' potential and foster an informed public dialogue. The society aims to bridge the gap between research and clinical applications, supporting innovation in the treatment of various diseases and conditions through stem cell-based approaches, while adhering to ethical standards and scientific rigor. For more information about SCSS and events see stemcell.org.sg.

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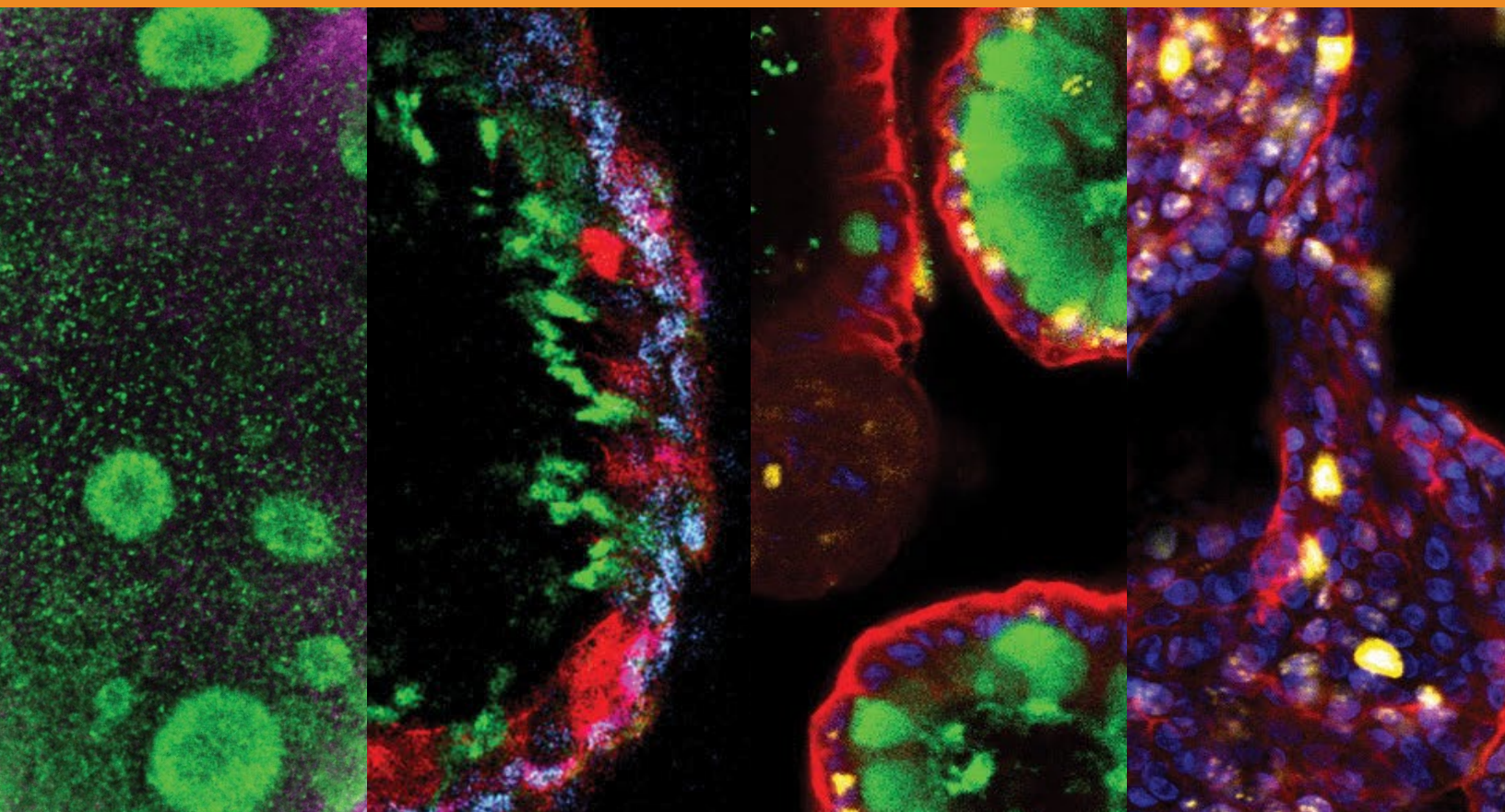

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All times are listed in Singapore Time (SGT)

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Pick up your name badge in the designated area during the posted hours below. Name badges are required for admission to all sessions, social events, meals/breaks, and the Exhibit & Poster area. Badges can be picked up during the following times:

Registration Desk Hours | Ground Floor Entrance
Nanyang Technological University, Lee Kong Chian School of Medicine, Clinical Sciences Building

Wednesday, 11 December	8:00 AM – 5:00 PM
Badge Pick-Up	
Exhibitors ONLY:	8:00 AM – 11:00 AM
Attendees:	11:00 AM – 5:00 PM
Thursday, 12 December	8:30 AM – 4:00 PM
Friday, 13 December	8:30 AM – 3:00 PM

ISSCR PROGRAM AGENDA

There will be no printed program book for the 2024 Singapore International Symposium. You can access the online version of the program agenda here: bit.ly/3YAWD5N to find the most up to date schedule as times are subject to change.

LIVESTREAMING

Livestream will not be available for this event. However, registrants can access the audio and slide recordings on-demand after the event on ISSCR.digital with their ISSCR credentials. If you have trouble logging in, first try resetting your password. If the problem persists, please direct questions to isscrdigital@isscr.org.

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Please bring found items to the ISSCR Registration Desk during posted hours. If you lose an item, visit the registration desk during posted hours for assistance.

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Each poster will be presented during a 45-minute session in the 4th floor foyer of the Nanyang Technological University, Lee Kong Chian School of Medicine, Clinical Sciences Building. **Poster presenters must adhere to the scheduled date and time of their poster display and presentation.**

Wednesday, 11 December – Poster Sessions I & II

Poster Session I (ODD Poster Numbers)

Poster Set-up:	2:20 PM – 2:50 PM
Poster Presentation:	5:00 PM – 5:45 PM
Poster Take-down:	6:30 PM

Poster Session II (EVEN Poster Numbers)

Poster Set-up:	2:20 PM – 2:50 PM
Poster Presentation:	5:45 PM – 6:30PM
Poster Take-down:	6:30 PM

Thursday, 12 December – Poster Session III & IV

Poster Session III (ODD Poster Numbers)

Poster Set-up:	3:20 PM – 3:40 PM
Poster Presentation:	5:15 PM – 6:00 PM
Poster Take-down:	6:45 PM

Poster Session IV (EVEN Poster Numbers)

Poster Set-up:	3:20 PM – 3:40 PM
Poster Presentation:	6:00 PM – 6:45 PM
Poster Take-down:	6:45 PM

Poster presenters are responsible for removing their posters upon completion of their presentation time. Any posters not removed at the end of the session will be discarded.

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STEM CELL REPORTS

STEM CELL REPORTS

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Stem Cell Reports is an open access forum, peer-reviewed journal communicating basic discoveries in stem cell research, in addition to translational and clinical studies. *Stem Cell Reports* focuses on manuscripts that report original research with conceptual or practical advances that are of broad interest to stem cell biologists and clinicians. *Stem Cell Reports* participates in Cell Press Multi-Journal Submission, allowing authors to simultaneously submit their papers for consideration by multiple journals at once.

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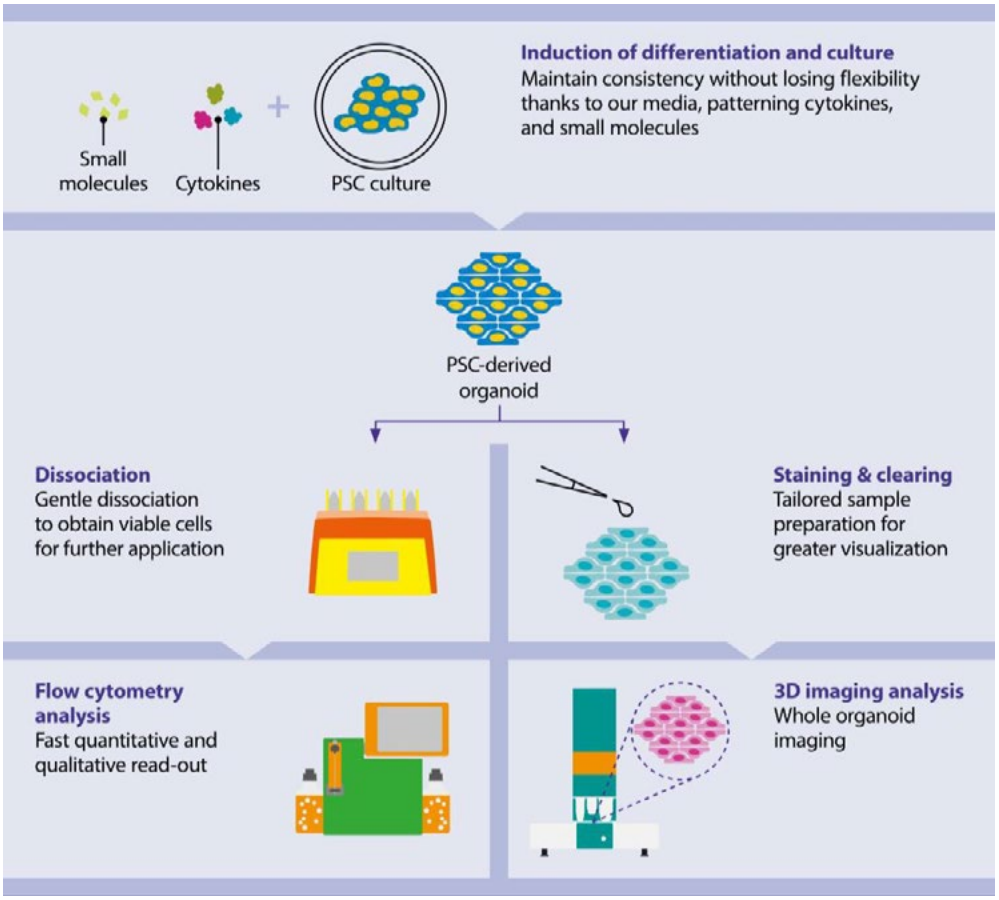

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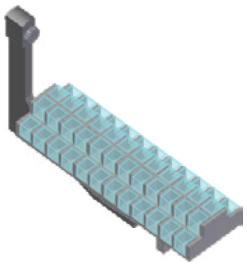

▶ See 48 organoids imaged in 3D here

SPEAKER ABSTRACTS

All times are listed in Singapore Time (SGT)

WEDNESDAY, 11 DECEMBER 2024

1:10 PM – 2:35 PM
EVOLUTIONARY DEVELOPMENT AND REGENERATION ACROSS SPECIES

1:10 PM – 1:45 PM

OPENING KEYNOTE:
UNDERSTANDING THE IN VIVO DYNAMICS OF DISTINCT MUSCLE STEM CELLS SYSTEMS DURING GROWTH AND REGENERATION AND THEIR DIFFERENT NICHES ACROSS THE VERTEBRATE PHYLOGENY

Peter Currie

Australian Regenerative Medicine Institute, Australia

While the function of adult tissue-resident stem cells during regeneration and disease have received much attention, the mechanistic basis of stem cell driven organ growth remains relatively poorly defined. Consequently, our understanding of processes that drive organ development remains piecemeal. Understanding stem cell dynamics within the organ systems that deploy them to generate growth is critical for the success of ongoing efforts to generate complex functioning organs from organoid rudiments in vitro. Thus, we have sought to understand the stem cell processes that regulate and drive myotomal growth from the template of the embryonic myotome using zebrafish as our main model system. Here we compare and contrast modes of myotomal growth and muscle regeneration and their molecular regulation in distinct niches across the phylogeny, in attempt to generate a fuller understanding of the way muscle stem cells act within different vertebrate clades.

Funding Source: National Health and Medical Research Council Grant 2016338 Australian Research Council Grant DP240101647.

1:45 PM – 2:00 PM

SKELETAL MUSCLE REGENERATION—LEADS FROM CROSS-SPECIES ANALYSIS

Hong-Wen Tang[1], Kah Yong Goh[2], Wen Xing Lee[2], Sze Mun Choy[1], Gopal Krishnan Priyadarshini[1], Kenon Chua[1], Qian Hui Tan[3], Shin Yi Low[1], Hui San Chin[1], Chee Seng Wong[1], Shu-Yi Huang[4], Nai Yang Fu[1], Jun Nishiyama[1], and Nathan Harmston[3]

[1]Duke-NUS Medical School, Singapore, [2]Program in Cancer and Stem Cell Biology, Duke-NUS Medical School, Singapore, [3]Yale-NUS College, Singapore, [4]National Taiwan University Hospital, Singapore

Muscle diseases commonly result in the loss of muscle mass, function, and regenerative capacity, severely limiting mobility and diminishing quality of life. Since muscle stem cells (MuSCs) play a critical role in muscle repair, targeting regulators of muscle regeneration represents a promising therapeutic strategy. However, the molecular mechanisms driving this process remain poorly understood. Through a genetic screen in Drosophila, we identified the transcription factor Deformed Epidermal Autoregulatory Factor 1 (Deaf1) as a novel regulator of muscle regeneration. Our findings demonstrate that Deaf1 binds to the promoter regions of PI3KC3 and Atg16l1, repressing their expression and thereby inhibiting autophagy. Consequently, Deaf1 depletion triggers autophagy, which disrupts MuSC survival and differentiation. On the other hand, Deaf1 overexpression suppresses autophagy, leading to protein aggregation and cell death. These observations suggest that both Deaf1 depletion and overexpression impair muscle regeneration, emphasizing the need for precise regulation of Deaf1-mediated autophagy during myogenesis. Importantly, we discovered that Deaf1 expression is dysregulated in sarcopenic and cachectic MuSCs. By manipulating Deaf1 expression, we were able to mitigate muscle atrophy and restore muscle regeneration in mouse models of sarcopenia and cancer cachexia. Together, these findings highlight a crucial role for Deaf1 in muscle regeneration and offer new insights into potential therapeutic strategies for combating muscle atrophy.

SPEAKER ABSTRACTS

Funding Source: This work was supported by Singapore's Ministry of Education (2022-MOET1-0004), National Academy of Medicine grant (MOH-001189-00), and National Medical Research Council (NMRC) (MOH-001208-00).

2:00 PM – 2:25 PM

UNLOCKING HUMAN ORGAN REGENERATION: LEARNING FROM THE PLANARIAN APSC (ADULT PLURIPOTENT STEM CELLS) SYSTEM

Kiyokazu Agata

National Institute for Basic Biology, Japan

Planarians possess an extraordinary ability to regenerate, enabled by their unique adult Pluripotent Stem Cells (aPSC, neoblast). These cells allow planarians to regenerate fully functional organs, like the brain and pharynx, even from small body fragments lacking these organs. Notably, planarians can precisely regenerate only the specific structures that have been lost; for instance, if dopaminergic neurons are selectively eliminated from the brain, they regenerate solely those neurons. Similarly, when the tip of the pharynx is partially removed, only the lost portion is precisely restored, allowing for the recovery of a fully functional brain or pharynx. This regenerative capability appears to be underpinned by a unique aPSC system that can proportionally induce growth or degrowth in response to nutritional conditions. In this system, aPSC alone retains proliferative ability and is immortal, while differentiated cells have limited lifespans, enabling continuous cellular turnover even in mature organs. Additionally, the fate of aPSC is controlled by a positional information system, allowing for proportional growth and degrowth to meet the organism's needs.

Recently, with the development of iPS cells by Dr. Shinya Yamanaka, human aPSCs have also been successfully generated. If human iPS cells possess similar characteristics to planarian aPSCs, planarian studies may provide substantial insights into future human organ regenerative medicine. Here, I will present evidence that planarian aPSCs exhibit properties remarkably similar to those of iPS cells and discuss how the planarian aPSC system is regulated.

3:05 PM – 4:30 PM
TISSUE MAINTENANCE: SESSION I

3:05 PM – 3:30 PM

RECONSTRUCTING THE COMPLEX HUMAN AIRWAY NICHE: INTERPLAY OF EPITHELIAL PROGENITORS, FIBROBLASTS, AND IMMUNE CELLS

Mu He

The University of Hong Kong, Hong Kong

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 huge 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

SPEAKER ABSTRACTS

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.

3:30 PM – 3:45 PM

DEVELOPMENT OF IMMUNE COMPETENT LUNG ORGANOIDS FOR MODELLING AIRWAY MAITENANCE AND REPAIR

Nicholas Hannan, Rizal Azis, Carlos Sainz, Liam Reed, Ana Serna, Sara Cuevas-Ocana, Sal Jones, and Amir Ghaemmaghami

University of Nottingham, UK

Collectively, respiratory diseases are a major cause of death worldwide. While COPD, asthma, viral infections and lung cancer are major causes of lung disease, exposure to environmental air pollution is a significant driver of airway dysfunction. Air pollution was responsible for >9 million premature deaths in 2015, with an associated economic impact of >US$ 4.6 trillion. Currently, animal models are the primary platform used in respiratory research, however they do not always recapitulate the human physiological response. Additionally, most human cell models focus only on epithelial cells leaving a significant gap in our understanding of cell-cell interactions in tissue maintenance and repair. We have addressed this issue by creating immune competent alveoli organoids that are comprised of hIPSC-derived alveolar type II cells (ATIIs), macrophages, dendritic cells, fibroblasts, and endothelial cells differentiated using a xeno and serum-free platform. This platform allows us to reconstruct alveolar tissue composition and model airway dysfunction caused by cell interaction with environmental particulate matter. To validate our platform each individual cell type has been characterised by flow cytometry, q-PCR and bulk RNA sequencing, for expression of mature cell markers and relevant GeneOntology, GSEA and Reactome profiling. Cell-specific enrichment analysis also show statistically significant cell and tissue-specific hits for individual cell types. We then assembled immune competent alveoli organoids to understand how cells respond to exposure to particulate matter. Cytokine assays demonstrated an increase in inflammatory markers linked with recruitment of immune cells, angiogenesis and pro- and anti-inflammatory signalling. Single-cell sequencing showed changes in numbers of endothelial and immune cells as well as distinct populations of fibroblasts when compared to untreated organoids. Interestingly we see activation of alveolar stem cells involved in repair of damaged lung epithelium. Our model allows for the construction of complex, multi-cell type organoids that are immune competent and can respond to environmental stimuli. This may lead to a deeper understanding of alveolar homeostasis and repair and identify new ways to treat respiratory dysfunction and disease.

Funding Source: MRC, BBSRC, NC3Rs.

3:45 PM – 4:10 PM

REGIONAL SKIN REGENERATION AND DISEASE PATTERNS

Ting Chen

National Institute of Biological Sciences, China

Skin is the largest organ of the human body and can be viewed as a two-dimensional organ from a holistic perspective. Various functional units of the skin, including hair follicles, the epidermis, and pigmentation, exhibit genetically-based regional differences that have evolved to enhance species survival. In addition to its role in normal development and homeostasis, many human skin disorders present with distinctive regional patterns that aid in clinical diagnosis. Our research focuses on understanding the cellular and molecular mechanisms that regulate skin regeneration and disease patterns at the whole-body level.

SPEAKER ABSTRACTS

THURSDAY, 12 DECEMBER

9:00 AM – 10:20 AM
TISSUE MAINTENANCE: SESSION II

9:00 AM – 9:25 AM

DECIPHERING STEM CELL ROLES IN TISSUE MAINTENANCE AND CANCER IN THE GI TRACT

Nicholas Barker

Institute of Molecular and Cell Biology, Singapore

Isolating endogenous gastric stem cells and their cancer counterparts is challenging due to a lack of useful surface markers. We recently identified Aqp5 as a membrane marker that facilitates enrichment of both human and mouse pyloric stem cells. Using mouse models and organoid assays, we further identify Aqp5-expressing populations in gastric cancers as cancer stem cells (CSC's), identifying them as a potential therapeutic target. Ongoing investigation of AQP5 roles in conferring key cancer traits using organoid, lineage tracing and orthotopic transplantation models will be discussed. In addition, we will discuss the recent discovery of new colon stem cell markers that facilitate the evaluation of colon stem cells as cancer origins and the development of advanced models of regional colon cancer.

Funding Source: This work is supported by A*STAR CRF funding, & the Singapore Ministry of Health's National Medical Research Council (NMRC) Open Fund-Individual Research Grant (OFIRG19may-0069).

9:25 AM – 9:40 AM

MECP2 REGULATES PRIMARY CILIA FUNCTION IN NEURONAL MAINTENANCE

Anthony Flamier[1], Margaux Brin[1], Marion Guillon[1], Yi Liu[2], Laetitia Laurent[1], Richard A. Young[2], and Rudolf Jaenisch[2]

[1]*CHU Sainte-Justine, Université de Montréal, Canada,* [2]*Whitehead Institute, USA*

Mutations in the methyl-DNA-binding protein MECP2 are the primary cause of Rett syndrome (RTT), a severe neurodevelopmental disorder predominantly affecting females. While MECP2's role in transcriptional regulation is complex, encompassing both activation and repression of gene expression, its exact functions remain partially understood. Utilizing an integrated multi-omic approach—comprising CUT&Tag for chromatin profiling, transcriptomics for gene expression analysis, and proteomics for protein interaction mapping—we sought to delineate the impact of MECP2 mutations on neuronal function and maintenance. Our comprehensive analysis revealed that MECP2 binds to CpG-rich promoter-proximal regions of over 4,000 genes, including numerous autism spectrum disorder risk genes, in conjunction with RNA polymerase II (RNA Pol II). This binding suggests that MECP2 acts as a cofactor for RNA Pol II, enhancing transcription at these sites. Notably, MECP2 mutations led to reduced expression of these co-occupied genes, underscoring its role in facilitating transcriptional activation. Further investigation into the temporal dynamics of transcriptional dysregulation in MECP2-mutant neurons revealed that defects in gene expression emerge early during neuronal differentiation. Single-nucleus RNA sequencing of brain organoids derived from MECP2-mutant iPSCs identified significant dysregulation of genes associated with primary cilia, essential organelles involved in cellular signaling and homeostasis. This dysregulation was phenotypically manifested by shortened primary cilia, suggesting a novel aspect of RTT pathology linked to ciliary dysfunction. Given the critical role of primary cilia in maintaining cellular signaling pathways and tissue homeostasis, our findings propose that MECP2 mutations contribute to RTT pathogenesis by impairing primary cilia function, thereby affecting neuronal maintenance and potentially accelerating aging-related neurodegenerative processes. This study not only elucidates MECP2's dual role in transcriptional regulation but also highlights primary cilia as a potential therapeutic target for enhancing tissue maintenance and mitigating aging-associated neuronal degeneration in RTT. In conclusion, our integrative multi-omic approach has provided significant insights into the molecular mechanisms underpinning MECP2 function and its role in neuronal health. By identifying early-onset primary cilia defects as a critical component of RTT pathogenesis, we pave the way for novel therapeutic strategies aimed at correcting ciliary dysfunction and improving outcomes

SPEAKER ABSTRACTS

for individuals with Rett syndrome. These findings have broader implications for understanding the maintenance and repair of neuronal tissues and the interplay between genetic regulation and cellular aging.

9:40 AM – 9:55 AM

AMP-ACTIVATED PROTEIN KINASE IN MAMMARY GLAND TISSUE HOMEOSTASIS

Annapoorni Rangarajan

Indian Institute of Science, India

Mammary gland development during puberty, and thereafter during cycles of pregnancy and lactation, is an energy intensive process. It involves extensive proliferation, self-renewal and differentiation, and is fuelled by mammary gland stem cells. AMP-activated protein kinase (AMPK) is an energy-sensor and central metabolic regulator that regulates diverse range of biological processes including cell growth, polarity, and autophagy. AMPK is also implicated in various metabolic disorders, cardiovascular diseases, and cancer. Prior work from our lab has identified a key role for AMPK in mammosphere formation, stress survival and EMT using models of human breast cancer cell lines. We hypothesize that AMPK plays a critical role in regulating mammary gland stem cells during normal development and breast cancer. The mouse serves as an excellent model system for investigating mammary gland development and obtaining mechanistic insights into breast cancer. Using MMTV-Cre induced transgenic mice model, we developed a conditional AMPK KO mice to begin to address the role of AMPK in normal mammary gland development and stem cell biology to get insights into mammary gland tissue homeostasis as well as breast cancer. Our data shows that AMPK KO mice show hyperproliferation, increased epithelial content and show increased milk production, suggesting that AMPK plays a homeostatic role during pregnancy and lactation by regulating epithelial cell proliferation and lactogenesis.

9:55 AM – 10:20 AM

TRACING CELL ORIGINS IN TISSUE MAINTENANCE AND REGENERATION

Bin Zhou

Center for Excellence in Molecular Cell Science, Chinese Academy of Sciences, China

Recent studies of lineage tracing methodologies have revolutionized our understanding of cellular origins, fates, plasticity, and heterogeneity in organ development, regeneration, and diseases. This presentation will introduce two recently developed genetic lineage tracing approaches and research advances, giving examples of their novel applications in studying cell origins and fate plasticity. Specifically, the dual recombinase-mediated genetic lineage tracing approach has been used to unravel regional hepatocyte proliferation in the liver lobule and elucidate transient hepatic progenitor cells in liver repair and regeneration. Intercellular genetic lineage tracing technology is generated to monitor cell-cell communication and contact histories in vivo, the application of which identifies the role of stem cell-contacting niche stromal cells in tissue homeostasis and diseases. This presentation will also analyze and discuss the major considerations, potential solutions, and future research directions for applying these new technologies.

10:40 AM – 12:00 PM
TISSUE REPAIR—PRINCIPLES OF REGENERATION

10:40 AM – 11:05 AM

THE EMBO KEYNOTE LECTURE: GUIDING CELL FATE DURING INTESTINAL EPITHELIAL REPAIR

Kim Jensen

Novo Nordisk Foundation Center for Stem Cell Biology—reNEW, University of Copenhagen, Denmark

The intestinal epithelium is essential for digestion and absorption of nutrients. Adult stem cells located at the bottom of crypts are responsible for the life-long replenishment of the epithelium by giving rise to differentiated offspring. Upon damage the tissue has to rapidly repair the injured areas. Yet the mechanisms

SPEAKER ABSTRACTS

guiding the regenerative process are still poorly understood. Interestingly, multiple cell populations can contribute to tissue repair, which is entail a process of cellular reprogramming, where cells begin to express gene regulatory pathways associated with a more primitive fetal intestinal state. If we can understand the mechanisms that direct these state transitions, we speculate that we can enhance the process of tissue regeneration and provide new therapeutic options for treating ulcerative disorders. Recent work from my team has begun to map the pathways controlling and coordinating these state transitions using both compound and crispr/cas9 screens identifying a series of regulators of cell fate and the regenerative state, which will be the focus of my lecture.

11:05 AM – 11:20 AM

PROFOUND CELLULAR DEFECTS ATTRIBUTE TO MUSCULAR REGENERATION FAILURE AND PATHOGENESIS IN RHESUS MONKEY MODEL OF DUCHENNE MUSCULAR DYSTROPHY

Ping Hu[1], Xin Fu[2], Yongchang Chen[2], Wei Shuai[3], and Hui Zhao[1]

[1]Guangzhou Laboratory, China, [2]Medical School, Shanghai Jiaotong University, China, [3]Kunming University of Science and Technology, China

Duchenne muscular dystrophy (DMD) is a genetic disease due to the mutation of DMD gene, that leads to continuous injury of muscle and failure of regeneration. Several animal models in mice and dogs have been established and used for the development of drugs. However, they cannot faithfully recapitulate the regeneration defects and pathogenesis at the early stage of the disease due to the species differences. Nonhuman primate provides a model to closely mimic human beings in many aspects. By breeding the initial heterogenous monkey for two generations. The Rhesus monkey DMD model recapitulates the typical morphologies in human patients and enables us to explore the pathogenic mechanism at the early stage of the disease. Single cell sequencing analysis using muscles from DMD monkey revealed that inflammation and fibrosis occurred at the very early stage of DMD. Furthermore, muscle stem cells (MuSCs) displayed multiple dysfunctions including exit of quiescence, abnormal proliferation and differentiation, elevated apoptosis and fibrosis. These results revealed that MuSCs have profound pathogenic changes even at the early stage of DMD. The defects fo stem cells leads to failure of regeneration even at the early stage of the diseas and suggest that DMD is also a stem cell disease.

11:20 AM – 11:35 AM

IN VITRO MODELLING OF RESPIRATORY INFECTIONS WITH HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED ALVEOLAR EPITHELIAL TYPE 2 CELLS USING A NOVEL CHEMICALLY DEFINED PLATFORM

Ana Serna Valverde[1], Liam Reed[2], Sara Cuevas Ocaña[2], Carlos Sainz Zuñiga[2], Amanda Tatler[3], Gisli Jenkins[4], and Nicholas Hannan[2]

[1]University of Nottingham, UK, [2]Cancer and Stem Cells, University of Nottingham, UK, [3]Respiratory Medicine, University of Nottingham, UK, [4]National Heart & Lung Institute, Imperial College London, UK

The alveolar sacs, essential for gas exchange in the lungs, consist of two main cell types: alveolar epithelial type 1 (AT1) and type 2 (AT2) cells. AT2 cells play a key role in producing pulmonary surfactant, preventing alveolar collapse during breathing, and acting as progenitors that can self-renew and differentiate into AT1 cells to maintain alveolar structure during homeostasis or after injury. However, genetic and environmental risk factors can impair AT2 cells, diminishing their ability to repair lung tissue and triggering fibrotic pathways, which can lead to lung disease. Studying these effects has been challenging due to a lack of suitable in vitro models for human AT2 cells. Recent advances in generating AT2 cells from human induced pluripotent stem cells (hiPSCs) have provided insights into lung development and disease mechanisms. However, these models are limited by variability in AT2 cells produced, largely due to their reliance on animal-derived components like Matrigel®. To address this, we developed a novel xeno-free hiPSC-directed differentiation protocol to generate AT2 cells. This protocol mimics in vivo development and uses fluorescent-activated cell sorting to enrich NKX2.1-expressing lung progenitors using the surface marker carboxypeptidase M. These progenitors were matured in 3D culture using a human recombinant laminin-111 and

SPEAKER ABSTRACTS

peptide hydrogel mixture, forming spheroids containing homogeneous AT2 cells expressing surfactant proteins. The cells were further purified using an antibody against the surface marker NaPi2b, a protein expressed by surfactant protein C-producing cells. Purity was validated through RT-qPCR, bulk RNA sequencing, and single-cell RNA sequencing. To test the model's relevance for disease research, we exposed the AT2 cells to H1N1 influenza A virus. Bulk RNA sequencing revealed stress-related responses, including inflammation, immune activation, and oxidative stress, as well as changes in genes involved in AT2 cell function. These results confirm the protocol's reliability for studying lung diseases in vitro. This model can be genetically modified to explore genotype-phenotype relationships in conditions like idiopathic pulmonary fibrosis, aiding in the development of therapies to limit fibrosis progression.

11:35 AM – 12:00 PM

RECONSTRUCT TISSUE MICROENVIRONMENT FOR STUDYING KIDNEY DISEASES

Yun Xia

Nanyang Technological University, Singapore

Robust stem cell-based organoid models open a new avenue for understanding human organ formation and disease pathogenesis. Human pluripotent stem cell-derived kidney organoids recapitulate multiple spatiotemporal processes of morphogenesis observed in the developing human kidney, but manifest only rudimentary function. One significant limitation associated with the current generation of organoids is that generic cell types, such as vasculature and immune cells, are severely underrepresented. Our laboratory focuses on developing methodologies to generate kidney organoids that contain a comprehensive repertoire of diverse cell types including both tissue-specific cell types and generic cell types. This effort aims to enhance our understanding and investigation of the collective behaviour of this multicellular system during development and disease pathogenesis. We aim to recreate a tissue microenvironment and intercellular crosstalk that closely resemble native conditions, which are essential for tissue structural complexity, functional maturation, and pathogenesis. To accomplish this, we are employing multidisciplinary experimental frameworks, including multicellular self-organization, genetic perturbation, xenotransplantation, and single cell analysis. We wish to dissect disease-specific phenotypes and mechanisms with an emphasis on identifying targetable pathways for therapeutic intervention.

Funding Source: Singapore Ministry of Health National Medical Research Council (MOH-001214 and OFLCG22may-0011), Singapore Ministry of Education (MOE-T2EP30220-0008, MOE-T2EP30223-0031).

1:00 PM – 2:00 PM

INNOVATION SHOWCASE
THE NEXT GENERATION OF INTESTICULT GROWTH MEDIA: ADVANCING PHYSIOLOGICALLY-RELEVANT INTESTINAL ORGANOIDS

Presented by STEMCELL Technologies

Martin Stahl

STEMCELL Technologies, Canada

Intestinal organoids are a valuable tool for basic and applied research, and drug discovery. Although intestinal organoids represent an advancement on previous culture methods, conventional organoid media favor the proliferative stem cell niche, often at the expense of more functional differentiated cell types. However, inducing differentiation in these cultures results in the loss of the progenitor population. To address this issue, we developed a next-generation IntestiCult™ medium that promotes the growth of human intestinal organoids without inhibiting the development of physiologically-relevant phenotypes and morphology. Human intestinal organoids generated by this medium possessed both proliferative and differentiated cell types. We observed increased marker expression and differentiation for enterocyte, goblet, Paneth, and rare enteroendocrine and tuft cells in proportions similar to those found in vivo, while maintaining a robust stem cell population and long-term organoid expansion. These organoids recapitulated each of the duodenal, ileal, and colonic intestinal regions, and exhibited a distinct budded morphology with crypt-like structures. These organoid cultures exhibited physiologically-relevant and donor-dependent responses to functional assays,

SPEAKER ABSTRACTS

including drug toxicity screens, transport assays, and forskolin-induced swelling assays. demonstrating novel applications for physiologically-relevant organoids in both basic and applied research with unique, donor-specific phenotypes.

2:00 PM – 3:20 PM
TISSUE REPROGRAMMING TO A REGENERATIVE STATE

2:00 PM – 2:25 PM

EXPLORING THE CELLULAR AND MOLECULAR MECHANISMS CONTROLLING STRETCH-MEDIATED TISSUE EXPANSION IN VIVO

Mariaceleste Aragona[1], Caroline Aguilera Stewart[2], Ceyhun Alar[2], Leslie Bargsted[2], and Alejandro Sifrim[3]

[1]University of Copenhagen, Denmark, [2]Novo Nordisk Foundation Center for Stem Cell Medicine, reNEW, Denmark, [3]KU Leuven Institute for Single Cell Omics (LISCO), Department of Human Genetics, Belgium

Stretch-mediated tissue expansion is commonly used to grow extra skin for reconstructive surgery. To study the temporal consequences of stretching the skin in vivo, we developed a mouse model that uses miniaturised prosthesis placed subcutaneously on mouse back skin. We have previously demonstrated that stretching induces skin expansion by creating a transient bias in the renewal activity of epidermal stem cells, while a second subpopulation of basal progenitors remains committed to differentiation. However, to ensure harmonious growth, the two main skin compartments, the epidermis, and the dermis, must both adjust their behaviour. It remains unexplored which mechanisms allow stretch-mediated tissue expansion on the dermis and in particular in fibroblasts, the main cell type in the dermis. Here, we have generated a comprehensive atlas of the gene expression and cellular changes occurring in space and time during stretch-mediated tissue expansion in the entire skin. Based on this atlas, we are elucidating how cell types in the skin communicate and the signaling crosstalk involved in tissue expansion.

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

2:25 PM – 2:40 PM

STAB WOUND INJURY ELICITS TRANSIT AMPLIFYING PROGENITOR-LIKE PHENOTYPE IN PARENCHIMAL ASTROCYTES

Jovica Ninkovic[1], Priya Maddhesiya Maddhesiya[1], Christina Koupourtidou[1], Alessandro Zambusi[1], Klara Novoselc[1], Judith Fischer-Sternjak[2], Tatiana Simon[1], Cora Olpe[3], Sebastian Jessberger[3], and Magdalena Götz[1]

[1]Ludwig-Maximillan University of Munich (LMU), Germany, [2]Helmholtz Center Munich, Germany, [3]University of Zurich, Switzerland

Astrocytes exhibit dual roles in central nervous system (CNS) recovery, offering both beneficial and detrimental effects. Following CNS injury, a subset of astrocytes undergoes proliferation, de-differentiation, and acquires self-renewal and neurosphere-forming capabilities in vitro. This subset of astrocytes represents a promising target for initiating brain repair processes and holds potential for neural recovery. However, studying these rare plastic astrocytes is challenging due to the absence of distinct markers. In our study, we characterized these astrocytic subpopulations using comparative single-cell transcriptome analysis. By leveraging the regenerative properties observed in radial glia of zebrafish, we identified and characterized injury-induced plastic astrocytes in mice. These injury-induced astrocytic subpopulations were predominantly proliferative and demonstrated the capacity for self-renewal and neurosphere formation, ultimately differentiating exclusively into astrocytes. Integration with scRNAseq data of the subependymal zone (SEZ) allowed us to trace the origins of these injury-induced plastic astrocytic subpopulations to parenchymal astrocytes. Our analysis revealed that a subset of these injury-induced astrocytes shares transcriptional similarities with endogenous transient amplifying progenitors (TAPs) within the SEZ, rather than with neural stem cells (NSCs). Notably, these injury-induced TAP-like cells exhibit distinct differentiation trajectories, favoring gliogenic over neurogenic differentiation. In summary, our study identifies a rare subset of injury-induced, proliferative plastic astrocytes with neurosphere-forming capacities. These cells originate from reactive astrocytes and resemble TAPs in their transcriptional profile. This study enhances our understanding of astrocyte plasticity post-injury that can be utilized for the repair purposes.

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2:40 PM – 2:55 PM

INTEGRATION OF SPATIAL AND DEVELOPMENTAL PLASTICITY FORMS A DYNAMIC REPAIR RESPONSE IN MOUSE AND HUMAN INTESTINAL EPITHELIUM

Sakura Kirino[1], Fumiya Uefune[2], Kensuke Miyake[2], Ryuichi Okamoto[3], and Shiro Yui[1]

[1]Institute of Science Tokyo, Japan, [2]Center for Stem Cell and Regenerative Medicine, Institute of Science Tokyo, Japan, [3]Department of Gastroenterology and Hepatology, Institute of Science Tokyo, Japan

Intestinal epithelium represents the plasticity to escape from the loss of intestinal stem cells (ISCs) upon injuries. Conventionally, it has been reported that the ISC pool reconstructed by the spatial plasticity in which differentiated cells are reprogrammed back to LGR5 +ve crypt basal columnar cells (CBCs). In recent years, revival stem cells (RSCs) exhibiting a fetal signature have been identified as a new stem cell pool during injury, indicating the plasticity along the developmental time-axis. Considering that the fetal phenotype has a pathological significance in colorectal cancers, a systematic view how these two types of plasticity integrate with each other is essential not only to define the inflammatory-dependent fate reversion but also to refine cancer pathophysiology. To grip this issue in one unified system, we need to clarify the relationship between ISC pool of CBCs/RSCs and differentiated fractions by focusing on whether CBCs and RSCs can convert with each other, or whether the developmental plasticity intermediates in the process of spatial plasticity. Using lineage-tracing models and mouse and human organoids systems of fetal reversion, we confirmed the transition between CBCs and RSCs, including a high reversion potential in CBCs. Sorting assay at the same time also indicated the reversion event in non-CBCs and absorptive enterocytes were found in the lineage flow to RSCs in trajectory analysis. Thus, we validated the reversion potential of the lineage, confirming that the spatial and developmental plasticity integrate in the process. The results of this study present a simple model that RSCs basically originate from CBCs. RSCs are also supplied from a differentiated absorptive lineage to form a coordinated integration of spatial and developmental plasticity. Our approach provides a basis how to reveal the process of the plasticity in other types of secretary cell lineages to illustrate the full shape of the plasticity of intestine.

Funding Source: Japan Science and Technology Agency(JST) Forrest Program (JPMJFR2012 to S. Yui) JST SPRING (JPMJSP2120 to S. Kirino).

2:55 PM – 3:20 PM

ENHANCED LIVER REGENERATION VIA TARGETED MRNA DELIVERY FOR PARTIAL IN VIVO REPROGRAMMING

Hyuk-Jin Cha[1], Beom-Ki Jo[2], Young Seok Song[2], Woohyun Song[3], Hee-Ji Eom[2], Yon Jae Lee[4], Jumee Kim[2], Seunghee Hong[3], and Seung-Woo Cho[5]

[1]Seoul National University, South Korea, [2]College of Pharmacy, Seoul National University, South Korea, [3]Department of Biochemistry, College of Life Science and Biotechnology, Yonsei University, South Korea, [4]Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University, South Korea, [5]Yonsei University and Cellartgen, South Korea

Recent studies suggest that injury-induced dedifferentiation, which leads to the formation of 'injury-responsive cells', contributes significantly to tissue repair across various organs, including the liver. Utilizing Yamanaka factors (Oct4, Sox2, Klf4, and c-Myc: OSKM) for in vivo partial reprogramming generates 'injury-responsive cells' in the intestine, mirroring those derived from injury-induced dedifferentiation. Thus, the transgene induction of OSKM or viral delivery of Oct4, Sox2, and Klf4 shows promise in facilitating tissue regeneration in the intestine, liver, skeletal muscle, and retina. Herein, we demonstrated that transient OSKM induction produces two distinct liver progenitor-like cell populations. One of these populations resembles liver progenitor-like cells (LPLCs) generated by acute acetaminophen (APAP) injury without triggering immune responses. To explore in vivo reprogramming as a viable strategy for tissue regeneration, we employed lipid nanoparticles (LNP) carrying OSKM mRNA (OSKM mRNA-LNP) to stimulate LPLCs formation. Notably, the production of Sox9+ LPLCs, and OSKM-induced dedifferentiation, was closely correlated with successful tissue regeneration in the liver post APAP injury. Thus, the OSKM mRNA-LNP approach represents a promising therapeutic intervention for the repair of acute liver injuries.

SPEAKER ABSTRACTS

3:50 PM – 5:05 PM
TISSUE AGING: SESSION I

3:50 PM – 4:15 PM

HEMATOPOIETIC STEM CELLS THROUGH THE AGES: A LIFETIME OF ADAPTATION TO ORGANISMAL DEMANDS

Emmanuelle Passegué

Columbia University Medical Center, USA

Hematopoietic stem cells (HSC) are responsible for lifelong blood production, but their function deteriorates with age. Understanding the mechanisms underlying HSC aging is vital for developing interventions preventing or delaying hematopoietic deterioration. I will start by reviewing our previous work articulating a conceptual model whereas aging degrades the interactions between HSCs and their bone marrow niche environment thereby triggering a set of adaptive responses with both beneficial and damaging consequences. Understanding this complex array of co-regulations is key for developing effective anti-aging rejuvenation approaches. I will then detail recent work identifying nucleolar stress response engagement as a cytoprotective response providing HSC resilience during aging. We previously found that aging HSCs accumulate nucleolar DNA damage histone marks (γH2AX) as a consequence of replication stress. The nucleolus is the site of ribosome biogenesis that also integrates responses to diverse cellular stresses, and HSCs are known to rely on precise regulation of protein translation to maintain their regenerative potential. Here, we connect nucleolar γH2AX in old HSCs with induction of a cytoprotective nucleolar stress response. We show that triggering nucleolar stress in HSCs leads to impaired protein translation and delayed cell cycle progression, with the resulting p53-mediated nucleolar stress response being essential for preserving HSC functionality and the residual regenerative potential of old HSCs. Moreover, we establish the connection between nucleolar stress response engagement in old HSCs and maintenance of functional quiescence under stress in an activation-resistant subset of old HSCs. These findings unveil new mechanisms underlying HSC aging, demonstrating the crucial protective role of nucleolar stress response, and raising the exciting possibility that nucleolar stress signaling could be harnessed to improve the output of old HSCs in humans.

4:15 PM – 4:30 PM

IMMUNE MODULATORY STRATEGIES TO RESTORE REGENERATIVE CAPACITY IN AGED ORGANS

Joana Neves, Neuza Sousa, Marta Bica, Tiago Costa, Nuno Barbosa-Morais, and Pedro Sousa-Victor

Instituto de Medicina Molecular (iMM), Portugal

Aging is accompanied by a decline in regenerative capacity in multiple tissues, however the contribution of immune aging to regenerative failure is just starting to be explored. Here, using the mouse skeletal muscle as a model of tissue regeneration, we applied a combination of single cell RNA sequencing, flow cytometry and functional analysis to understand how immune aging impacts stem cell function and regenerative success. Our results revealed a complex landscape of immune cells and states associated with muscle regeneration, and an unrecognized heterogeneity within the myeloid-response, challenging established notions of immune modulation in the context of tissue repair. Importantly, our data pinpoints key age-related alterations in immune cell types and signaling associated with regenerative decline, which can be leveraged to improve regenerative capacity in aging. We uncover a new mechanism of immune modulation operating during skeletal muscle regeneration that is disrupted in aged animals and relies on the regulation of macrophage function. The immune modulator MANF is induced following muscle injury in young mice, but not in aged animals, and its expression is essential for regenerative success. Regenerative impairments in the aged muscle are associated with defects in the repair-associated myeloid response similar to those found in MANF-deficient models and could be improved through MANF delivery. We propose that immune modulatory interventions targeting defects in the aged immune response, such as restoring MANF levels, are viable strategies to improve regenerative capacity in the aged skeletal muscle.

Funding Source: ERC Consolidator Grant (101126073) EMBO Installation Grant (IG4448).

SPEAKER ABSTRACTS

4:30 PM – 4:55 PM

STEM CELL SENESCENCE AND ITS BYPASS: A CLUE FOR TISSUE AGEING AND CANCER

Emi Nishimura

The University of Tokyo, Japan

The accumulation of an individual's life-long environmental exposure, known as the "exposome," significantly impacts health. Somatic tissues functionally decline with age, exhibiting typical aging phenotypes, including hair graying and cancer. However, which genotoxin induces which phenotype and the cellular processes responsible are still largely unknown. We previously identified melanocyte stem cells (McSCs) in mammalian hair follicles as somatic stem cells that generate pigment-producing melanocytes. Subsequently, we reported that those cells are depleted with aging and multiple types of genotoxic stress, resulting in hair-graying phenotypes. However, the exact fate of DNA-damaged stem cells has been 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. On the other hand, senolytic drugs have failed to rescue hair graying. Our data demonstrate that the individual clone fate of stem cell expansion versus exhaustion cumulatively determines a tissue phenotypic trade-off between aging and cancer, based on the exposome type, through the stem cell niche.

Funding Source: AMED, JSPS.

FRIDAY, 13 DECEMBER 2024

9:00 AM – 10:25 AM
TISSUE AGING: SESSION II

9:00 AM – 9:25 AM

PROGRAMMING AND REPROGRAMMING OF AGING

Guanghui Liu

Institute of Zoology, Chinese Academy of Sciences (CAS), China

The research team, dedicated to unraveling the mechanisms, early detection, and intervention of aging, has established a comprehensive study system that encompasses both primate organ aging and human stem cell senescence. They have created a multi-tiered "aging atlas" spanning organ, cellular, and molecular levels, and developed methods to assess biological age within the Chinese population. Their work suggests that heterochromatin erosion is a critical factor in driving aging and has demonstrated that the reactivation of endogenous retroviruses contributes to the acceleration of aging. The team has also pioneered a variety of innovative strategies for aging intervention. These efforts have enhanced our understanding of aging mechanisms and paved the way for the development of early warning systems, strategic interventions, and clinical applications for age-related diseases.

9:25 AM – 9:40 AM

NOT A RANDOM PROCESS—PREDICTABLE REMODELLING OF TRANSCRIPTION FACTOR NETWORKS IN AGING ACROSS CELL TYPES VIA HIJACKING OF A DEVELOPMENTAL MECHANISM

Christian Nefzger

University of Queensland, Australia

A mechanistic link between aging and development is largely unexplored. Transcription factor (TF) networks control cell identity and function. While epigenetic changes have been implicated as a key driver of aging, how they manifest at the TF network level is largely unclear. By profiling age-related chromatin and transcriptional changes across 22 mouse cell types, and analyzing them alongside previous mouse and

SPEAKER ABSTRACTS

human organismal maturation datasets (29 cell types), we uncovered a transcription factor binding site (TFBS) pattern common to both processes and shared across cell types. Early-life candidate cis-regulatory elements (cCREs), which progressively lose accessibility during maturation and aging, are enriched for cell-type identity TFBSs. Conversely, cCREs gaining accessibility throughout life have a lower abundance of cell identity TFBSs but elevated activator protein 1 (AP-1) levels. We associate TF redistribution toward these AP-1 TFBS-rich cCREs, in synergy with mild downregulation of cell identity TFs, as driving early-life cCRE accessibility loss and altering developmental and metabolic gene expression throughout life. Such remodeling can be triggered by elevating AP-1 (either directly or via different stimulus/stress processes) and through declining epigenetic repression (e.g., via depletion of repressive H3K27me3). Our data indicates that during organismal maturation, AP-1-linked chromatin opening (with hormonal co-factors) disrupts cell identity TFBS-rich cCREs to reprogram the transcriptome and cell function (e.g., to slow growth and early-life processes as part of development). Ongoing AP-1-linked chromatin opening in aging (e.g., via stress/inflammation) hijacks this process by further disrupting cell identity TFBS-rich cCREs, thereby fueling age-related identity loss. This can be summarized as the "Stimulus-Induced Programming Hijacks Ontogeny" (SIPHON) model for age-related chromatin and transcriptome reprogramming—a potential explanatory framework for many of aging's predictable phenotypes. Collectively, our study provides compelling evidence that chromatin and TF-network remodeling in aging reflects the predictable degrading effects of a mechanism initially critical for organismal maturation, rather than undirected loss of epigenetic information.

Disclaimer: The work has been published in part in Cell Metabolism in August 2024 (DOI: 10.1016/j.cmet.2024.06.006). This is compatible with the ISSCR Abstract Submission Agreement as it was published less than 6 months ago at the time of abstract submission.

9:40 AM – 10:05 AM

AGE-RELATED CHANGES TO STEM CELLS AND THE STEM CELL NICHE

Leanne Jones[1], Nicholas J. Jackson[1,2], Jordan Kryza[1,2], Lauren Shechtman[1], Anthony Vo[1], Orianna Narson[1], Beth Dunham[1], Turan Aghayev[1], Gregor Bieri[1], Jeroen Roose[1,3], and Saul Villeda[1,3,4]

[1]University of California, San Francisco, USA, [2]University of California, Los Angeles, USA, [3]Bakar Aging Research Institute, USA, [4]Eli and Edythe Broad Center for Regenerative Medicine, University of California, San Francisco, USA

Adult stem cells support tissue homeostasis and repair throughout the life of an individual. Numerous changes occur with age that result in altered stem cell behavior and reduced tissue maintenance and regeneration. Changes can be cell autonomous including changes in cell cycle progression, decreased bioenergetic efficiency, increased DNA damage, and epigenetic alterations. In addition, changes to the local and systemic environments occur that result in decreased stem cell activity or alterations in commitment or differentiation potential. Our work has defined novel components of the local 'niche' and characterized age-related changes that contribute to loss of tissue homeostasis over time in different tissues and organisms. Given the importance of the niche in regulating stem cell behavior, we wanted to explore the impact of an environmental intervention, such as voluntary exercise, on stem cell-niche interactions in the mammalian small intestine and colon. Our findings suggest that exercise enhances regeneration in the mammalian small intestine and reverses numerous hallmarks of aging, such as the decline in proliferation within stem cell containing crypts and a differentiation bias toward the secretory lineage. Morphological changes are accompanied by a shift in gene expression such that intestinal stem cells and supportive niche cells (Paneth cells) in aged, exercised mice resemble those from young, sedentary cohorts. The impact of exercise was more pronounced in male mice when compared to female cohorts. Our experiments will expand on the usefulness of exercise as a non-invasive intervention

SPEAKER ABSTRACTS

to rejuvenate both endogenous stem cell pools and the local niche, as well as provide a new paradigm to identify mechanisms regulating intestinal homeostasis and regeneration.

10:55 AM – 12:50 PM
IMAGING TOOLS AND THEIR APPLICATION TO UNDERSTANDING HOMEOSTASIS, AGING AND DISEASE

10:55 AM – 11:20 AM

UNDERSTANDING REGENERATION THROUGH SPATIOTEMPORAL OMICS

Ying Gu

BGI-Shenzhen, China

The current progresses of single-cell sequencing and spatiotemporal transcriptome enable scientists not only can discover the specific cell types in tissue, but also can detect the cell distribution in the spatial dimension. Taking advantages of a newly developed spatial-temporal transcriptomics approach—SpaTial Enhanced REsolution Omics-sequencing (Stereo-seq) by BGI-research, with the highest profiling resolution to data, we studied the cellular and molecular dynamics during organ regeneration process in Axolotl, an animal that can regenerate damaged appendages and multiple internal organs, including the brain. Employing Stereo-seq, we generated a group of spatial transcriptomic data of telencephalon sections that covered six developmental and seven injury-induced regenerative stages. We discovered a sub-population of progenitor cells may then proliferate to cover the wound area and subsequently replenish lost neurons. Interestingly, we also observed that regeneration of the axolotl telencephalon exhibited similar neurogenesis patterns to development, suggesting that brain regeneration partially recapitulates the development process. Our spatial transcriptomic data highlights the cellular and molecular features of the axolotl telecephalon during development and injury-induced regeneration.

11:20 AM – 11:45 AM

FILMING THE FATE OF CELLS CARRYING MUTATIONS IN CANCER DRIVER GENES

Jacco van Rheenen

Netherlands Cancer Institute, Netherlands

Cells with mutations in driver genes are abundantly present in tissues of healthy individuals, yet they rarely develop into tumors. The underlying protection mechanisms that prevent tumor formation are largely unknown. Over the years, we have developed high-resolution intravital microscopy techniques to visualize and study the behavior and fate of individual cells in breast and intestinal tissues in living mice. In my talk, I will show how we have used these intravital technologies to study the mechanisms in breast tissues that both prevent and mediate the formation of tumors by cells with oncogenic mutations. Our findings highlight that the menstrual cycle drives the cellular turnover within the mammary ductal network, leading not only to loss but also the dispersal of mutant cells throughout the network. Intriguingly, this dispersal increases the risk of tumorigenesis. Furthermore, we showed that the menstrual cycle also drives periods of proliferation and cell death in tumor tissue. Importantly, the proliferative phase was accompanied by enhanced chemosensitivity. Collectively, our study reveals the menstrual cycle as an important infradian rhythm that plays a crucial role in tumor initiation and modulating chemosensitivity.

11:45 AM – 12:00 PM

SPATIAL MULTIOMIC LANDSCAPE OF THE HUMAN PLACENTA AT MOLECULAR RESOLUTION

Johain Ounadjela[1], Koseki Kobayashi-Kirschvink[2], Ke Zhang[2], Kang Jin[2], Andrew Russell[2], Andreas Lackner[3], Claire Callahan[1], Francesca Viggiani[1], Kushal Dey[4], Karthik Jagadeesh[2], Theresa Maxian[3], Anna-Maria Prandstetter[3], Naeem Nadaf[2], and Qiyu Gong[2], Ruth Raichur[2], Morgan Zvezdov[5], Mingyang Hui[1], Xinwen Liu[5], Wei Min[5], Martin Knofler[3], Fei Chen[2], Jian Shu[1], and Sandra Haider[3]

[1]Massachusetts General Hospital, USA, [2]Broad Institute of MIT and Harvard, USA, [3]Medical University of Vienna, Austria, [4]Weill Cornell Medicine, USA, [5]Columbia University, USA

SPEAKER ABSTRACTS

Successful pregnancy and healthy human embryo development rely directly on the placenta's complex, dynamic gene regulatory networks, both within placental subtypes and at the maternal-fetal interface (MFI), that underlie stemness, proliferation, differentiation, invasion, immune tolerance, and communication. These cellular and molecular mechanisms are notoriously challenging to elucidate and make this organ arguably the least understood in the human body. Additionally, disruption of this vast collection of intercellular and intracellular programs and pathways leads to pregnancy complications and developmental defects. In the present study, we generated a comprehensive, spatially resolved, multimodal cell census elucidating the molecular architecture of the first trimester human placenta. We utilized paired single-nucleus (sn) ATAC (assay for transposase accessible chromatin) sequencing and RNA sequencing (RNA-seq), spatial single-nucleus ATAC and RNA sequencing (Slide-tags), and in situ sequencing and hybridization mapping of transcriptomes at molecular resolution (STARmap-ISS and STARmap-ISH) to spatially reconstruct the joint epigenomic and transcriptomic regulatory landscape. Paired analyses unraveled intricate tumor-like gene expression and transcription factor motif programs potentially sustaining the placenta in a hostile uterine environment; further investigation of gene-linked cis-regulatory elements revealed heightened regulatory complexity that may govern trophoblast differentiation and placental disease risk. Complementary spatial mapping techniques decoded these programs within the placental villous core and extravillous trophoblast cell column architecture while simultaneously revealing niche-establishing transcriptional elements and cell-cell communication. Finally, we computationally imputed genome-wide multiomic single-cell profiles and spatially characterized the placental chromatin accessibility landscape. This spatially resolved single-cell multiomic framework of the first trimester human placenta serves as a blueprint for future studies investigating cellular and molecular programs that regulate early placental development and pregnancy.

Funding Source: This work was supported by funds from Massachusetts Life Science Center, Broad Institute of MIT and Harvard, Massachusetts General Hospital, and Austrian Science Funds nos. P34588 and P36159.

12:00 PM – 12:15 PM

MADE TO BE BROKEN: THE ARCHITECTURE AND REGENERATIVE FUNCTIONS OF SKIN FRACTURE PLANE IN AFRICAN SPINY MICE

Hanseul Yang

Department of Biological Sciences, KAIST, Korea

In most mammals, wound-induced tissue regeneration is incomplete and results in fibrotic scarring. The mechanisms by which adult stem cells lose their regenerative potential and the trade-off of incomplete regeneration remain unknown. Interestingly, the African spiny mouse (Acomys spp.) is unique among mammals in its ability to autotomize its skin to escape predators and completely regenerate missing skin parts, similar to salamanders. For skin autotomy, the skin of Acomys spp. is mechanically soft and fragile, which raises several questions: How does the skin of Acomys spp. endure normal life? Does skin fracture occur randomly or in a controlled manner? How does skin fracture influence subsequent regenerative processes? Recently, we discovered a unique diamond-shaped pattern in the skin of Acomys spp, characterized by collagen fibers at the boundary, hair follicles at the center, and adipocytes in the middle. When subjected to force, fractures propagate through the collagen boundary, suggesting that the structure guides tissue destruction in Acomys spp. skin, akin to the fracture plane of Lizard tails. Immunofluorescence analyses revealed that the fracture plane is enriched in fibroblasts and macrophages which are crucial for collagen production and immune regulation, respectively. It is notably devoid of blood vessels, which may help minimize bleeding during fracture formation, implying that the fracture plane is pre-adapted for skin tearing in Acomys spp. To investigate when and how the fracture plane develops, we performed histological analyses of postnatal skin and utilized in utero lentiviral epidermal-specific DKK1 overexpression. Our findings indicate that hair follicle morphogenesis (particularly spiny hairs) and fracture plane development are functionally linked. In addition, ultrastructural analyses revealed that the distinct node and edge structures of the fracture plane facilitate well-controlled skin tearing processes. Finally, to determine the impact of the fracture plane on tissue regeneration, we compared ripped (through the fracture plane) and

SPEAKER ABSTRACTS

cut (independent of the fracture plane) skin injuries. Ripped wounds undergo more efficient regenerative healing, both in terms of the quantity and quality of hair follicle neogenesis. In sum, for the first time in mammals, we have identified the fracture plane of Acomys spp. skin, which is specially designed for both programmed tissue destruction and following tissue regeneration. This study will provide insights into the evolutionarily basis of superior regenerative potential of Acomys spp.

Funding Source: This study is supported by SUHF and National Research Foundation of Korea grants (2019R1A6A1A10073887).

12:15 PM – 12:40 PM

TITLE NOT AVAILABLE AT TIME OF PUBLISHING

ABSTRACT NOT AVAILABLE AT TIME OF PUBLISHING

2:00 PM – 4:40 PM
REPAIRING TISSUES USING STEM CELLS AS MEDICINE

2:00 PM – 2:25 PM

APPLICATIONS AND FUTURE OF HEMATOPOIETIC STEM CELL EXPANSION TECHNOLOGY

Satoshi Yamazaki

University of Tokyo, Japan

Hematopoietic stem cells (HSCs) are thought to reside in the bone marrow microenvironment and can provide all the blood and immune cells in our bodies. Historically, the existence of HSCs was experimentally proven in the 1970s, and further development of flow cytometry has led to a leap forward in clonal analysis. On the other hand, the technology to expand HSCs in vitro has been difficult to achieve for many years. However, we have reported the successful ex vivo amplification of mouse HSCs in 2019 and human HSCs last year recently. Furthermore, we have reported advanced gene therapy concepts by integrating genome editing technologies with single cell expansion systems of HSCs. In this symposium, we would like to present an update on HSC expansion technology and introduce the mysteries of the expansion conditions. In addition, we would like to share the possibility of the maturation inducing function of HSCs.

2:35 PM – 2:55 PM

INNOVATION SHOWCASE
SCALABLE PRODUCTION OF PLURIPOTENT STEM CELL-DERIVED NATURAL KILLER (INK) CELLS FOR CANCER IMMUNOTHERAPY

Presented by Thermo Fisher Scientific

Roland Leathers

Thermo Fisher Scientific, Switzerland

Pluripotent stem cells (PSCs) offer a promising avenue for cell-based therapies, particularly in cancer treatment. However, their clinical application necessitates overcoming challenges in commercial manufacturing, including scalable workflows and suitable reagents. We have developed CTS™ StemScale™ PSC Suspension Medium, designed for large-scale PSC cultivation in clinical settings.

Natural Killer (NK) cells are pivotal in allogeneic immunotherapy, with clinical trials indicating a need for 10^6 to 10^8 NK cells per dose. Traditional NK cell production methods are inefficient and logistically complex.

Our study introduces a feeder-free protocol using CTS StemScale for generating PSC-derived NK (iNK) cells. This involves differentiating PSC spheroids into CD34+CD90+ hematopoietic progenitors, which then mature into CD56+ iNK cells, suitable for cryopreservation and later expansion in CTS™ NK-Xpander™ Medium, enhancing the production of mature, functional NK cells with CD56+CD3- and CD56+CD16+ phenotypes.

These iNK cells demonstrate significant cytotoxicity against K562 cancer cells and patient-derived 3D colon tumoroids, confirming their therapeutic potential. Our method not only simplifies but also scales up iNK cell production, facilitating broader clinical use. Thus, CTS StemScale streamlines cytolytic iNK cell production, proving the viability of feeder-free PSC cultures for large-scale, clinically applicable cell therapies.

2:35 PM – 3:05 PM

SCSS—DR. SUSAN LIM AWARD FOR OUTSTANDING YOUNG INVESTIGATOR 2024

SPEAKER ABSTRACTS

3:05 PM – 3:20 PM

LONG-TERM BIOCOMPATIBLE IN VIVO TRACKING OF CARDIOVASCULAR PROGENITORS USING AGGREGATION-INDUCED EMISSION FLUORESCENT NANOPARTICLES

Lynn Yap

Nanyang Technological University, Singapore

Stem cell-based regenerative medicine has shown significant promise for treating cardiac diseases in preclinical trials. Our lab is at the forefront of this field, having pioneered the transplantation of cardiovascular progenitors (CVPs) derived from pluripotent stem cells into both small and large animal models of myocardial infarction (MI). A crucial component of this approach is the ability to track the transplanted cells within the damaged heart. Typically, in vivo bioluminescent imaging is employed to non-invasively monitor live animals by detecting light emitted from luciferase-expressing reporter cells. However, this technique has notable limitations, including shallow penetration depth and the need for genetic modifications to incorporate the luciferase reporter. In this study, we hypothesized that we could safely label and monitor the viability and location of CVPs using fluorescence nanoparticles in a mouse model. We report employing aggregation-induced emission near-infrared-I (NIR-I) fluorescent lipid polymer nanoparticles to track live CVPs, assessing their viability and biocompatibility within the mouse model. Additionally, we aim to elucidate the mechanism of nanoparticle uptake by conducting uptake inhibition studies, flow cytometry, cell toxicity assessments, and microscopy imaging. These fluorescent nanoparticles exhibit high efficiency in cell internalization, bright fluorescence signals, and excellent biocompatibility with CVPs, making them ideal for labeling CVPs without impairing their function or viability. The use of methyl-β-cyclodextrin to inhibit uptake suggests that lipid raft-mediated endocytosis may be a key mechanism for CVP-nanoparticle internalization. For in vivo analysis, we compared the cell viability and biocompatibility of NIR-labeled and luciferase-labeled CVPs by injecting these cells into the muscles of immunocompromised mice and tracking them over a period of 7 weeks. Imaging results indicated that both luciferase and NIR-I fluorescent nanoparticles performed comparably in tracking cells in vivo. This study highlights the potential of fluorescence nanoparticles as non-invasive probes for long-term tracking in both in vitro and in vivo, offering promising opportunities for regenerative medicine.

Funding Source: MOE SUG, MOE Tier 1 grant RG38/24 and NRF-CRP24-2024.

3:20 PM – 3:35 PM

HOW LESSONS FROM DEVELOPMENTAL BIOLOGY ENABLED THE GENERATION OF HUMAN ARTERY AND VEIN ECS FROM PLURIPOTENT STEM CELLS

Lay Teng Ang, Kyle Loh, and Kristy Red-Horse

Stanford University, USA

Generating pure populations of artery and vein ECs from human pluripotent stem cells (hPSCs) in vitro has remained a longstanding challenge. To surmount this challenge, we drew on knowledge of vascular development established through pioneering in vivo studies of mouse, chicken, zebrafish, frog, and other model systems. We developed a method to sequentially differentiate hPSCs into primitive streak, lateral mesoderm, and subsequently either artery or vein ECs. This differentiation was remarkably fast and efficient, yielding >90% pure artery or vein ECs within 3–4 days. While this in vitro differentiation reconfirmed the roles of classical signals (e.g., NOTCH) in arteriovenous development, it also yielded new signals (e.g., TGFβ) and emphasized that they must be turned on and off in a temporally-dynamic way every 24 hours. In unpublished work, we have also differentiated hPSCs into plexus-like ECs capable of bidirectional differentiation into both artery and vein ECs. In sum, we mapped how hPSCs develop into artery vs. vein ECs in vitro and the signals driving the initial bifurcation of arterial and venous identities in humans. This knowledge enables the generation of an ample supply of molecularly distinct human arterial and venous ECs, which could be used to model human vascular diseases in vitro and vascularize organoids or engineered tissues for regenerative medicine.

Funding Source: Additional Ventures.

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13 December 2024

3:35 PM – 4:00 PM

ORGANOID-BASED THERAPY FOR INFLAMMATORY BOWEL DISEASE

Ryuichi Okamoto

Institute of Science Tokyo, Japan

Inflammatory bowel disease (IBD), encompassing ulcerative colitis (UC) and Crohn's disease (CD), is a chronic inflammatory disorder of the gastrointestinal tract. Its pathophysiology is complex, involving immune dysregulation, microbiota changes, and environmental factors. In Japan, the prevalence of UC and CD is rising, affecting approximately 300,000 individuals. Recent advances in IBD treatment, particularly the treat-to-target (T2T) strategy and biologic therapies like anti-TNF-α antibodies, have significantly improved outcomes. The primary treatment goal has shifted toward achieving "mucosal healing"—the full restoration of intestinal structure and function—which is now seen as essential for long-term remission and improved lifetime prognosis. Many IBD patients can achieve mucosal healing through optimized therapy, leading to sustained remission. However, a subset of refractory patients fails to attain mucosal healing, resulting in a unfavorable prognosis. Thus, novel therapies promoting mucosal regeneration are urgently needed for these patients to achieve long-term remission. Recent breakthroughs in intestinal stem cell (ISC) biology allow the expansion of murine ISCs by constituting organoids in vitro. Furthermore, patient-derived organoids can now be cultured from endoscopic biopsy specimens. Preclinical studies suggest that these expanded organoids serve as a promising cell source for regenerative therapy, potentially treating refractory ulcers in IBD patients. This talk will present the concept of organoid-based regenerative therapy, currently in clinical trials for UC patients, and explore the role of regenerative medicine in IBD treatment.

Clinical Trial ID: Approved by the Certified Special Committee for Regenerative Medicine, TMDU, under the approval No. RM2018-002. Also registered at the jRCT website (jrct.niph.go.jp/ge/reports/detail/2273) under the reference No. NA8140003.

4:00 PM – 4:35 PM

CLOSING KEYNOTE: STEM CELL DYNAMICS IN INTESTINAL REGENERATION AND DISEASE

Helen Abud[1], Diana Micati[2], Wing Hei Chan[2], Sara Hlavca[2], Shanika Amarasinghe[2], Max Tailler[2], Stuart Archer[2], Ha Do[2], Andrew Pattison[1], Christine Georges[3], Rebekah Engel[3], Paul McMurrick[3], Edward Giles[1], and Thierry Jarde[1]

[1]Monash University, Australia, [2]Department of Anatomy and Developmental Biology, Monash University, Australia, [3]Cabrini Hospital, Australia

The epithelial lining of the intestinal tract is highly vulnerable to infection, inflammation and tumorigenesis. Stem cells that reside within crypts and drive daily epithelial renewal have a remarkable capacity to promote epithelial repair following injury. Regeneration of the intestinal epithelium following damage is dependent on niche signals from surrounding cells that drive epithelial cell plasticity and fetal reprogramming that ultimately leads to replenishment of the epithelium. These same signals are commonly perturbed in disease states and during tumorigenesis where stem cells can promote tumour growth and augment epithelial plasticity in response to chemotherapeutic treatments. Our work utilises conditional genetic mouse models, injury assays and patient-derived intestinal organoids to decipher stem cell dynamics within the epithelium. We also incorporate single cell and spatial technologies to interrogate the molecular mechanisms that control these processes. Our studies have revealed that members of the EGF family of growth factors play a key role in intestinal regeneration and reveal that NRG1 is a potent mediator of changes in epithelial cell fate. We have also defined that reversion to a regenerative fetal cell fate is a key feature of colorectal tumours that are resistant to chemotherapy. This work reveals critical regenerative signals and cell states that could lead to innovative treatment strategies for inflammatory bowel disease and colorectal cancer.

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POSTER ABSTRACTS

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WEDNESDAY, 11 DECEMBER 2024

5:00 PM – 5:45 PM
POSTER SESSION I

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STIMULATION OF ADULT MELANOCYTE STEM CELLS IN HAIR FOLLICLE WITH KITL LEADS TO SKIN PIGMENTATION AND INHIBITS HAIR GRAYING

Hitomi Aoki

Gifu University, Japan

Kitl, a ligand of the receptor tyrosine kinase Kit, plays a key role in melanocytes development. Mutant animals with reduced Kit signaling develop white spot or white hair, and conditional deletion of Kit in the melanocyte lineage leads to white hair, indicating that Kit functions cell-autonomously in melanocytes. Constitutive expression of Kitl in the epidermis maintains melanocytes in the normally melanocyte-free interfollicular epidermis for life and prevents hair graying, suggesting that Kitl-Kit signaling is involved in the maintenance of adult melanocyte stem cells (aMSCs). Kit neutralizing antibody Ack2 eliminates melanoblasts, whereas aMSCs maintained in the follicular bulge niche are resistant to Ack2 and are retained, suggesting that they are maintained in a Kit-independent manner. Thus, the precise function of Kitl signaling in aMSC is poorly understood. To investigate in detail the effects of Kitl expression in postnatal and adult mice, we generated genetically engineered mice expressing a Kitl transgene under the control of a doxycycline-inducible promoter. Whole-body expression of Kitl in postnatal and adult mice activated MSCs and led to pigmented epidermis. Induction of Kitl expression in the epidermis after birth or in adults also resulted in epidermal pigmentation. Transient Kitl expression had long-term effects on aMSCs and their progeny, leading to skin pigmentation. Intermittent Kitl expression in adults repeatedly repigmented the epidermis in response to Kitl induction. Radiation-induced hair graying in adults was suppressed by conditional Kitl expression from the time of radiation exposure. These results indicate that Kitl expression affects the self-renewal capacity and subsequent differentiation and proliferation of MSCs, as well as melanocyte migration from hair follicles to the epidermis in postnatal and adult epidermis. Our findings suggest the potential application of Kitl in the treatment of vitiligo and hair graying.

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IMMUNE-EVASIVE HUMAN IPSC-DERIVED ENDOTHELIAL CELLS AS OFF-THE-SHELF THERAPIES FOR VASCULAR REGENERATION

Da-Hyun Kim

Sungshin Women's University, South Korea

For treating the patients with organ failure, one of the best regenerative approach is to use primary cells for autologous transplantation, but the substantial time required for cell expansion has hindered its clinical application. In this regard, cells differentiated from human-induced pluripotent stem cells (hiPSCs) can be promising substitutes because they can be readily prepared. However, allogeneic transplantation of hiPSC-derived cells has the potential to elicit the immune responses in recipients, thereby leading to graft rejection. To address these challenges, we investigated strategies to obtain hypoimmunogenic stem cells by engineering the immune-related genes. First, to evade T cell-mediated responses, major histocompatibility complex (MHC) class I and II genes were inactivated. CD24, one of 'don't eat me signals', was inserted in the cells since depletion of MHC class renders cells susceptible to NK cell attack. Indeed, activation of not only T cells but also NK cells was markedly decreased in the universal hiPSC-derived endothelial cells (U-ECs) compared to wild type hiPSC-derived endothelial cells (WT-ECs), indicating the immune-evading capabilities of hypoimmunogenic stem cells. We confirmed that U-ECs survived for longer periods with better functions than WT-ECs after being transplanted into the humanized mice generated by injecting CD34+ human hematopoietic stem cells into NSG mice. These findings

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suggest that U-ECs produced from hypoimmunogenic iPSCs can be utilized as an off-the-shelf cell therapy for patients with ischemia. Beyond 2D-cultured cell therapy, we next aimed to develop 3D-engineered vascularized liver tissues assembled with hepatoblasts and endothelial cells, both differentiated from universal iPSCs. These off-the-shelf liver tissues also showed reduced immune rejection in vitro while maintaining their functionalities, suggesting that this approach can serve as a potential organ substitute for patients in need of transplants. Taken together, in this study, we lay the foundation for regenerative approaches with off-the-shelf hiPSCs, including cell and tissue therapy.

Funding Source: This work was supported the Korea National Research Foundation (NRF) grant (No. 2021R1C1C2010636).

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HUMAN CORNEAL EPITHELIUM SECRETOMES IN ADSC DIFFERENTIATION TOWARDS CORNEAL EPITHELIUM

Taty Anna Kamarudin, Shen Lip Yam, Abdul Malik Setiawan, Fairus Ahmad, Adila A Hamid, Jen Kit Tan, Abdul Kadir, and Abdul Karim

Universiti Kebangsaan, Malaysia

Adipose derived stem cells (ADSC) showed potentials as an alternative source for tissue engineering toward ectodermal lineage cells such as corneal epithelium. Corneal differentiation of ADSC involves several important pathways, such as BMP4, TGFbeta, and Wnt-signalling. These pathways may be altered by the addition of supplements in the culture media and the morphological changes could be observed histologically. However, the molecular and cellular responses of these cells during differentiation, that may involve various secretomes and exosomes being released into or taken up from the media is still poorly understood. Thus, this study was aimed to elucidate the secretomes from a human corneal epithelium (hTCEpi) and ADSC lines, the proteomics analyses outcomes were then used to explain the ADSC differentiation process especially via co-culture method. The condition media from hTCEpi (CMC) and ADSC (CMA) were collected for secretome identification, quantification and proteomics analyses. ADSC was cultured for 15 days in a differentiation medium supplemented with the CMC to induce corneal differentiation. Corneal differentiation was assessed by gene and protein expression of OCT4, CK3, ABCG2, and Ecadherin. Spectrophotometry and proteomics analyses identified a total of 1113 proteins from both ADSC and hTCEpi secretomes. Most of which are involved in integrin and growth factors signalling pathways. There were increased in CK3, ABCG2 and CDH1, but decreased OCT4 expressions. Together, these findings suggest that hTCEpi secretomes may induce corneal differentiation in ADSC mainly via the integrin and growth factors signalling pathways.

Funding Source: Research funding by grants from the Faculty of Medicine, Universiti Kebangsaan Malaysia: FF-2019-540, GGP-2019-008 and FF-2023-375.

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PERI-METANEPHROS STROMAL IGF SIGNALING ORCHESTRATES KIDNEY ORGANOGENESIS IN HUMAN AND MOUSE

Akinori Mitsui[1], Hikaru Eto[1], Takanori Takebe[1], Sachiko Sekiya[2], and Jinhua Piao[1]

[1]Kyowa Kirin Co., Ltd, Japan, [2]Institute of Research, Tokyo Medical and Dental University, Japan

Kidney organogenesis is initiated upon intermixing with the ureteric bud and metanephric mesenchyme, navigated by the timed and localized specific-stromal supports. However, the developmental contribution of peri-metanephric stroma-derived factors is not systematically studied. Here, spatial and single cell transcriptomics of embryonic kidneys revealed the enrichment of insulin-like growth factor 2 (IGF2) in cortical stromal progenitors and peri-Wolffian mesenchyme during ureteric bud and metanephros induction. Using human pluripotent stem cell-derived organoids, IGF2 orchestrates nephrogenic and ureteric differentiation in the absence of the traditional patterning factor. Inhibition of endogenous IGF signaling in the embryonic kidney explants resulted in global growth arrest and ureteral peristalsis retardation with multifaceted differentiation defects affecting podocyte

POSTER ABSTRACTS

and collecting duct epithelium. Transplanted IGF2-induced human kidney organoids develop functional vasculatures and continued to mature, transitioning into fetal kidney phenotype. These findings underscore a pleiotropic role of stromal IGF2 in kidney development, suggesting its use in balanced differentiation mechanism.

Funding Source: This research was supported by Kyowa Kirin Co., Ltd. sponsored research grants, Agency for Medical Research and Development under Grant Number JP22gm1210012, and JP22bm1123009.

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EFFECT OF ANDROGRAPHOLIDE ON CELLUAR VIABILITY, MITOCHONDRIAL FUNCTION, AND STEMNESS IN MCF7 BREAST CANCER CELLS

Natthima Suwan, Sirinya Jenjittikul, Yovipat Senaweenin, Sasipat Teerawongsuwan, and Ruttachuk Rungsiwiwut

Srinakharinwirot University, Faculty of Medicine, Thailand

Andrographolide, derived from Andrographis paniculata, has shown a promising anti-neoplastic property. However, its effects on pluripotency and cancer stemness are largely unknown. Due to pluripotency and cancer stemness contributing to treatment failure and relapse, exploring these effects in plant-derived substances like andrographolide is essential. In this study, we aim to investigate the effects of andrographolide on cell viability, mitochondrial function, apoptosis markers, pluripotent genes, and stemness in the MCF7 cell line. MCF7 cells were treated with andrographolide (7.5µM–120µM) for 24 or 48 hours. Cell viability was assessed using an MTT assay, and the result showed a decreased cell viability in a dose- and time-dependent manner. Mitochondrial membrane potential was measured by TMRE staining and showed a reduction consistent with mitochondrial dysfunction. RT-qPCR was performed to analyze gene expressions, demonstrating TOMM20 and caspase-3 downregulation, despite MCF7 typically being caspase-3 deficient. BAX upregulation in the 60 µM group suggested an apoptosis induction. Interestingly, OCT4 was upregulated dose-dependently with a 13.52-fold in the 60 µM group. Gene correlation analysis showed a negative TOMM20 and OCT4, suggesting downregulation of TOMM20 is associated with increased pluripotent gene expression. A negative correlation between the OCT4 and BAX relationship and a positive correlation between OCT4 and BAX indicated the complex relationship between apoptosis and stemness response. Immunocytochemistry confirmed the cytoplasmic localization of TOMM20. Reduced mammosphere and colony formation in the treatment groups indicated reduced proliferation and self-renewal. These findings suggest that andrographolide exerts its anti-neoplastic effect by mitochondrial dysfunction and apoptosis. A paradoxical OCT4 gene expression with decreased proliferation and self-renewal may indicate a survival or adaptive response in residual cells. Downregulation of TOMM20 and upregulation of OCT4 have been associated with enhanced cancer stem-like properties and drug resistance, posing a challenge in clinical cancer treatment. Further research is necessary to clarify the long-term effects of andrographolide on cancer stem cell stemness.

Funding Source: This research and presentation expense was funded by the Faculty of Medicine, Srinakharinwirot University, MED-STUDENT-50 (grant number 091/2567).

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CONTROLLED SLOW RELEASE OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN ALGINATE AND HYALURONIC ACID MICROBEAD SYSTEM TO PROMOTE WOUND HEALING IN PUNCH-INDUCED WOUND RAT MODEL

Hwan Jun Choi

Soonchunhyang University, Plastic and Reconsructive Surgery, South Korea

Wounds with compromised vascularity and hypoxia may be healed with the additional growth factor to promote vascularity. Among the different angiogenic growth factors, vascular endothelial growth factor (VEGF) is a crucial and important candidate. To address this issue, a combination of two different polymers, alginate (ALG) and hyaluronic acid (HA) in 80:20 ratio

POSTER ABSTRACTS

composition is used to optimize the bead system along with the 5 IU heparin (Hep) by crosslinking into calcium chloride (CaCl2). Encapsulation of Vascular endothelial growth factor (VEGF) in the bead system shows delayed cumulative release in phosphate buffer saline (PBS). For in vitro studies, ALG–HA/VEGF150 improves endothelial Vascular cell adhesion protein 1 (VCAM1) and endothelial nitric oxide synthase (eNOS) expression markers in CPAE cells. In vivo evaluation of the bead system shows around 68% of wound closure 2 weeks post-implantation in 8 mm punch wound models. The treatment group shows decreased epithelial gap between the ends of the wound and neo-epidermal regeneration. The objective of our study is to fabricate a dual polymer bead system for wound dressing that can be degraded in 5 to 6 days; the beads could be slowly releasing VEGF growth factor and to investigate the effect of VEGF incorporation into the bead system to improve wound healing. From clinical aspects, our bead system might be reducing pain and lowering dressing cost for the patient and convenience for the healthcare provider in future.

Funding Source: This work was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government (MSIT) (2020R1A2C1100891), and supported by Soonchunhyang University research fund.

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PHOTOMODULATION ALLEVIATES CELLULAR SENESCENCE OF AGING MOUSE MESENCHYMAL STEM CELLS

Jianbo Wu

Southwest Medical University, China

Mesenchymal stem cells (MSCs) therapies are emerging as a promising approach to therapeutic regeneration. Therapeutic persistence and reduced functional stem cells following cell delivery remain critical hurdles for clinical investigation due to the senescence of freshly isolated cells and extensive in-vitro passage. The mRNA expression levels of senescence markers were significantly increased in the later passage of adipose-derived stem cells (ASCs). We show that light activation reduced the expression of senescent genes, and SA-β-Gal in all cells at passages. Moreover, the light-activated ASCs-derived exosomes decrease the expression of senescence, and SA-β-Gal in the later passage cells. We further investigated the photoreceptive effect of Opsin3 (Opn3) in light-activated ASCs. Deletion of Opn3 abolished the differences of light activation in reduced expression of senescent genes, increased Ca 2+ influx, and cAMP levels. ASCs can undergo cellular senescence in-vitro passage. Photomodulation might be better preserved over senescence and Opn3-dependent activation in aged mesenchymal stem cells. Light-activated mesenchymal stem cells (-derived exosomes) could be served as a new protective paradigm for cellular senescence in-vitro passage.

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ACCUMULATION OF AUTOPHAGOSOMES IN THE CENTRAL ZONE OF HUMAN LENS EPITHELIAL CELLS: A STUDY ON BIOLOGY OF ADULT STEM CELLS IN AGE-RELATED CATARACT PATHOGENESIS

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Cataract is the leading cause of blindness worldwide. We have earlier demonstrated the loss of adult tissue resident stem cells in the central zone of human cataractous lens epithelium based on the absence of SOX2+ GJA1- stem cells and a significant reduction in the sphere forming ability. In continuation, this study aims to analyse the cellular changes in the central zone of cataractous lens epithelium. Donor lenses from both normal and cataractous eyes were obtained from the eye banks of the Aravind Eye Care System. Cellular alterations were evaluated by (i) confocal and transmission electron microscopy (TEM) to identify morphological changes, (ii) the TUNEL assay for detecting apoptosis, (iii) western blotting to analyse the expression of autophagy markers—ATG5, p62, and LC3 I/II, and (iv) Sudan Black B (SBB) staining to detect lipid-associated modifications. Confocal

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analysis of cataractous lens epithelial whole mounts revealed vacuoles containing particles in the cytoplasm of cells (39.8 ± 13.1%), specifically in the central zone of the cataractous lens. These vacuoles exhibited autofluorescence across all excitation wavelengths (UV to far red range). TEM analysis further verified the presence of autophagic vacuoles containing intracellular deposits. TUNEL assay results were negative, indicating that these deposits were not associated with apoptotic cell death. The increased expression of p62, LC3 I and II, and decreased expression of ATG5 confirmed the accumulation of autophagosomes in the central zone cells. SBB staining revealed dark black, granular aggregates within the cytoplasm of cells in the central zone of the cataractous lens epithelium, indicative of lipid accumulation. In conclusion, impaired autophagy; accumulation of autophagosomes, autolysosomes; and deposition of autofluorescent lipid components in the central zone of cataractous lenses indicated a probable role of these cells in the development of age related-cataract. Further studies are necessary to investigate the molecular mechanisms underlying these changes and their association with cataract pathogenesis.

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THE ROLE OF SUMO PATHWAY IN NEURAL STEM CELL REACTIVATION AND BRAIN DEVELOPMENT

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A delicate balance between neural stem cell (NSC) quiescence and proliferation is important for adult neurogenesis and homeostasis. Small ubiquitin-related modifier (SUMO)-dependent post-translational modifications cause rapid and reversible changes in protein functions. However, the role of the SUMO pathway during NSC reactivation and brain development is not established. Here, we show that the key components of the SUMO pathway play an important role in NSC reactivation and brain development in Drosophila. Depletion of SUMO/Smt3 or SUMO conjugating enzyme Ubc9 resulted in notable defects in NSC reactivation and brain development, while their overexpression led to premature NSC reactivation. Smt3 protein levels increase with NSC reactivation, which is promoted by the Ser/Thr kinase Akt. Warts/Lats, the core protein kinase of the Hippo pathway, can undergo SUMO- and Ubc9-dependent SUMOylation at Lys766. This modification attenuates Wts phosphorylation by Hippo, leading to the inhibition of the Hippo pathway, and consequently, initiation of NSC reactivation. Moreover, inhibiting Hippo pathway effectively restored the NSC reactivation defects induced by SUMO pathway inhibition. Overall, our study uncovered a novel role for the SUMO-Hippo pathway during Drosophila NSC reactivation and brain development.

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EFFICIENT GENERATION OF HUMAN AIRWAY ORGANOIDS FROM PLURIPOTENT STEM-CELL VIA ISOLATED EPITHELIAL PROGENITORS

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Human organoids derived from reprogrammed and tissue stem cells offer an in vitro platform for studying human development, tissue regeneration, and disease modeling. Current differentiation protocols primarily generate airway epithelial cells from human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), but fail to recapitulate complex tissue-like structure and pose difficulties in being adopted as a manipulable pipeline. We present an improved method for generating complex human airway organoids derived from iPSCs. We utilize carboxypeptidase M (CPM) and EpCAM as surface marker to enrich airway progenitors and further induced the epithelial niches with FGF and WNT signaling, resulting in multiple cell types with the proximal airway identities with tissue-like architecture. Using different iPSC cell lines, we demonstrate the feasibility of our improved protocol to form complex airway organoids with increase cellular

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heterogeneities. This approach has the potential to lead to a scalable, propagable, and easily manipulated platform for studying the pathology of respiratory diseases and for anti-viral drug screening.

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ADENYLYL CYCLASE ISOFORM 1 PLAYS A KEY ROLE IN ADIPOGENIC AND OSTEOGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS

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Renewal of cells and tissues of the body occurs throughout the entirety of a person's life. The activity of postnatal stem cells plays a key role in this process. In stromal tissues, this role is performed by multipotent mesenchymal stromal cells (MSCs), which ensure the maintenance and renewal of bone, fat, connective and other tissues. These processes are controlled through a exact balance between signals for differentiation and maintenance of stemness. To maintain this balance, on the one hand, a system of response to systemic stimuli, informing the tissue about the state of the whole organism, and, on the other hand, correct communication between cells, ensuring synchronization of the tissue response is required. In both processes, the system of secondary messengers that ensure the transmission of intracellular signals is of decisive importance. For stromal cells, the signaling system of cyclic AMP, synthesized by the enzyme adenylate cyclase (AC), plays a significant role. As an example, disruption of adenylate cyclase signaling in bone tissue leads to the development of such conditions as pseudohypoparathyroidism and McCune-Albright syndrome. This disease affects cell differentiation processes, causing fibrous dysplasia of bone tissue. Mammalian cells express nine membrane isoforms of ACs (AC1-9) involved in Gs-protein-coupled receptor signaling and one soluble AC10. At the same time, the exact roles of these isoforms in governing functions of MSC are still unclear. In presented work we identified that AC1 plays a critical role in the processes of MSC differentiation. We observed a significant increase in ADCY1 expression during MSC differentiation in both adipogenic and osteogenic direction. Interestingly, osteogenesis led to an increase of full-length 120 kDa AC1 protein, while adipogenesis is associated with an increase of shorter 35–40 kDa splice variant of AC1. Using CRISPR/Cas9 D10A genome editing system we obtained a AC1 knockout MSC cell line. These cells demonstrate disrupted both adipogenic and osteogenic differentiation, suggesting a crucial role of AC1 signaling is these processes.

Funding Source: This study was supported by the Russian Science Foundation grant 19-75-30007.

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AN UNEXPECTED ROLE OF IL10 IN MESODERM INDUCTION AND DIFFERENTIATION FROM HUMAN PLURIPOTENT STEM CELLS

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Mesoderm induction is a crucial step for vascular cell specification, vascular development and vasculogenesis. However, the cellular and molecular mechanisms underlying mesoderm induction remain elusive. In the present study, a chemically-defined differentiation protocol was used to induce mesoderm formation and generate functional vascular cells including smooth muscle cells (SMCs) and endothelial cells (ECs) from human induced pluripotent stem cells (hiPSCs). Zebrafish larvae were used to detect an in vivo function of interleukin 10 (IL10) in mesoderm formation and vascular development. A three dimensional approach was used to create hiPSC-derived blood vessel organoid (BVO) and explore a potential impact of IL10 on BVO formation. A murine model hind limb ischemia was applied to investigate a therapeutic potential of hiPSC-derived cells treated with or without IL10 during differentiation. We found that IL10 was significantly and specifically up-regulated during mesoderm stage of vascular differentiation. IL10 addition in mesoderm induction media dramatically increased mesoderm induction and vascular cell generation from hiPSCs, whereas an opposite effect was observed

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with IL10 inhibition. Mechanistic studies revealed that IL10 promotes mesoderm formation and vascular cell differentiation by activating signal transducer and activator of transcription 3 signal pathway. Functional studies with an in vivo model system confirmed that knockdown of IL10 using morpholino antisense oligonucleotides in zebrafish larvae caused defective mesoderm formation, angiogenic sprouting and vascular development. Additionally, our data also show IL10 promotes blood vessel organoid development and enhances vasculogenesis and angiogenesis. Importantly, we demonstrate that IL10 treatment during mesoderm induction stage enhances blood flow perfusion recovery and increases vasculogenesis and therapeutic angiogenesis after hind limb ischemia. Our data, therefore, demonstrate a critical role for IL10 in mesoderm formation from hiPSCs and during zebrafish vascular development, providing novel insights into mesoderm induction and vascular cell specifications.

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SINGLE-CELL RNA SEQUENCING RESOLVES THE EFFECTS AND POTENTIAL MECHANISMS OF UBC ON THE MICROENVIRONMENT OF SPERMATOGONIAL STEM CELLS IN NOA PATIENTS

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Non-obstructive azoospermia (NOA) is a severe spermatogenesis disorder responsible for approximately 10–15% of male infertility cases. Spermatogonial stem cells (SSCs), located in the basement membrane of seminiferous tubules, undergo development and differentiation into spermatozoa. The disruption of SSC development and the abnormal microenvironment surrounding these cells in NOA patients contribute significantly to spermatogenesis disorders. However, the underlying mechanisms remain poorly understood. In this study, we analyzed testicular tissue samples from three NOA patients and three control individuals, with ethical approval. Single-cell RNA sequencing (scRNA-seq) revealed a marked absence of germ cells in the NOA group. In both NOA and control groups, sertoli cells exhibited the highest number of differentially expressed genes. Further functional and signaling pathway enrichment analysis indicated that mitochondrial dysfunction or impairment in sertoli cells might be a crucial factor affecting the SSC microenvironment. We identified several differentially expressed genes related to mitochondrial function, including Ubiquitin C (UBC), in sertoli cells through scRNA-seq. To investigate the role of UBC, we constructed UBC-specific siRNA and transfected it into mouse sertoli cells (TM4). Down-regulation of UBC expression led to a significant inhibition of TM4 cell proliferation and migration, accompanied by cell cycle arrest. Additionally, UBC down-regulation resulted in decreased mitochondrial membrane potential, disrupted electron transfer in the mitochondrial respiratory chain, elevated ROS levels, and a reduction in mitochondrial number in TM4 cells. These findings suggest that UBC down-regulation adversely affects mitochondrial function, thereby impacting TM4 cell biology. To further elucidate the impact of UBC down-regulation on SSC development, we co-cultured TM4 cells with SSCs. ELISA results indicated that UBC inhibition reduced the secretion of GDNF, a critical protein for SSC support, from TM4 cells. CCK-8 and EdU assays demonstrated a significant decrease in SSC survival rates in the presence of UBC-inhibited TM4 cells, though SSC proliferation levels remained unaffected. These results imply that UBC expression changes in sertoli cells may influence SSC survival through modulation of GDNF expression, thereby impacting the SSC developmental microenvironment. Our study utilized scRNA-seq to delineate the alterations in major cell types within testicular tissues of NOA and control groups, highlighting potential factors affecting the SSC microenvironment. We identified UBC as a key regulator affecting mitochondrial function and energy metabolism in sertoli cells, which in turn influences SSC support and may lead to irreversible apoptosis of spermatogonial stem cells. This research offers new insights and potential targets for the diagnosis and treatment of non-obstructive azoospermia.

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Clinical Trial ID: This study was approved by the Medical Ethics Committee of Guangzhou Huadu District Maternal and Child Health Care Hospital (Ethics Approval No. 2023-017). The Center for Reproductive Medicine at Guangzhou Huadu District Maternal and Child Health Care Hos.

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NOVEL MOLECULAR MECHANISMS IN THE REACTIVATION AND ASYMMETRIC CELL DIVISION OF DROSOPHILA NEURAL STEM CELLS

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Neural stem cells (NSCs) have the ability to be reactivated from a reversible quiescent state, self-renew and undergo differentiation via asymmetric division. Dysregulation in the balance between quiescence, self-renewal and differentiation can lead to several neurodevelopmental disorders. Here, we demonstrate novel roles of two critical Golgi proteins, Arf1 and its guanine-nucleotide exchange factor (GEF) Sec71 in regulating Drosophila quiescent NSC (qNSCs) reactivation and NSC asymmetric division. In Drosophila, qNSCs extend a primary protrusion, which is a hallmark of qNSCs. We have unraveled that qNSC protrusions can be regenerated upon injury. This regeneration relies on the Golgi apparatus which acts as the major acentrosomal microtubule-organizing centre in qNSCs. Furthermore, the Golgi-resident GTPase Arf1 and its GEF Sec71 promote NSC reactivation and regeneration via a novel Patronin-Arf1/Sec71-Msps pathway through the regulation of microtubule growth and NSC reactivation. Interestingly, in contrast to its role in regulating non-centrosomal microtubule growth in quiescent neuroblasts we find that Arf1 and Sec71 can regulate neuroblast polarity independent of its known function in microtubules in quiescent neuroblasts. Here, we show that the Golgi proteins Arf1 and ARFGEF2/Sec71 control asymmetric division of Drosophila NSCs by physically anchoring myosin II regulatory light chain, Sqh, to the NSC cortex. Arf1 can physically associate with Sqh and Vibrator, a type I PITP that stimulates phospholipid PI4K activity for PI(4)P production. Further, Arf1 and Sec71 are required for PI(4)P localization to the cell cortex of neuroblasts. Our data provides the first evidence that the Golgi proteins Arf1 and its GEF Sec71 can regulate promote NSC reactivation and regeneration via the regulation of microtubule growth and further neuroblast polarity and asymmetric division through phospholipid-dependent non-muscle myosin II cortical localization.

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STEM CELLS-DERIVED EXOSOMES: TINY TITANS FOR ALZHEIMER'S DISEASE

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Alzheimer's Disease (AD) is progressive degenerative disease, generally characterized by disruption of basic functions, such as swallowing, walking, attention and memory. Apolipoprotein E (APOE)4 homozygotes people are more prone to develop AD which is independent of Alzheimer's amyloid-b and tau pathology. APOE4-driven decrease in exosome levels in the post-mortem brains of neuropathologically healthy humans as well as in humanized APOE mice is well reported. We developed in vitro AD patient (Homozygous for ApoE e4 risk variant) iPSC-derived "tripartite synapse" model i.e. co-culture of Oligodendrocytes (MOG+), Astrocytes (GFAP+) and Microglia (TMEM119+). Increased GFAP/S100β ratio indicated increased astrocyte activation. Expression of extracellular proteins ITGAV, EFEMP1, and SIRPA confirmed synaptic development. Increased Ca2+ oscillations (Spikes/Min) of the neurons indicated functional tripartite synapse formation in our co-culture model. Mutation status of AD patient, homozygous for ApoE e4 risk variant, was confirmed by sanger sequencing and PCR. AD patient's iPSC line expressed OCT4, NANOG, SOX2; exhibit normal karyotype and 100% genomic loci matched with source PBMC as

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analysed by STR. Further, this well-characterized tripartite synapse model was treated with human umbilical cord derived mesenchymal stem cell derived exosomes (Stem cell-Exos). A dose dependent reduction in β-and γ-secretase, acetylcholinesterase, GSK3β, CDK5, and activated α-secretase activities was observed. Downregulated mRNA expression of BACE1, PSEN1, CDK5 and GSK5 and GSK-3β was observed after exosome treatment. Stem Cells-Exos downregulated IL-1β, TNF-α, and IL-6, which are associated with neuronal inflammation. Overall, our findings suggest that stem cell derived exosome treatment ameliorated the neurodegeneration of AD patient derived in vitro tripartite synapse model.

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COMPARATIVE ANALYSIS OF STEMNESS BETWEEN DERMAL PAPILLA CELLS AND HUMAN DERMAL STEM/PROGENITOR CELLS

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Adult stem cells (ASCs) have great applicative potential in tissue regeneration. The comparative studies of ASCs from different niches are necessary to understand the characteristics of each population for their potential therapeutic applications. In this study, the proliferation ability, stem cell marker expressions, and differentiation potential of skin-derived ASCs were compared between hair follicle dermal papilla cells (HFDPCs) and human dermal stem/progenitor cells (hDSPCs). The cell division capacity of hDSPCs was significantly increased compared with HFDPCs, and the differentiation capacity into adipocytes, chondrocytes, and osteoblasts was significantly increased in hDSPCs. On the contrary, HFDPCs showed significantly increased the expression of dermal papilla–related markers compared with hDSPCs. To analyze why these two types of ASCs have different properties, I analyzed intracellular signaling by protein kinase assay. Protein kinase assays showed that the phosphorylation of ERK1/2, c-JUN, CREB, YES, and GSK3α/β is significantly changed in HFDPCs and hDSPCs compared with dermal fibroblasts. HFDPCs have increased expression of markers related to hair regeneration compared with hDSPCs, on the other hand hDSPCs are more multipotent than HFDPCs. The five above mentioned phosphorylated signaling proteins (ERK1/2, c-JUN, CREB, YES, and GSK3α/β) are responsible for the characterization of HFDPCs and hDSPCs. The different characteristics of each skin-derived ASC might be a major factor influencing their effective use for tissue regeneration and therapeutics.

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DERIVATION OF GRANULOSA-LIKE CELLS FROM HUMAN ENDOMETRIAL IPSCS FOR POTENTIAL HORMONE THERAPY

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This study aimed to derive granulosa cell-like cells from iPSCs generated from discarded endometrial stromal cells, exploring their potential as a novel source of autologous estradiol production. iPSCs were created using human endometrial cells obtained from five benign hysterectomies, and granulosa cells were successfully differentiated in a stepwise manner, inducing estradiol production. The differentiation process involved forming embryoid bodies from iPSCs, followed by mesodermal lineage induction with BMP4, WNT3a, Activin A, and bFGF, and further differentiation using BMP4, Follistatin, and bFGF. Markers indicative of granulosa cell differentiation (AMH, FOXL2, FSHR, AMHR2, LHR, CYP19A1) were analyzed via qPCR and flow cytometry. After a 12-day differentiation period, the cells were seeded, and an E2 assay was performed using ELISA after adding androstenedione. Results showed that brachyury expression, a marker for primitive streak-mesendoderm, was 21.7% for iPSCs and 30% for H9 cells on day 6, decreasing by day 12. The expression of granulosa cell markers increased significantly after differentiation, with AMH expression in iPSCs reaching 10.8 (SE ± 0.11) compared to 2.4 (SE ± 0.1) in H9-derived cells. Estradiol levels were measured at 2,119.7 pg/ml (SE ± 211.9) for iPSCs and 1,364.3 pg/ml (SE ± 107.9) for H9, demonstrating the successful derivation of functional granulosa-like cells from human endometrium-derived iPSCs, with significant implications for hormone therapy applications.

Funding Source: This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea Government (MSIT) (No. NRF-2017R1D1A1B03036168).

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ABERRANT SPLICING IN WERNER SYNDROME IPS CELL-DERIVED MESENCHYMAL STEM CELLS RESULTS IN REDUCED WOUND HEALING CAPACITY VIA INCREASED EXPRESSION OF SOLUBLE FLT1

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Werner syndrome (WS), caused by mutations in the RecQ type helicase gene WRN, is an autosomal recessive disorder that develops accelerated aging-associated symptoms after puberty. Refractory skin ulcers are characteristic of WS and frequently result in lower limb amputation, but the pathogenesis of the disease is not yet clear. Recently, regenerative medicine with mesenchymal stem cells (MSCs) for skin ulcers has attracted attention, and we conducted experiments to investigate the utility of WS-derived MSCs in wound healing and elucidate their pathogenesis in this study. We established iPS cells from normal subjects and WS patients, differentiated them into MSCs (iMSCs), and injected them around wounds in a mouse model of

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intractable skin ulcers to investigate their healing effect. iMSC culture supernatants were used for proteomics of angiogenic factors, and subsequent studies were conducted on the molecules that showed significant differences. Similar analyses were also performed on urine samples from normal subjects and WS patients. Compared to the normal iMSC-treated group (NM group), the WS-iMSC-treated group (WS group) showed significantly delayed wound healing and significantly reduced blood vessel volume in skin sections. iMSCs culture supernatant proteomics showed a reduced VEGF content in WS group, alongside an increased expression of soluble FLT1 (sFLT1), a splicing variant of the VEGF decoy receptor FLT1. As splicing factor (SF) abnormalities were considered in the background, RNA-seq of iMSCs showed decreased expression of hnRNPA1, a major SF. Therefore, overexpression of hnRNPA1 in WS-iMSCs resulted in a significant reduction in sFLT1 expression. Co-immunoprecipitation also suggested the binding of hnRNPA1 protein to FLT1 pre-mRNA, suggesting that hnRNPA1 is involved in FLT1 splicing. Furthermore, wound healing was significantly delayed in mice treated with NM-iMSCs overexpressing sFLT1. In addition, urinary sFLT1 levels were significantly higher in both elderly and WS patients, suggesting a link between sFLT1 and general aging. sFLT1 may be a useful biomarker for general aging, as the impaired wound-healing effect of WS-iMSCs was attributed to increased sFLT1 due to decreased hnRNPA1 expression. It was considered that sFLT1 may be useful as a biomarker for general aging.

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UNDERSTANDING CHOROID PLEXUS DIFFERENTIATION USING ORGANOIDS DERIVED FROM HUMAN STEM CELLS

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Choroid plexus (CP), an epithelial structure within the brain ventricles, produces cerebrospinal fluid and serves barrier functions in the brain. Primary neoplasms of CP are rare intracranial tumors that predominantly occurs in childhood. CP tumors can be categorized into benign CP papilloma, and malignant CP carcinoma (CPC) frequently associated with poor outcomes. These patients have worst prognosis even after chemotherapy, surgery and radiation. The development of safer and more effective therapies for CPC requires a better understanding of its biology. However, the small patient population, scarcity of patient specimens and lack of accurate experimental models have hindered these efforts. In order to address the existing gap between research and therapeutic development, this study utilized CP organoids derived from human induced pluripotent stem cells (iPSC) and embryonic stem cells (ESC). Specifically, human iPSC line IMR-90 and human ESC line H9 were used to develop CP organoids based on previously published protocols. After 30 days, cystic structures filled with CSF-like fluid were grossly evident. Immunofluorescence data revealed epithelial characteristics similar to those of CP epithelium in humans and mice, whereas the expression of, ADP-ribosylation factor-like protein 13B (ARL13B), a cilia marker, indicated the presence of multiciliated cells thus recapitulating the human CP epithelial phenotype. The expression of CP markers such as Aquaporin 1 (AQP1), Zonula Occludens-1 (ZO-1), and Orthodenticle Homeobox 2 (OTX2) were detected in CP organoids. Results from qPCR and western blot experiments further confirmed their expression at RNA and protein levels, respectively. Analysis of single cell RNAseq data along with in situ hybridization studies showed the expression of regulators of ciliogenesis including Forkhead box protein J1 (FOXJ1) and TAp73 in epithelial structure. Future work includes analysis of gene expression in diverse cellular compartments at different stages of CP organoid development. Knowledge of regulatory mechanisms of CP differentiation may hopefully facilitate the development of potential therapeutic strategy in a subset of CPC.

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BRAIN-WIDE ENGRAFTMENT OF HUMAN MONOCYTES, BUT NOT MICROGLIA, INDUCES A CHRONIC PROINFLAMMATORY STATE, PROMOTING SYNAPTIC AND BEHAVIORAL DYSFUNCTION

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Aims Hematopoietic stem cell transplantation (HSCT) is increasingly being tested as a potential therapy for neurological disorders. The premise of this approach is that HSCT-derived monocytes may infiltrate the brain and differentiate into “microglia-like” cells. Recent advances in the differentiation of induced pluripotent stem cells (iPSCs) into microglia, provide a potential new source of therapeutic microglia. However, many questions remain regarding the similarities and differences between microglia and monocytes (MNs) and which of these cell types may be optimal for therapeutic application. To compare the engraftment potential and transcriptional and functional profiles of iPSC-microglia (iMGs) and human monocytes following transplantation into the brain, we developed a novel xenotransplantation-compatible mouse model that lacks endogenous murine microglia (hFIRE mice). Human iMGs and blood monocytes from four male patients were transplanted into adult male hFIRE mouse brains. Four months later brains were examined by spatial RNA sequencing, TMT-MS proteomics, histological and biochemical approaches. Immunohistochemical analysis revealed near-complete chimerism of human iMGs and human monocytes throughout the brain. Four months after transplantation, monocyte-derived cells express several microglial-related makers and yet continue to exhibit significant differences in marker expression and cell morphology. In addition, spatial sequencing, multiplex cytokine ELISAs, and proteomics reveal important and persistent differences between engrafted monocytes and microglia including a chronic elevation of proinflammatory cytokines in monocyte-transplanted mice. Also, monocyte engrafted mice exhibit significantly distinct behavior deficits suggestive of elevated anxiety compared with Microglia engrafted hFIRE mice. Taken together, these results offer novel insights with important implications for the development of CNS-wide microglial replacement therapies. Collectively, our data suggest that HSCT transplantation induces important additional safety concerns beyond those observed with iMG transplantation and that peripherally-derived monocytes fail to become microglia.

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DEVELOPMENT OF CD34+ CELLS MAINTAINANCE MEDIUM FOR HUMAN INDUCED PLURIPOTENT STEM CELLS-DERIVED HEMATOPOIETIC STEM CELLS

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The hematopoietic system plays the vital role for keeping human survival and healthy. Hematopoietic stem cells (HSCs) transplantation has been the curative treatment for many hematologic disorders, and umbilical cord blood (UCB), bone marrow (BM) and peripheral blood (PB) have served as the allogeneic transplantation sources for decades. To overcome the disadvantages associated with donor variations, the induced pluripotent stem cells (iPSCs)-derived HSCs may become a promising solution. These cells theoretically have the potential to address issues related to donor-matching and provide a continuous, unlimited source of cells. Here we have developed a complete serum-free differentiation and proliferation culture system for iPSCs-CD34+ cells. In the first step, the CD34+ cells were differentiated from iPSCs with high efficiency (>90%). To obtain more iPSCs-CD34+ cells, the medium was modified by adding TPO, IL-3, SCF, IL-6, Flt3, insulin, transferrin, HSA, UM729 and SR1 to support proliferation. The induced CD34+ cells proliferated approximately 7-fold in cell numbers within 7 days while maintaining high CD34+ expression (>60%). When the medium composition was replaced with GMP-grade ingredients, the proliferation capacity of iPSC-CD34+ cells showed a slight decrease (6.3-folds), but the percentage of CD34+ increased (>80%). These results were superior to those achieved with commercially available HSC GMP media.

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After differentiation, the iPSC-CD34+ cells demonstrated fully multipotent capability, as evidenced by formation CFU-E, -GM and -GEMM colonies in methylcellulose-based CFU assay. In contrast, the expanded iPSCs-CD34+ cells exhibited remarkable ability for lymphoid lineage differentiation, showing only CFU-GM expression. This study indicates that the CD34+ derived from iPSCs can be efficiently generated and proliferated using our chemical-defined serum-free culture system. These cells hold potential as a starting point cells for research and immunotherapy applications.

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EXPLORING MONOCYTE-MEDIATED NEUROINFLAMMATION IN ALZHEIMER'S DISEASE USING ENGINEERED HUMAN BRAIN ORGANOID CHIPS

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Recent evidence increasingly links the pathogenesis of Alzheimer's disease (AD) to neuroinflammation. Peripheral monocytes, key mediators of inflammation in the human immune system, remain understudied in AD pathogenesis, partly due to the lack of suitable human models. To address this gap, we developed engineered human brain organoid (hBO) chips derived from human embryonic stem cells (hESCs) to model dynamic AD neuroinflammation mediated by monocytes. By incorporating 3D-printed scaffolds into hBO cultures within standard 96-well plates, we developed hBO chips with tubular hBOs, largely reducing necrosis and enhancing organoid viability. Using these engineered hBO chips, we observed the notable alterations of monocytes from AD patients, including higher infiltration rates, reduced amyloid-beta (Aβ) clearance, and heightened inflammatory responses. Meanwhile, AD monocytes induced elevated astrocyte activation and neuronal apoptosis, highlighting their promoting effects in AD neuroinflammation. Notably, the expression of IL1B and CCL3 was significantly increased at both the transcriptional and protein levels, underscoring the central role of these cytokines and chemokines in monocyte-mediated AD neuroinflammation. Our findings shed light on the contribution of monocytes to AD pathogenesis, while the presented engineered hBO chips offer a user-friendly, adaptable platform for studying neuroinflammation and for developing new therapeutics targeting neuroinflammatory diseases.

Funding Source: Indiana University Departmental start-up funds.

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DYNAMIC CONTROL OF STEM CELL COMPETENCY TO GENERATE TRANSIT-AMPLIFYING PROGENITORS DURING DROSOPHILA BRAIN NEUROGENESIS

Cheng-Yu Lee, Cyrina Ostgaard, and Arjun Rajan

Cell and Developmental Biology, University of Michigan, USA

Emerging evidence suggests that intermediate progenitors, stem cell progeny that function to generate differentiated cells, are heterogeneous in developmental and proliferative capacity. The mechanisms allowing stem cells to generate distinct intermediate progenitor subtypes are unknown. We investigate stem cell (neuroblasts) competency to generate distinct intermediate progenitor subtypes (ganglion mother cell [GMC] and intermediate neural progenitor [INP]) in Drosophila larval brains. Each asymmetric division of a type I neuroblast generates a GMC that produces two neurons. By contrast, a type II neuroblast always generates an INP that produces 5–6 GMCs. Surprisingly, cell-type-specific enhancers of genes essential for INP generation and function remain accessible in type I neuroblasts suggesting that both neuroblast subtypes are competent to generate INPs. Mis-expressing type I NB-specific transcription factor Asense or its downstream-effector Prospero drives type II NB progeny to bypass an INP identity and directly assume a GMC identity. Removing asense or prospero function increases the efficiency of induced INP generation by type I NBs. We conclude that the Asense-Prospero transcriptional cascade promotes proper neural patterning by limiting neuroblast competency to generate INPs.

Funding Source: National Institute of Neurological Disorders and Stroke grants (R01NS111647, R01NS107496 and R01NS134942), Sontag Foundation Distinguished Scientist Alumni Award.

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MONOAMINE MODULATION MITIGATES TDP-43 MISLOCALISATION IN A TARGETED DRUG SCREEN FOR AMYOTROPIC LATERAL SCLEROSIS

Noor Ul Ain Akram, Bryan Ng, Shi Yan Ng, and Caroline Wee

*Agency for Science, Technology and Research (A*Star), Singapore*

Amyotrophic lateral sclerosis (ALS) is an insidious neurodegenerative disorder that results in the degeneration of motor neurons in the motor cortex, spinal cord, and brainstem. Mutations in (different genes including) TARDBP (which codes for) coding for TDP-43 are known to cause familial ALS, while close to 97% of sporadic ALS cases exhibit TDP-43 proteinopathy wherein TDP-43 mislocalizes from the nucleus and aggregates in the cytoplasm. Currently, the most common FDA-approved treatments improve lifespan by only up to 3 months hence there is an urgent need for disease-modifying therapeutics focusing on TDP-43 proteinopathy. To address this, we developed a targeted drug screen using motor neurons derived from a human induced pluripotent stem cell (iPSC) line with TARDBP G298S mutation knocked-in via CRISPR-Cas9. We found that modulators in the monoamine pathway displayed dose-dependent effects to mitigate TDP-43 mislocalization accompanied by improvements in motor neuron survival. Additionally, we identified protein targets for pathway enrichment analysis and potential protein-protein interactions of the top-performing drugs in mitigating TDP-43 mislocalization using various bioinformatic tools to highlight novel cellular pathways and uncover common targets that can ameliorate TDP-43 proteinopathy in ALS. Protein-protein interactions predicted by STRING help identify nodes/hubs of interest guiding future pathway studies and the development of disease-modifying therapeutics for ALS.

Funding Source: Singapore Therapeutics Development Review (STDR) (Implemented by A*STAR, Singapore-MIT Alliance for Research and Technology (SMART), EDCC and NHIC.

WEDNESDAY, 11 DECEMBER 2024

5:45 PM – 6:30 PM
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HIGH RESOLUTION SPATIAL TRANSCRIPTOMICS PROFILING OF HUMAN UPPER AIRWAY

Jiayi Zheng[1], Mu He[2], Shuxin Xiao[2], Chi Wai Michael Chan[3], and Hiu Ha Rachel Ching[3]

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Recent advancements in spatial transcriptomics have revolutionised the exploration of cell-cell interactions within airway biology, enabling identification of new niche population based on their spatial localisation. However, most respiratory spatial datasets have been focusing on distal region of the lung, while the few upper airway ones are limited by their resolution below single-cell level. Our group has addressed this gap by utilising Stereo-Seq on tracheal biopsy samples, to provide a high-resolution reference map for the field, and dissect the heterogeneity of secretory cells in submucosal gland cells (SMGs). We were also able to identify distinct population of plasma cells within the SMGs, enriching the current understanding of immune-epithelium interaction in upper airway context. Furthermore, we extended our study to include ex vivo influenza infections of upper airway samples using different viral strain. Leveraging spatial transcriptomics, we were able to identify cell-type specific virus localisation in the upper airway. Our findings also identified diverse cellular responses and interactions, revealing differences in host cell interactions in response to different influenza strains.

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PHF16 IS REQUIRED FOR MOUSE INTESTINAL REGENERATION FOLLOWING IRRADIATION DAMAGE

Sein Kim and Jun-yeong Ahn

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Great self-renewal capacity of intestinal stem cells (ISCs) enables rapid turnover of the intestinal epithelium by constant replenishment of differentiated progeny. But it also sensitizes ISCs to irradiation or chemical damage. Following damage-induced ablation of ISCs, revival stem cells (revSCs) emerge from dedifferentiation of epithelial cells and contribute to re-establishment of homeostasis. Although regulatory mechanisms for emergence of revival stem cells have been extensively studied, it remains unclear how regenerative state returns to homeostatic state. Here, we found that epigenetic regulator plant homology domain (PHD) finger protein 16 (Phf16) restores homeostasis of the intestinal epithelium after initial damage-induced repair. Phf16$^{-/Y}$ mice showed defects not in normal homeostasis, but only in intestinal regeneration upon irradiation damage. At 7 days post-irradation(dpi), Phf16$^{-/Y}$ mice underwent severely compromised regeneration with reduced numbers of Olfm4+ ISCs and hyperactivated YAP and fetal gene expressions. Single-cell RNA sequencing and pseudotime analysis identified that Phf16$^{-/Y}$ mice had revSCs remain in a fetal-like state instead of giving rise to differentiated cell types and returning to homeostasis at 7dpi. Our findings collectively demonstrate that Phf16 plays an important role in exiting a fetal-like state of revSCs during crypt regeneration.

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THE REGULATORY ROLES OF MELATONIN ON THE INTESTINAL REGENERATION

Yoo Jin Seo, Ji-Su Ahn, Su-Jeong Oh, Jeong Hyun Yu, Seong Hui Kim, Yunji Lee, Hee-Jeong Park, Jiwon Yang, Min-Jung Kang, and Hyung-sik Kim

Pusan National University, South Korea

Enteroendocrine cells (EECs), constituting merely 1% of the intestinal epithelium, represent a profound endocrine system in terms of hormone and bioactive molecule production. This research examines the influence of EEC-derived melatonin (M) on intestinal homeostasis and regenerative capabilities using intestinal organoids (IOs) and transgenic models. It was observed that melatonin hindered the budding process and proliferation capacity in murine IOs without inducing cell death. Notably, based on the bulk sequencing results and gene set enrichment analysis, M administration may activate pathways associated with injury response and a fetal-like regenerative process. Further, single-cell RNA sequencing revealed an increase in a specific subset of EECs with regenerative potential (characterized by high PAX6 and low Neurog3 expression) following M treatment. Recently, it has been shown that Prostaglandin E2 (PGE2) is one of the major paracrine signals to orchestrate the repair process during intestinal injury by inducing Clu/Ly6a+ regenerative stem cell (RSC) population in the intestinal epithelium. Intriguingly, M treatment was found to enhance the PGE2-driven augmentation of Ly6a+ RSC pools within IOs. To determine whether M administration can aid intestinal regeneration in vivo, we treated M to dextran sulfate sodium (DSS)-exposed mice and found that continuous treatment of M could improve the clinical symptoms of colitis. Finally, we further explored the impact of endogenous M by examining knockout mice deficient in AANAT, the rate-limiting enzyme crucial for M synthesis. It was revealed that the recovery pace from DSS-induced damage was slower in AANAT knockout mice compared to their wild-type counterparts. Therefore, these findings illuminate the potential of EEC-derived hormones in modulating the RSC population and augmenting the endogenous regenerative capacity.

Funding Source: Korean Fund for Regenerative Medicine (KFRM) grant (22A0205L1-11) and National Research Foundation of Korea(NRF) grant (RS-2024-0034006) supported this project.

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GENERATION OF A XENO-FREE IPSC-DERIVED MESENCHYMAL STROMAL CELLS (IMSCS) THROUGH THE NEURAL CREST LINEAGE AS A POTENTIAL SOURCE FOR CGMP-GRADE MSC-BASED CELL THERAPY

Anggia Putri, Claryssa Bianca, Ricky Sanjaya, Naufalia Faza, Andrian Faisal, and Halida Widyastuti

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Mesenchymal stromal cells (MSCs) are multipotent progenitor cells with strong therapeutic potential due to their self-renewal capacity and immunomodulatory effects. Although MSCs can be isolated from sources like bone marrow, umbilical cord, and adipose tissue, their clinical application is hindered by scalability challenges due to limited proliferative capacity, cell heterogeneity, and donor variability. To address these limitations, we developed an alternative source of MSCs by deriving it from induced pluripotent stem cells (iPSCs), which offers a promising solution due to their greater expandability, and potentially continuous supply. In this study we developed a robust, xeno-free differentiation protocol using human platelet lysate (hPL). We had previously reprogrammed iPSCs from umbilical cord-derived MSCs (UC-MSCs) using a non-integrating, RNA-based method. The characterized iPSCs were then differentiated into MSCs via WNT signaling pathway activation using a combination of TGFβ and GSK3β inhibitors. This approach efficiently generated >90% neural crest-like progenitor cells, which were further differentiated into iPSC-derived MSCs (iMSCs). The resulting iMSCs exhibited stable proliferation over 30 population doublings and maintained consistent gene expression and surface marker profiles. Moreover, genomic stability was preserved, as shown by karyotyping, confirming no genetic alterations occurred during the differentiation process. Comprehensive characterization per ISCT guidelines confirmed the expression of MSC-specific markers (CD105, CD73, CD90) and additional markers (CD29, CD44), while lacking hematopoietic markers. Our iMSCs have functional properties comparable to traditional MSCs, retaining their trilineage differentiation potential into osteocytes, adipocytes, and chondrocytes. This xeno-free protocol provides an up-scalable source of iMSCs, a promising alternative to primary MSCs sources for regenerative medicine and highlighting its potential for future clinical applications, including cGMP-grade MSC-based cell therapy production.

Funding Source: This work was supported and fully funded by PT. Kalbe Farma, Tbk Indonesia.

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MODULATION OF AIRWAY EPITHELIAL RESPONSES TO INFLUENZA INFECTION BY MACROPHAGE-DERIVED CYTOKINES

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The University of Hong Kong, Hong Kong

Airway epithelial cells (AECs) play a pivotal role in maintaining homeostasis of the respiratory system. As the first line of the host defence system, AECs facilitate rapid response to pathogenic invasion, including viral infections. Meanwhile, they mediate pathogen clearance via the crosstalk with resident immune cells, particularly macrophages. The anti-viral inflammatory responses mediated by airway immune cells against different viral strains have been widely documented. However, how AECs respond to immune cell signals and are protected upon viral infection remains understudied. To better understand how AECs communicate with immune cells under infection, we employed influenza 415 (H1N1) and G1h (H9N2) to infect human airway organoids with or without co-cultured macrophages. Utilizing single-cell RNA analysis, strain-specific airway epithelial landscapes were observed in response to viral infections. In this study, we identified core gene modules upregulated by AECs upon infection, including the IL1 pathway as well as the IFN pathway in AEC-macrophage co-cultures. In addition, we observed that in the presence of macrophages, viral infection induced ciliated cell loss was ameliorated. Consistent with this observation, pro-inflammatory signatures were upregulated in multiple epithelial cell types under the stimulation of macrophage-derived cytokines. The

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data define the cellular responses generated by human AECs following viral infection and contribute to a more comprehensive understanding towards the epithelial-immune axis.

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REJUVANATION OF SENESCENT MESENCHYMAL STEM CELL BY MITOPHAGY REACTIVATION

Kiyoshi Sato and Hiroyoshi Kawakami

Tokyo Metropolitan University, Japan

Regenerative therapy and bioartificial tissues/organs require a large quantity of human cells. However, current cell manufacturing processes are accompanied by cellular senescence, leading to the deterioration of cell proliferation and function together with the secretion of senescence-associated secretory phenotype (SASP) factors. Therefore, suppression of replicative senescence during expansion/production culture is one of the crucial issues for the development of regenerative medicine. We herein report dual drug-encapsulated liposomal nanoparticles to suppress cellular senescence in human adipose tissue-derived mesenchymal stem cells (AT-MSCs), a promising cell source for regenerative medicine and stem cell-based therapy, by removal of dysfunctional mitochondria from cultured AT-MSCs. We found that the nanoparticle treatment induces mitophagy, recovers cell proliferation ability, and reduces senescent makers in the cells via mitophagy reactivation. SASP secretion from the cells also improved by the treatment. Our findings may contribute to the production and availability of MSCs suitable for medical applications and tissue engineering.

Funding Source: The Japan Society for the Promotion of Science (JSPS) KAKENHI (JP21K19920 and JP23H03743). Tokyo Metropolitan Government Infectious Disease Research Project and Advanced Research Grant Number (R4-1).

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NOGO RECEPTOR ANTAGONIST, LOTUS GENE TRANSFER BY ADENO-ASSOCIATED VIRUS, FACILITATES AXON REGROWTH AND FUNCTIONAL RESTORATION IN SPINAL CORD INJURY MODEL MICE

Junpei Matsubayashi, Yuki Kawaguchi, Yutaka Kawakami, Kiwa Kobayashi, and Kohtaro Takei

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The regeneration of the central nervous system (CNS) is extremely limited after spinal cord injury (SCI). After SCI, several axonal growth inhibitors derived from myelin debris and a glial scar bind to Nogo receptor-1 (NgR1) or Paired immunoglobulin-like receptor B (PirB), thereby failing CNS regeneration. Lateral olfactory tract usher substance (LOTUS) contributes to axon regrowth and synaptic formation as an endogenous NgR1 and PirB antagonist. Previous studies reported that neuronally LOTUS-overexpressing transgenic mice and LOTUS-overexpressing human iPSC-NS/PCs transplanted mice showed axonal regeneration and functional recovery after SCI. Therefore, LOTUS is expected to be useful for SCI therapy as an endogenous axonal regenerative substance. However, the expression level of LOTUS drastically decreases after SCI. The decrease in LOTUS expression can be considered one of the major causes of delayed functional restoration following SCI. In addition, to apply the beneficial effects of LOTUS to clinical treatment, establishing a non-invasive and adaptive therapeutic strategy is desirable. Herein, we evaluated the therapeutic effects of LOTUS gene treatment using an adeno-associated virus (AAV) vector in contusive SCI model mice. First, we confirmed the treatment of AAV-LOTUS suppresses neurite outgrowth inhibition induced by NgR1-ligands in cultured neurons. Next, we performed intrathecally induction of AAV-LOTUS in SCI model mice. AAV-LOTUS-transduced mice expressed higher levels of LOTUS in the injured spinal cord. Histological analysis revealed that AAV-LOTUS promotes the regrowth of raphespinal serotonergic fibers beyond the epicenter and suppresses axonal dieback of corticospinal tract fibers after SCI. Moreover, overexpression of LOTUS via AAV significantly restored

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functional motor and sensory activity, as determined by the basso mouse scale (BMS) locomotion score and grid-walking test 28 days after SCI. These findings suggest that overexpression of the LOTUS gene using AAV compensates for LOTUS expression, inhibiting NgR1 and PirB functions and promoting neuronal regeneration after SCI. Thus, transduction of AAV-LOTUS could be a promising therapeutic strategy for treating humans with acute SCI.

Funding Source: This research was supported by AMED under Grant Number JP24bk0104175h0001.

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THE EFFICIENCY OF CULTURE MEDIA FOR CONVERSION PRIMED TO NAÏVE STATE OF RHESUS MONKEY EMBRYONIC STEM CELLS

Rangsun Parnpai[1], Ratree Moorawong[2], Preeyanan Anwised[2], Worawalan Samruan[2], Jittanun Srisutush[2], Irene Aksoy[3], and Pierre Savatier[3]

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Pluripotent stem cells (PSCs) capable of stable self-renewal in the naïve state of pluripotency hold significant potential for developmental and biotechnological applications. Unlike PSCs in the primed state, naïve PSCs can form blastoïds in vitro and generate germline chimeras in vivo. Therefore, establishing PSC lines that can stably self-renew in the naïve state is a major objective in various mammalian species, In this study, we examined the efficiency of two different culture media in converting primed rhesus monkey embryonic stem cells (rhESCs) to the naive pluripotent state under feeder-free conditions. These two media contain activin A, LIF, the PKC inhibitor Gö6976, and the tankyrase inhibitor XAV939, differing only in the conditioned medium added: either derived from mouse embryonic fibroblasts of the OF1 strain (OF1-MEFs-CM) or from human Wharton's jelly mesenchymal stem cells from the umbilical cord (hWJ-MSCs-CM). We assessed key markers of naive pluripotency, cellular morphology, and gene expression profiles. Our results showed that conversion of rhESCs in both conditioned media led to strong expression of core pluripotency genes, including NANOG and SOX2, while expression of TBXT, a primed state marker, was lost. Cells converted with hWJ-MSCs-CM on day 9 exhibited significantly higher expression of naive genes, including KLF4, KLF17, ESRRB, TFAP2C, DPPA2, and DPPA5. In contrast, conversion using OF1-MEFs-CM resulted in notably higher DPPA5 expression on both day 9 and day 21 compared to hWJ-MSCs-CM. Additionally, epigenetic analysis revealed an increase in H3K14 acetylation and a decrease in H3K9 trimethylation. These findings provide valuable insights into optimizing culture conditions for achieving and maintaining naive pluripotency in non-human primate ESCs, with important implications for developmental biology, regenerative medicine, and comparative stem cell research across species.

Funding Source: This work was funded by the Program Management Unit for Human Resources & Institutional Development, Research and Innovation (PMU-B), Government of Thailand (Grant Number: B16F640104).

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PHF16$^{-/Y}$ MOUSE INTESTINAL ORGANOIDS ARE LOCKED IN AN UNDIFFERENTIATED REGENERATIVE STATE

Joowon Cha and Jun-Yeong Ahn

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The process of injury-induced intestinal regeneration is dynamic and involves the reprogramming of differentiated cells to a fetal-like state followed by the restoration of homeostasis. This complex process is governed by precisely regulated transcriptional mechanisms. The Yes-associated protein/transcriptional co-activator with PDZ-binding motif (YAP/TAZ) signaling is critical for initiating regenerative activity, while the retinoic acid receptor (RAR)/retinoic X receptor (RXR) signaling is essential for exiting the regenerative state. However, the molecular mechanisms coordinating these pathways to restore intestinal homeostasis after injury remain unclear. In this study, we use Phf16$^{-/Y}$ intestinal organoids to demonstrate that Plant Homeodomain

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(PHD) finger protein 16 (Phf16) epigenetically regulates both RAR/RXR and YAP/TAZ activities to swiftly restore intestinal epithelium homeostasis. RNA sequencing and ATAC-seq analyses of wild-type (WT) and Phf16$^{-/Y}$ intestinal organoids reveal that Phf16 promotes epithelial homeostasis by activating RAR/RXR target genes through HBO1-mediated histone H3K14 acetylation. Concurrently, Phf16 counteracts YAP/TAZ activity by facilitating the ubiquitination of CDC73. Our findings uncover previously unknown epigenetic mechanisms that tightly regulate intestinal regeneration, providing new insights into chronic intestinal disorders such as inflammatory bowel disease (IBD) and cancers associated with these regenerative processes.

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REGENERATIVE EFFECTS OF BACOPA SEEDED WITH NEURAL STEM CELLS DERIVED FROM MICE

Rohit Sarda

Sikkim Manipal Institute of Medical Sciences, Sikkim Manipal University, India

Repairing of neuronal tissue injury reduces the successful functional outcome. It is important for the neuronal cells to regenerate at the injury site and establish proper neuronal tracts on site. Further, the newly formed cells must anatomically align with the existing cells or achievement of functionality. Stem cells, with addition to other herbal agents, have been made use of in many studies. In Ayurveda, Bacopa is frequently used for the treatment of cognitive impairment. The purpose behind conducting the study is that Bacopa extract is able to induce structural changes in Central Nervous System (CNS) repair and to check the feasibility of using Bacopa as a regeneration and differentiation agent to cure the injured site. The study outcomes can provide application-oriented, clinical and therapeutic implications. The NSCs were extracted from newborn albino rats, and Bacopa extract was procured in the name of 'Brahmi'. After the growth of NSCs in media, aqueous suspension of Bacopa alcoholic extract measuring 2µl was added to the media in culture plates. Bacopa seeded Stem Cells at Site of injury shows insignificant necrosis. Diffused cell infiltration is present which less than Sham (control) tissue was. Number of neural heads increases on sit of injury, better anastomosis appeared between neural cells. Vacuolation were absent in around the tissue which was seen during histological study. This is a significant outcome in terms of biological applications. This study is a first-of-its-kind research in this domain and provides the future researchers with further opportunities in nervous tissue injury. it may clinically revolutionize the concepts of central and peripheral nervous tissue treatment in future, especially if stem cells are used.

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STEM CELL-DERIVED MODELS REVEAL ENTRECTINIB'S ROLE IN ATTENUATING LPS-INDUCED NEUROINFLAMMATION AND COGNITIVE IMPAIRMENT THROUGH JNK, P38, AND AKT PATHWAY INHIBITION

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Entrectinib, an FDA-approved TRK inhibitor, is known for treating cancers such as non-small cell lung cancer (NSCLC) by crossing the blood-brain barrier (BBB). However, its effects on neuroinflammation and memory functions remain unclear. This study aimed to investigate the effects of Entrectinib on neuroinflammatory responses using stem cell-derived microglia and neuronal models. In vitro experiments were conducted on stem cell-derived microglial cultures treated with 200 ng/ml LPS, followed by 1 µM Entrectinib. Western blotting, real-time PCR, and immunocytochemistry were used to assess inflammatory markers, cellular signaling, and phagocytic activity. Entrectinib significantly reduced LPS-induced proinflammatory cytokines (Il6, Tnfα, Ccl2) and suppressed JNK, P38, AKT, NF-κB, and STAT3 signaling pathways. Entrectinib also shifted microglial polarization by decreasing CD16/32 and increasing CD206 expression, enhancing phagocytic function. In stem cell-derived neuronal models, Entrectinib ameliorated LPS-induced neuroinflammation and improved synaptic plasticity, suggesting a rescue of cognitive deficits.

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These findings highlight Entrectinib's potential in mitigating neuroinflammation and memory impairments, suggesting its therapeutic use in neuroinflammation-associated diseases.

Funding Source: (G-LAMP) Program of the National Research Foundation of Korea (NRF) grant funded by the Ministry of Education (RS-2023-00301850), NRF funded by the Ministry of Science and ICT (RS-2024-00351023).

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REGULATION OF HEMATOPOIETIC CELL FATE IN ACTIVATED MESENCHYMAL STEM/STROMAL CELLS

Yo Mabuchi

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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 the basis of BM. BM transplantation therapy promotes platelet generation and early neutrophil differentiation, thereby reducing the risk of infection. Myeloid differentiation can also be induced through the administration of specific factors, such as granulocyte colony-stimulating factor and granulocyte/macrophage colony-stimulating factor, at the time of HSPC transplantation. However, it remains unclear which factors contribute to lineage determination in BM or transplanted cells. This study aimed to unravel the mechanisms underlying the effects of MSCs on HSPC differentiation. We found that activated PDGFRα+Sca-1+ (PαS-MSCs) cells secreted CCL2 and regulated the differentiation ability of HSPCs to produce myeloid cells. When CCL2 was knocked out of PαS-MSCs cells, the ability of HSPCs to differentiate into myeloid cells was significantly reduced. The main cell cluster secreting CCL2 stimulated by lipopolysaccharide was PαS-MSCs cells, which regulate the induction of differentiation by targeting granulocyte/macrophage progenitors. Further, the MSCs acted as sensors against inflammation and infection and propagated inflammatory signals to hematopoietic cells in vivo. This supports a model in which human MSCs, through CCL2 signaling, can promote myeloid cell differentiation and support the recovery of essential immune cell compartments.

Funding Source: This work was financially supported by the AMED (grant numbers 23bm1223011h0001); the JST, CREST Japan (grant number JPMJCR2124).

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MULTILINEAGE-DIFFERENTIATING STRESS-ENDURING CELLS IN THE TREATMENT OF ISCHEMIC STROKE: A SYSTEMATIC REVIEW

Alexandra Gleave[1], Adeel Khan[2], and Aryan Shah[2]

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Muse (Multilineage-differentiating Stress-Enduring) cells were discovered by researchers when they identified a small subpopulation of mesenchymal stem cells exhibiting remarkable stress resistance. While thrombolysis and mechanical thrombectomy offer some recovery after ischemic stroke, their use is limited to a narrow therapeutic window. Rehabilitation can provide additional functional improvements but is limited by neuroplasticity. Stem cells have been explored in randomized clinical trials (RCTs) for conditions like ischemic stroke, though the results have been mixed. In animal models, stem cells often fail to survive long enough to replace damaged neuronal tissue after stroke. With their unique stress-resistant properties, Muse cells are proposed to survive better in tissue and thus be more effective than other stem cells. This is the first systematic review investigating the safety and efficacy of Muse cells for ischemic stroke. Three studies met the inclusion criteria: one RCT and two preclinical animal studies. In preclinical studies, Muse cells transplanted into the brains of mice after lacunar stroke differentiated into neuronal and oligodendrocyte cells. These cells integrated into the brain's circuits and improved functional recovery, with no signs of tumour formation or migration to other organs. In the second study, mice that received intravenous Muse cells similarly showed long-term functional improvements without tumorigenesis. In the human RCT, participants who received intravenous allogeneic Muse cells (n=25) experienced greater recovery at 12 and 52 weeks compared to the placebo group (n=10). By week 52, 40% of the treatment group achieved a modified Rankin Scale (mRS) score of ≤2, indicating slight or no disability,

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compared to 10% in the placebo group. The difference in mRS scores persisted over the 52-week follow-up, with no significant differences at baseline. These findings suggest that Muse cells could offer a safe and effective treatment for ischemic stroke. However, this review is limited to only three studies, including one RCT, revealing that Muse cell research is in its infancy. Further research, including RCTs, is required to understand their clinical potential better, hoping that they may eventually become a competitive treatment for ischemic stroke.

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ACTIVATION OF THE JAK2-STAT3 SIGNALING PATHWAY BY FGF4 AND ASCORBIC ACID ENHANCES DIRECT REPROGRAMMING INTO MATURE CARDIOMYOCYTES

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Direct reprogramming represents a transformative therapeutic approach to convert somatic cells, such as fibroblasts, into cardiomyocytes without transitioning through a pluripotent stem cell state. Despite this potential, the efficiency of direct reprogramming into fully functional cardiomyocytes remains suboptimal, often leading to partial conversion and immature cardiomyocyte stages. This study aims to overcome these limitations by employing growth factors, including Fibroblast Growth Factor (FGF4) and Ascorbic Acid (AA), in combination with key transcription factors (MGT; Mef2c, Gata4 Tbx5, and MGTMM; MGT, MESP1, and MYOCD) to promote the structural and functional maturation of induced cardiomyocytes (iCMs). Our investigation revealed that the combination of FGF4 and AA significantly enhances cardiomyocyte-specific markers, leading to organized sarcomere formation and improved cardiac ion channel functionality. Transcriptomic analysis further demonstrated the upregulation of essential signaling pathways, particularly JAK2-STAT3 and TGFB, which are integral to extracellular matrix (ECM) synthesis and cardiomyocyte maturation. Knockdown of JAK2-STAT3 signaling resulted in diminished expression of mature cardiomyocyte markers, confirming its pivotal role in iCM maturation. These findings suggest that activating the JAK2-STAT3 pathway through FGF4 and AA treatment enhances the direct reprogramming of fibroblasts into functionally mature cardiomyocytes. The development of this reprogramming strategy holds promise for novel drug screening methods and patient-specific regenerative therapies for cardiovascular diseases.

Funding Source: This research was supported by a grant(RS-2024-00331852) from Ministry of Food and Drug Safety in 2024.

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ENGINEERED HUMAN BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS EXPRESSING ACE2 AS A DECOY STRATEGY TO COUNTERACT SARS-COV-2 INFECTION

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Acute Respiratory Distress Syndrome (ARDS) remains a critical complication in ICU patients with COVID-19, driven by SARS-CoV-2 and its evolving variants, which continue to pose global health risks. SARS-CoV-2 utilizes the ACE2 receptor for cell entry, either through TMPRSS2 or clathrin-mediated pathways, with Omicron variants favoring the endosomal route. ACE2 not only facilitates viral entry but also plays a crucial role in modulating inflammatory responses, making it a promising therapeutic target. We hypothesize that human bone marrow-derived mesenchymal stromal cells (hMSCs) engineered to express ACE2 (hMSCACE2) can act as effective viral decoys, preventing viral entry while preserving endogenous ACE2 activity, thereby protecting lung function. Lentiviral transduction successfully induced ACE2 expression in hMSCs, confirmed by confocal microscopy, without

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compromising their therapeutic potential. In vitro, hMSCACE2 exhibited strong decoying capacity, reducing SARS-CoV-2 infection. In vivo studies in K18-hACE2 mice demonstrated that hMSCACE2 significantly reduced viral load, minimized lung injury, and improved clinical outcomes compared to controls. Histological analysis showed reduced inflammation and preservation of ACE2 expression in treated lungs. Proteomic analysis revealed that hMSCACE2 treatment modulated key molecular pathways, including suppression of inflammatory responses, mitigation of endoplasmic reticulum stress, and restoration of lipid metabolism. These findings highlight the protective effects of hMSCACE2 by disrupting viral replication and reducing lung tissue damage. Overall, hMSCACE2 offers a dual mechanism—acting as a viral decoy and supporting lung protection, making it a promising therapeutic strategy for COVID-19-associated ARDS.

Funding Source: This work was supported by the Canadian Institutes of Health Research (CIHR, OV3-170344, SBC-171482 and VS1-175560 to HZDEcoy).

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EFFICACY AND SAFETY OF MUSE CELLS IN HUMAN AND ANIMAL MODELS: A SYSTEMATIC REVIEW

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Muse cells are a subpopulation of mesenchymal stem cells (MSCs) with stress resistance. Unlike MSCs, Muse cells are pluripotent, forming cells from all three germ layers. They have a lower risk of tumor formation and immune rejection compared to embryonic and induced pluripotent stem cells and are thought to have superior regenerative capacity. This systematic review examined research from Medline(R), Cochrane, and OVID databases for primary research on Muse cells, identifying four human randomized controlled trials (RCTs). In an ischemic stroke RCT, 40% of participants treated with Muse cells achieved minimal or no disability by 52 weeks, compared to 10% in the placebo group. Muse cells were well-tolerated, with one case of unrelated status epilepticus. In an amyotrophic lateral sclerosis (ALS) Phase II trial, no significant functional improvements were observed, though some inflammation markers decreased. Phase I trials on spinal cord injury and myocardial infarction reported no serious adverse events. Given the limited human data, 23 animal studies were included, all reporting positive outcomes of Muse cell treatment without adverse effects. Conditions validated by two or more studies include myocardial infarction, spinal cord injury, and ischemic stroke. Muse cells reduced myocardial infarct size by 30–40% and improved cardiac function by 20–30%. In spinal cord injury, motor function improved by 60–80% over 6 to 20 weeks. In ischemic stroke, Muse cells improved motor function by 30–40%. None of the preclinical studies directly investigated ALS or aging-related conditions. This review suggests that while Muse cells appear safe for human use, their efficacy remains uncertain. However, success in human trials has aligned with conditions supported by animal models, as in ischemic stroke. Future RCTs should focus on areas with preclinical support, including ischemic stroke, spinal cord injury, and myocardial infarction. The ALS trial may indicate the need for alternative treatment protocols, which should be explored in preclinical models. Given their low tumorigenesis and immune rejection risks and regenerative potential, Muse cells should also be investigated in models of aging. Animal studies should prioritize protocols feasible for human trials to improve translation.

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PLURIPOTENT STEM CELL EXTRACELLULAR VESICLES FUNCTION IN CARDIOMYOCYTE PROTECTION TO HYPERGLYCEMIA AND PROMOTE CELL PROLIFERATION

Joshua Tompkins[1], Imtiaz Rony[1], Pratibha Mishra[1], Debbie Hussey[1], Elizabeth Lizhar[1], Angel Gu[2], Alireza Shokrani[1], Jingjing Ye[3], Chang-Yi Chen[3], Yin Wang[4], Zhao Wang[5], Fouad Kandeel[2], and Rama Natarajan[1]

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Virtually all diabetics are at heightened risk for cardiovascular disease (CVD), and this represents a substantially growing global health threat. New therapies are thus needed to mitigate these current and future risks, especially treatments which reduce inflammation among the CVD system and promote tissue regeneration. Extracellular vesicles (EVs) represent important players in this space, both for their natural communication functions, and as specialized nanoscale delivery systems. Harboring functional cargo (e.g. miRNAs, proteins), EVs are decorated with an array of ligands and receptors, which reflect their cell of origin, and often aid in targeting recipient cells. Those derived from several stem cell sources have demonstrated significant potential in tissue regeneration, and with their early developmental role, human pluripotent stem cell (hPSC) EVs may promote both increased cell proliferation, and immunosuppression. These EVs also contain pluripotency factors and miRNAs which promote pluripotent signaling, particularly the miR-302 family, and present conventional EV markers CD63 and CD81. On the other hand, others have shown that brief transgenic overexpression of core pluripotency transcription factors can extend lifespan in mice, provide cardioprotection, and improve beta-cell function in vivo. Here, we present evidence that hPSC EVs may mimic these effects and demonstrate that hPSC-EV treated cardiomyocytes are protected from high glucose-induced hypertrophy and cardiac toxicity, more so than CM-derived EVs treatments. To facilitate clinical translation, we discuss our pipeline for the large-scale purification of Good Manufacturing Practice GMP grade hPSC EVs, suggested release testing for future clinical utility, and provide pilot data indicating that hPSC EVs promote proliferation in both CMs, and human donor isolated islets.

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YAP ACTIVATION REVERSES EPITHELIAL CELL SENESCENCE CAUSED BY SOFT MICROENVIRONMENTS

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Senescence is a cellular response to various stress conditions, featured by irreversible cell cycle arrest, senescence-associated beta-galactosidase (SA-β-GAL) accumulation, and increased production of senescence-associated secretory phenotype (SASP). With the emerging role of cellular senescence in age-related pathologies, elimination or rejuvenation of senescent cells has shown promising effects in the treatment of such diseases. Here, we report a new type of senescence, termed softness-induced senescence, in epithelial cells cultured on a soft microenvironment. Soft substrates diminish integrin-mediated cell-to-ECM interaction, and the inhibition of integrin signaling is sufficient to induce epithelial cell senescence. Mechanistically, the loss of cell-to-ECM interaction leads to a decrease in the activity of YAP and, thereby, cellular senescence. Furthermore, restoring YAP activity in senescent cells can alleviate senescence phenotype, like the production of SASP, indicating its therapeutic potential for targeting age-related diseases. Our work has identified the soft microenvironment-induced YAP inactivation as a critical mechanism that drives epithelial cell senescence.

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TGFβ2-DRIVEN IRON DYSREGULATION AND FERROPTOSIS IN SALIVARY GLANDS TRIGGER HYPOSALIVATION UNDER POSTMENOPAUSAL CONDITIONS

Hyung-Sik Kim, Su-Jeong Oh, Ye Young Shin, Yunji Lee, Seong Hui Kim, Jeong Hyun Yu, Hee-Jeong Park, Ji-Su Ahn, Min-Jung Kang, and Yoojin Seo

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Xerostomia, also known as dry mouth, is a salivary gland (SG) dysfunction characterized by reduced saliva secretion. Despite the high incidence of dry mouth in postmenopausal women, the underlying mechanisms and potential therapeutic interventions are less understood. In this study, using ovariectomized (OVX) mouse models, we identify ferroptosis as a central mechanism driving postmenopausal SG dysfunction. In OVX-SGs, the TGFβ signaling pathway is enhanced due to aberrant TGFβ2 expression in SG mesenchymal cells. Interestingly, treatment with TGFβ2 reduces iron-storing ferritin levels, leading to lipid peroxidation and ferroptotic death in SG epithelial organoids (SGOs). TGFβ2 promotes autophagy-mediated ferritin degradation, known as ferritinophagy. A significant overexpression of the type III TGFβ receptor (TGFβRIII) is observed in OVX-SGs and TGFβ2-treated SGOs. Finally, the administration of the ferroptosis inhibitor, Liproxistatin-1 (Lip-1), improves saliva secretion in OVX mice. These findings suggest a critical link between TGFβ signaling, ferroptosis, and SG injury, offering new therapeutic avenues for treating postmenopausal xerostomia.

Funding Source: This study was supported by the National Research Foundation of Korea, funded by the Ministry of Science and ICT (RS-2023-00223591 and RS-2024-00340037).

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EXPLORING HUMAN UMBILICAL CORD LINING EPITHELIAL CELLS AS A SOURCE FOR TREATING SKIN WOUND DEFECTS

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Treatment of large wounds such as burns can be complex. Cultured epithelial autografts (CEAs), an option for treating large burns, is based on Rheinwald and Green's (R&G) techniques for culturing human epidermal keratinocytes (HEKs). The technique is well established for cultivating epithelial cells and stem cells from biopsies. Human umbilical cord lining epithelial cells (CLECs) hold promise as epidermal substitutes due to their similarities with HEKs. In addition, they are derived non-invasively from tissues typically regarded as medical waste. Thus, further investigation is warranted to determine if CLECs can be used as alternative for HEKs in resurfacing large wounds. To investigate this, we compared (1) cell culturing characteristics of CLECs to that of HEKs by colony forming efficiency assays, growth potential and cumulative population doubling assessments; (2) characterised their gene and protein expressions; and (3) assessed the in vitro and in vivo functions using de-epidermalised dermis organotypic culture and nude mice flap model. In our lab, CLECs can be isolated from umbilical cords and grown in R&G's culture system all the time. However, cell yield varied from donor to donor. CLECs expressed some similar genes and proteins as HEKs in tissues and culture. When compared to HEKs, the initial growth of CLECs was comparable, but declined in later passages. Addition of small molecules, Rho-kinase inhibitors (ROCK inhibitor) Y-27632 improved the number colony forming cells within each CLEC passage but not from passage to passage. In vitro and in vivo assays demonstrated that CLECs have partial stratification, indicative of some barrier function. In conclusion, early passages of CLECs in R&G's system have sufficient growth potential to expand in culture. CLECs may be a suitable alternative for HEKs in autologous resurfacing of skin for rare diseases such as severe congenital aplasia cutis.

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Funding Source: This project was funded by National Medical Research Council, and the Lee Seng Teik and Lee Hoo Leng Distinguished Professorship in Plastic Surgery and Regenerative Medicine at Duke-NUS Medical School.

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DECIPHERING INTERMEDIATE EPIGENETIC STAGES OF DIRECT LINEAGE REPROGRAMMING INTO DOPAMINERGIC NEURONS FOR PARKINSON'S DISEASE

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Direct lineage reprogramming into dopaminergic (DA) neurons holds great promise for the more effective production of DA neurons, offering potential therapeutic benefits for conditions such as Parkinson's Disease. However, the reprogramming pathway for fully reprogrammed DA neurons remains largely unclear, resulting in immature and dead-end states with low efficiency. In this study, using single-cell RNA sequencing, we analyze the trajectory of reprogramming DA neurons at multiple time points, identifying a continuous pathway for their reprogramming. We identified intermediate cell populations crucial for resetting host cell fate during early DA neuronal reprogramming. Further longitudinal dissection revealed two distinct trajectories: one leading to successful reprogramming and the other to a dead end. Notably, we have identified Arid4b, a histone modifier, as a crucial regulator at this branch point, essential for the successful trajectory and acquisition of mature dopaminergic neuronal identity. Consistently, overexpressing Arid4b in the DA neuronal reprogramming process increases the yield of iDA neurons and effectively reverses the disease phenotypes observed in the PD mouse brain. Thus, gaining insights into the cellular trajectory holds significant importance for devising regenerative medicine strategies, particularly in the context of addressing neurodegenerative disorders like Parkinson's Disease.

Funding Source: This work was supported by 2021M3E5E5096464, RS-2024-00433755, and NRF-2022R1A6A1A03053343.

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NOVEL MATURATION FACTOR ENHANCES STRUCTURAL AND FUNCTIONAL MATURITY OF CARDIAC ORGANOIDS

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Korea University, South Korea

Human induced pluripotent stem cells (hiPSCs) can differentiate into cardiomyocytes, but these often display an immature phenotype despite high differentiation efficiency (~90%). Given that 13.7% of drugs are withdrawn due to cardiotoxicity and the limitations of animal models in predicting human responses, there is a growing need for human-derived cardiac organoids to improve drug toxicity assessments. This study aimed to enhance the maturation of cardiac organoids using a 3D culture model and a novel maturation factor. Comparative analysis revealed that these organoids exhibit molecular, biochemical, and physiological characteristics closely resembling human heart tissue, with cardiomyocytes and vascular cells constituting 50.7% and 21.7% of the organoids, respectively. The maturation factor was found to promote structural (organized sarcomeres, mature T-tubules), functional (adjusted beating frequency, enhanced ion channel expression), and metabolic maturation (increased mitochondrial density and ATP content). Additionally, it upregulated key signaling pathways (extracellular matrix-integrin, focal adhesion, LEFTY-PITX2) and mitophagy markers. These results suggest that cardiac organoids are a robust tool for evaluating drug safety and efficacy, providing more accurate predictions of human cardiac responses, facilitating early detection of cardiotoxicity, and potentially reducing failure rates and costs in drug development.

Funding Source: This research was supported by a grant(RS-2024-00331852) from Ministry of Food and Drug Safety in 2024.

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OLD MITOCHONDRIA INDUCE NICHE-INDEPENDENCE OF STEM CELLS BY PROMOTING NICHE RENEWAL

Daniel Borshagovski[1], Simon Andersson[2], Hien Bui[2], Emilia Kuuluvainen[2], Swetha Gopalakrishnan[2], Ella Salminen[2], Eija Jokitalo[2], Ville Hietakangas[2], and Pekka Katajisto[1,2]

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Cellular metabolism is emerging as a potent regulator of cell fate, raising the possibility that the recently discovered metabolic heterogeneity between newly synthesized and chronologically old organelles may impact stem cell fate in mammalian tissues. The small intestine is maintained by actively cycling intestinal stem cells (ISCs) that give rise to metabolically distinct progeny, including their Paneth cell niche. Here, we find that a subset of ISCs is enriched for old mitochondria (ISC mito-O). ISCs mito-O form organoids niche-independently, owing to their ability to recreate the Paneth cell niche, despite lack of reserve stem cell characteristics. Mechanistically, mitochondria in ISCs mito-O run a more active Tricarboxylic Acid Cycle, which generates intermediates for anabolic activity and co-factors for epigenetic modifiers. High mitochondrial production of alpha-ketoglutarate (aKG) in ISCs mito-O drives ten-eleven translocation (Tet) methylcytosine dioxygenase-mediated epigenetic changes that are required for differentiation towards the Paneth cell fate. Finally, aKG supplementation in vivo promotes Paneth cell turnover leading to niche renewal, which promotes recovery from chemotherapy-induced damage in aged animals. Our results reveal a transcriptionally indistinguishable subpopulation of intestinal stem cells whose old mitochondria metabolically regulate cell fate choices, and provide proof-of-principle for metabolically promoted replacement of specific aged cell types in vivo.

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IMPORTANCE OF HIGH-DENSITY MICROELECTRODE ARRAYS FOR RECORDING MULTI-SCALE EXTRACELLULAR POTENTIAL AND LABEL-FREE CHARACTERIZATION OF NETWORK DYNAMICS IN IPSC-DERIVED NEURONS

Zhuoliang (Ed) Li, Elvira Guella, and Marie Obien

MaxWell Biosystems AG, Switzerland

Advances in the development of microelectrode arrays (MEAs) for in-vitro electrophysiological recordings have enabled the characterization of multi-scale behavior in neuronal networks, ranging from subcellular level to network dynamics. Such devices are fundamental for studying the phenotype of neurological disorders and for drug discovery, providing unique insights into the complexity of neuronal networks. Electrode density, spacing, and size influence the signal quality, noise level, and sensitivity. To properly characterize the full behavior of neuronal networks, MEAs must combine single-cell and subcellular resolution with high-throughput assays, while maintaining sensitivity to small extracellular action potentials to describe the full range of network dynamics. In this study, the MaxOne and MaxTwo high-density (HD) MEA systems (MaxWell Biosystems, Switzerland) were used to record activity from induced pluripotent stem cell derived neurons, demonstrating the advantages of having 26,400 electrodes per well, which is key to increasing the statistical power of data collected longitudinally. HD-MEA recordings were compared with simulated low-density recordings, in which larger, low-density electrodes were mimicked by clustering adjacent electrodes on HD-MEAs. Additionally, the AxonTracking Assay, an automated tool for recording and analyzing individual axonal arbors from many neurons in parallel, was used to characterize the function and axonal structure of recorded cultures. Results indicated that higher density and smaller electrodes provided greater sensitivity, enabling the detection of smaller spikes, and covering the full spectrum of network behavior. The high-resolution analysis of network dynamics, coupled with the AxonTracking Assay's subcellular insights, provide powerful insights into drug screening and disease modelling.

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Funding Source: NEUREKA project, GA 863245, within the H2020 Framework Program of the European Commission. - HyVIS project, GA 964468, within the H2020 Framework Program of the European Commission.

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IN VITRO MODELING AND MAINTENANCE INTEGRITY OF BLOOD-BRAIN BARRIER IN ISCHEMIC STROKE

Youn Joo Moon

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The Blood–Brain Barrier (BBB) is a highly selective physiologic barrier essential for the central nervous system. Brain endothelial cells are the main components of the BBB and playing an important role in the maintenance of BBB integrity. A major hallmark of ischemic stroke is the breakdown of BBB, becoming increased BBB permeability, leading to neuroinflammaion by infiltrated immune cells. We constructed in vitro BBB models using triple co-culture system, composed of human astrocytes, pericytes and endothelial cells. BBB permeability were assessed by transendothelial electrical resistance (TEER) measurements. We tested the therapeutic effects of miR-17-5p and miRNA-93-5p antagonitic sequences whether it can facilitate the maintenance of BBB integrity after oxygen glucose deprivation (OGD) because our previous study has demonstrated that miR-17-5p and miRNA-93-5p antagonists protected endothelial cells from in vitro ischemic stroke models. Under OGD conditions, the viability of endothelial cells was significantly decreased and increased BBB permeability. The silencing of derived microRNAs effectively protected BBB integrity by preventing endothelial cells injury. Our findings indicated that miR-17-5p and miRNA-93-5p antagonists preserved the BBB integrity after ischemic stroke by modulating endothelial cells survival and that derived microRNAs may be potential therapeutic target for ischemic stroke induced BBB disruption.

THURSDAY, 12 DECEMBER 2024

5:15 PM – 6:00 PM
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REMUSCULARISATION OF THE CHRONICALLY INFARCTED RAT HEART USING SPECIES-MATCHED OR HUMAN PLURIPOTENT STEM CELL-DERIVED CELL THERAPY CAN RESTORE CARDIAC FUNCTION

Lay Ping Ong[1], Silvia Marchiano[2], Jonathan Lee[2], Elaheh Karbassi[2], Bonnie Chen[1], Semih Bayraktar[1], Leslie Blakely[2], Catherine Wilson[1], Alessandro Bertero[3], Charles Murry[2], and Sanjay Sinha[1]

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Ischaemic heart failure is due to a permanent loss of cardiomyocytes. Human pluripotent stem cell (hPSC)-derived cardiomyocytes alone can successfully remuscularize the heart when introduced shortly after a heart attack but carry no benefit in chronic heart failure. The chronically infarcted heart is adversely remodelled with a depleted blood supply. We reasoned that the hostile environment of chronically infarcted heart posed a greater engraftment challenge to hPSC-cardiomyocytes. Previously, we showed that combining hPSC-cardiomyocytes & hPSC-epicardium improved cardiac engraftment, neovascularization and cardiac function when delivered acutely after a heart attack. We posited that hPSC-epicardium can recapitulate its beneficial effects in chronic heart failure. Here, we found that adding hPSC-epicardium with hPSC-cardiomyocytes (combination cellular therapy, Epi+CM) stabilised the cardiac function of the chronically infarcted rat hearts. When we transplanted neonatal rat cardiomyocytes (NRVM)—a species matched and pro-regenerative cellular therapy, we altered the heart failure trajectory with increased ventricular function, voluntary exercise, and decreased BNP (B-type natriuretic peptide)—a biomarker of heart failure. Improved cardiac function in both groups were underpinned by robust cardiac grafts with mature myofibrils, improved graft-host electrical connectivity and host-derived neovascularization. Strikingly, the species-matched cardiac grafts displayed

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tissue-level organisation similar to the host myocardium. We conclude that remuscularization with partial restoration of the cardiac function of the chronically failing rat heart is possible. Species-mismatch likely masked some benefits of cardiomyocyte therapy, and that remuscularization of chronic human infarcts with human cardiomyocytes and epicardial cells may improve mechanical function.

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MECHANISMS OF ABNORMAL MUSCLE TISSUE FIBROPROLIFERATION IN FIBRODYSPLASIA OSSIFICANS PROGRESSIVA

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Fibroproliferation after muscle tissue damage is one of the safety systems for muscle tissue maintenance. However, excessive fibroproliferation leads to muscle fibrosis, fat infiltration, and heterotopic occification. Fibrodysplasia ossificans progressiva (FOP) is a rare genetic disorder characterized by progressive heterotopic ossification (HO) in soft tissues initiated by numerous proliferation-activated mesenchymal stromal cells (MSCs). However, the mechanisms underlying fibroproliferation remain unclear. In this study, we investigated the proliferation of MSCs derived from FOP patient-derived induced pluripotent stem cells (FOP-iPSCs) to elucidate the mechanisms underlying fibroproliferation. We found that bone morphogenetic protein (BMP)-9 mediated enhanced proliferation through aberrant activation of the transforming growth factor (TGF)-β signaling pathway in MSCs derived from FOP-iPSCs. In FOP model mice, elevated BMP-9 levels correlated with increased phosphorylation of Smad2/3 and increased cellular proliferation in affected tissues, while systemic BMP-9 neutralization and knockout attenuated flares and HO. Thus, BMP-9 aberrantly transduces TGF-β signaling and induces fibroproliferation and initiates HO. This study provides novel insights for the development of future FOP therapies.

Funding Source: iPS Cell Research Fund, AMED, JSPS, NSFC.

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PREDICTING STEM CELL BEHAVIOR IN MUSCULOSKELETAL TISSUE ENGINEERING AND REGENERATION USING A KNOWLEDGE GRAPH APPROACH: EXPLORING HUMAN IPSCS AND MOUSE C2C12 MODELS

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Musculoskeletal tissue regeneration relies on integrating complex biological, biochemical, and mechanical data to predict stem cell behavior and improve therapeutic outcomes. The musculoskeletal system, comprising bone, cartilage, muscle, tendon, ligament, and joint, requires precise approaches for effective tissue engineering. Current methods face limitations due to the scale of biological data and the need for accurate predictive models, making computational tools essential in regenerative medicine. We propose a knowledge graph (KG)-driven approach to organize and connect data from multiple sources, modeling relationships between biological entities like genes, proteins, signaling pathways, tissues, and environmental factors. Our KG, leveraging data from over 330,000 research papers, predicts stem cell behavior, providing a comprehensive framework for musculoskeletal tissue engineering. Tissue engineering hinges on four dimensions: stem cells (iPSCs, adult stem cells), scaffolds, inductive cues, and bioreactors, which are essential for guiding stem cell differentiation and tissue formation. Two case studies illustrate this approach: i) Prediction of differentiation in iPSCs (human) and C2C12 cells (mouse) for muscle tissue regeneration, comparing the effects of Human Serum Albumin (HSA) vs. Fetal Serum Albumin (FSA), which show varied influences on stem cell differentiation. ii) Semantic reasoning to predict stem cell therapy outcomes for osteoporosis, osteoarthritis, and sarcopenia, identifying pathways that enhance treatment strategies. This study highlights the integration of knowledge graphs and bioreactor data to optimize tissue engineering and stem cell therapies, especially for aging populations. These strategies could significantly improve patient outcomes and reduce the burden of musculoskeletal diseases.

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INTEGRATING ACCELERATED AGEING IN HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED GLIAL CELLS TO MODEL NEURODEGENERATIVE DISEASES

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Ageing is the strongest risk factor for most neurodegenerative diseases (NDD), and the main driver of increasing NDD prevalence due to a globally ageing population. Animal models have been widely used for NDD studies, although they do not naturally develop neurodegenerative pathologies linked to human NDD and differ inherently in their biology from humans. Human induced pluripotent stem cells (iPSCs) thus emerge as a unique experimental model that captures the genetic background of any donor while retaining the capabilities to self-renew and differentiate into any cell type in vitro. Yet, the lack of ageing-driven phenotypes in iPSC-derived cells presents a direct contradiction to recapitulating cellular ageing in studying age-related NDD. In this study, we will address the limitation by integrating accelerated ageing in cellular NDD models derived from patients with familial mutations. We will do so by employing CRISPR-Cas9-mediated gene silencing against individual targets which have been implicated in a range of biological processes that regulate ageing in vivo before differentiating these iPSCs into glial cells for ageing-related phenotype measurements and assays for functional decline. We hypothesise that accelerated ageing in vitro exacerbates cellular pathologies of patient cells, leading to more accurate disease modelling and providing opportunities to target ageing as a viable strategy to mitigate NDD pathogenesis.

Funding Source: Career Development Fund by the Agency for Science, Technology and Research in Singapore.

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EFFICACY AND SAFETY OF INTRACAVERNOUS ADMINISTRATION OF PLATELET-RICH PLASMA IN THE TREATMENT OF ERECTILE DYSFUNCTION IN KAZAKH POPULATION: RESULTS OF A SIX-MONTH FOLLOW-UP

Rinat Nugumanov[1], Yerbol Iskakov[1], Rustam Omarbayev[1], Yerkebulan Yermaganbetov[1], Timur Turgunbayev[1], Damir Akhmetkaliyev[1], Alexei Suchshenko[2], and Abai Baigenzhin[1]

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Erectile dysfunction (ED) is a significant problem worldwide, particularly in Kazakhstan. Out of the 1,550 men surveyed, aged 21 to 79 years, 784 (52.3%) were diagnosed with ED. A promising new strategy for the treatment of ED has emerged—intracavernous injection of autologous platelet-rich plasma (PRP). The aim is to assess the safety and effectiveness of intracavernosal PRP injections in Kazakh patients with moderate to severe ED. The study included 80 male participants aged 36 to 65 with moderate to severe erectile dysfunction (ED) nonresponsive to traditional treatments. The participants were divided into four groups: Group 1: continued conservative treatment with phosphodiesterase type 5 inhibitors (PDE5i). Group 2: received intracavernous injections of 4 mL of PRP. Group 3: received 6 mL of intracavernous PRP. Group 4: received a combination of 4 mL PRP and calcium chloride solution injected intracavernosally. In all groups except the control group, there was an improvement in the IIEF-EF and EHS scores after one and a half months, with a peak in the third month. However, these improvements were not statistically significant compared to the baseline values. In the sixth month, there was a negative trend in improvement. When comparing the effectiveness of different groups based on the IIEF-EF and EHS scores, there was a slight improvement in the group receiving 6 mL compared with the group receiving 4 mL. Also, the calcium chloride group showed slightly higher rates compared to the other two groups. However, in this study, there were no statistically significant differences between the two groups. PRP therapy appeared to be generally

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safe, with only minor side effects reported, such as short-term pain at the injection site, mild redness and swelling that disappeared after a few hours. No serious complications, like infection or severe allergic reactions, were observed. PRP treatment for moderate to severe erectile dysfunction (ED) appears to be safe, but its effectiveness is limited. Further research and development are needed to standardize the application of PRP in clinical practice. Prospective and randomized controlled trials with placebo control are needed to evaluate the efficacy of PRP for improving erectile function with greater certainty.

Funding Source: This research has been funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan (Grant No. AP23488303).

Clinical Trial ID: Local ethical committee approval was obtained (Protocol of the Local ethical committee No. 092/CI-86 dated October 27, 2023).

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MODULATING ADULT DUX4 EXPRESSION USING EMBRYONIC REGULATORS

Arnab Ray and Xue Shifeng

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Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant genetic disease affecting around a million people worldwide. FSHD is characterized by initial weakness in the muscles around the face, shoulder and upper arm regions, before progressing to the lower body. 20% of the patients require wheelchair assistance by the age of 50. Currently there are no approved therapies for FSHD. FSHD is caused by the aberrant expression of double homeobox 4(DUX4) in the muscle cells of the affected individuals. DUX4 is a primate-specific gene, however it has many similarities to Dux, the rodent ortholog. Physiologically both DUX4 and DUX play important roles in early embryogenesis, at the time of zygotic genome activation. Previously, several Dux repressors have been identified by researchers studying 2-cell-like cells (2CLCs), a rare totipotent cell population found in mouse embryonic stem cells (mESCs) that resemble the 2-cell embryo. In this project, we aim to identify repressors that can downregulate the expression of DUX4 in FSHD patients. We hypothesize that regulators that repress Dux will also repress DUX4. By performing an overexpression screen in myotubes derived from differentiating immortalized FSHD patient myoblasts, we identified regulators that were able to reduce DUX4 expression. Further studies revealed that overexpression of these regulators led to less apoptosis and increased differentiation in FSHD myotubes. Ongoing experiments will dissect the mechanisms by which these repressors interact with DUX4. Results of this study can provide new targets to be used for developing potential therapies for FSHD. It also provides a broader landscape of the regulatory mechanisms controlling DUX4 expression in both embryonic development and somatic tissues.

Funding Source: This work was support by the Ministry of Education, Singapore (T2EP30122-0015) and NUS.

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MAGNETIC NANOHYDROXYAPATITE AND OSTEOGENIC GROWTH PEPTIDE-INCORPORATED SILK FIBROIN-BASED HYDROGEL REGULATES OSTEOGENIC DIFFERENTIATION ON CPDLSCS

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Considerations of scaffold properties to resemble donor bone tissue architecture and enhance the osteoinduction are still an issue and, thus, a non-invasive injectable approach is proposed. Silk fibroin (SF) is a natural polymer obtained from silk-producing arthropods and can be fabricated into a potent injectable hydrogel. In addition, magnetic nanohydroxyapatite (MHAp) and osteogenic growth peptide (OGP) have been reported to have potential in improving bone regeneration and could be incorporated

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into a hydrogel. The knowledge of these components incorporated into SF-based hydrogel is still limited, especially for veterinary practice. Therefore, this study aimed to determine the osteogenic differentiation potential of MHAp and OGP-incorporated SF-based hydrogel in canine periodontal ligament stem cells (cPDLSCs). The synthesized MHAP was characterized and found that it has superparamagnetic property and is in nanoscale. At selected concentrations, the non-cytotoxic MHAp and OGP were able to be incorporated into SF-based hydrogel. Interestingly, the addition of MHAp into the hydrogel affected the morphology behavior of cPDLSCs into aggregated colonies. Additionally, the combination of both MHAp and OGP in the hydrogel revealed remarkable potential for osteogenic differentiation of cPDLSCs compared with combination of single component or its pristine hydrogel by the increased of ALP activity, gene expression, and biomineralization. Finally, the composite hydrogel presented promising potential for treating bone defects in veterinary practice. Next, dissemination of mechanism of composite hydrogel in enhancing osteogenic differentiation of cPDLSCs is necessary to perform, especially with proteomics analysis, to be further used for clinical applications.

Funding Source: SDP and TT are supported by the Second Century Fund (C2F), Chulalongkorn University for Doctoral Scholarship.

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NANOHYDROXYAPATITE HYDROGEL FOR PROMOTING OSTEOGENIC DIFFERENTIATION OF CANINE PERIODONTAL LIGAMENT STEM CELLS FOR ALVEOLAR BONE REGENERATION IN VETERINARY MEDICINE

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Canine periodontal disease poses significant challenges in veterinary dentistry. This disease often leads to alveolar bone loss and compromised oral health in dogs. Developing effective biomaterials for alveolar bone regeneration is crucial in veterinary dentistry. Currently, the development of composite biomaterial scaffolds has gained attention in bone tissue engineering thanks to their potential for facilitating bone repair and regeneration. Among this, nanohydroxyapatite (nHA) injectable hydrogels are off attention due to their potential to promote bone repair and regeneration, offering a promising approach for treating alveolar bone loss in canine periodontal disease. However, studies exploring the application of injectable nHA hydrogel for osteogenic differentiation in dog models are limited. In this study, the properties of nHA injectable hydrogel has been extensively explored to be an alternative strategy to facilitate bone regeneration. This study investigated the cytocompatibility and efficacy of a nHA injectable hydrogel in promoting osteogenic differentiation of canine periodontal ligament stem cells (cPDLSCs) in vitro. The nHA hydrogel was employed to investigate with cPDLSCs in this study and provided valuable insights into its effectiveness and safety prior to clinical use. Our findings indicate that the nHA injectable hydrogel is cytocompatible by sustaining the viability and proliferation of cPDLSCs. Additionally, the nHA injectable hydrogel enhances the osteogenic differentiation of cPDLSCs, as evidenced by increased expression of osteogenic markers, ALP activity, and mineralization. These findings contribute to the field of regenerative medicine by demonstrating nHA injectable hydrogels may serve as a promising biomaterial for alveolar bone regeneration through canine stem cell platforms. However, further research about reliable mechanisms should be evaluated to find its clinical feasibility for alveolar bone regeneration in veterinary clinical setting.

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IQGAP3 REGULATES STEM CELL ACTIVITIES TO REPAIR DAMAGED STOMICH VIA MYC-PATHWAY

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Adult stem cells repair damaged tissue through molecular pathways similar to carcinogenesis, suggesting that dysregulation of the tissue repair process may initiate early carcinogenesis. We previously identified the IQGAP3-expressing rapidly proliferating stem cells in the isthmus. Isthmus stem cells mainly contribute to maintaining homeostasis. Following tissue injury, however, we observed upregulation of stem cell markers, including IQGAP3, and oncogenic Myc pathways, especially in the fully differentiated chief cell population, implicating the contribution of IQGAP3 in tissue repair. To investigate the role of IQGAP3 in tissue repair, we generated a mouse model that conditionally knocks out (cKO) IQGAP3 in the Pgc-expressing cells in the stomach. We utilized High-dose tamoxifen (HDT) treatment to induce tissue damage and IQGAP3 cKO. IQGAP3-depleted mice showed reduced cell proliferation and chief cell population one month after damage. Transcriptomic analysis revealed suppressed Myc-related pathways and stem cell activity in IQGAP3-depleted stomachs, suggesting that IQGAP3 regulates tissue regeneration through the Myc pathway. To explore whether the Myc pathway is crucial for stem cell activity in the stomach, we performed organoid studies and found that a Myc inhibitor suppressed organoid formation and growth. We also established IQGAP3 cKO in the oncogenic KrasG12D-induced metaplasia mouse model to assess the role of IQGAP3 in carcinogenesis. Depletion of IQGAP3 expression in KrasG12D-induced metaplasia mice revealed impaired metaplastic gland formation with a lower number of CD44- and Myc-expressing cells. Our findings showed that IQGAP3 contributes to the stem cell activity via Myc-pathway for stomach regeneration and the formation of pre-cancerous metaplasia.

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ORGANOID ZOO-OPENING THE NEW HORIZON IN ANIMAL SCIENCE

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Organoid technology has revolutionized biomedical research by providing highly reliable in vitro human models. However, the use of organoids in animal science has not been fully explored to understand biodiversity and animal health, of which importance has been well appreciated during the COVID pandemic. The small intestinal organoids (sIOs), derived from adult intestinal stem cells, have shown robust growth and long-term expansion across a wide range of species, establishing them as a versatile platform for comparative biology in mammalian species. To explore the full potential of sIO, we successfully established sIOs from 25 animal species, including rodents, farm animals, wild species, and non-human primates. These sIOs provide continuously expanding, untransformed primary cell sources for each species. Fluorescent imaging confirmed the presence of both stem cells and differentiated cell types in all sIO cultures. Moreover, single-cell RNA sequencing revealed a broad range of cell types, highlighting evolutionarily conserved stem cell pools (e.g. revival stem cells and crypt base columnar cells) and differentiation trajectories to absorptive and secretory lineages. Notably, we observed species-specific traits. For instance, sIOs from spiny mouse exhibited enhanced regenerative capacity compared to a standard mouse, driven by conserved regeneration signaling pathways. Meanwhile, ferret sIOs demonstrated a high susceptibility to both human and

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avian influenza viruses. These results showcase the potential of organoid technology not only to deepen our understanding of animal biology but also to offer a novel platform for studying the biodiversity of various species, including non-experimental, wild, and rare animals.

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ORGAN-SPECIFIC EXPRESSION DATA STEERS CHOICE OF LAMININ FOR IN VITRO STUDIES IN BIOLOGICALLY RELEVANT DRUG DISCOVERY AND DEVELOPMENT PLATFORMS

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The Drug Discovery and Development (DDD) process incorporates multiple cell culture platforms to assess the safety and efficacy of lead compounds against a target for an indication. In recent years, with the advancement of stem cell research protocols, there has been shift in focus from 2D cell culture techniques to more complex patient-derived models. Human pluripotent stem cells (hPSCs) require an extracellular matrix (ECM) for maintenance, expansion, and differentiation; laminins, a large ECM protein family enriched within basement membranes of epithelial and endothelial tissues, are an essential part of stem cell niches. While laminins are increasingly implemented in hPSC research, standardised protocols using a biologically relevant ECM in DDD platforms have not been developed. Thus, we assessed protein and gene expression patterns of laminin isoforms in four distinct tissues most commonly used in DDD: the heart, liver, kidney and central nervous system (in vivo), before translation to proliferation and expression experiments in specified cell-types on laminin substrates (in vitro). We found the expression pattern is largely tissue- and cell type specific, for example, in the kidney. We also show that providing the relevant laminin isoform in vitro leads to increased standardization and functionality. For example, when using laminin as a substrate in culture, 43-times more dopaminergic neurons and > 80% functional cardiovascular progenitor cells (vs > 50% with competitor substrates), were observed. We also demonstrate how Biolaminins can be implicated in downstream microfluidic platforms to recreate the in vivo microenvironment when flow is introduced. Taken together, we demonstrate tissue- and cell-specific expression of laminin isoforms. In vivo expression data can guide scientists to recapitulate the ECM microenvironment of a given cell or tissue to promote optimal in vitro conditions—an important consideration as we aim to create more biologically-relevant cell-based models to move away from animal models. By doing so, current limitations relating to differentiation, reproducibility, and maturity of PSC-derived cells can be addressed in more sophisticated DDD applications, such as organ-on-a-chip platforms.

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COMBINATION OF HYPOXIA AND LIPOPLYSACCHARIDE INCREASE VIABILITY AND PROLIFERATION OF HUMAN UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS

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Human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) have been increasingly investigated for their therapeutic effect in various diseases. However, transplanted MSCs often encounter a hostile microenvironment characterized by inflammation and hypoxia leading to poor survival and limited engraftment. The aims of this study were to determine the effect of hypoxia and lipopolysaccharide (LPS) treatment on the viability and proliferation rate of hUC-MSCs.

Methods: MSCs were isolated from Wharton's jelly of human umbilical cord tissue using the explant method and cultured under hypoxic conditions induced by cobalt chloride (CoCl2) in combination with 10 ng/mL LPS supplementation for 24 h. Cell viability was examined by the Cell Counting Kit-8 (CCK-8) assay and proliferation was measured using the wound scratch migration assay and population doubling time.

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Results: While hypoxia or LPS treatment did not change the viability of hUC-MSCs, the combination of hypoxia and LPS administration increased cellular viability compared to control group. Similarly, hUC-MSCs cultured under hypoxic and LPS treatment demonstrated higher migration rate and required shorter time to double the cell population than those of hUC-MSCs cultured normoxic and without the addition of LPS. In conclusion, combination of hypoxia and LPS treatment enhanced the viability and proliferation of hUC-MSC. These results suggest that hypoxic condition and the LPS priming may modulate the therapeutic capacity of hUC-MSCs.

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EXPLORING THE EFFECT OF LIPID DYSREGULATION AND NEUROINFLAMMATION IN ALS ASTROCYTES

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Astrocytes, the most abundant glial cells in the CNS, play critical roles in maintaining neuronal health and brain homeostasis. Despite their critical roles, they can also contribute to neurotoxicity through their secretory activities, including the release of pro-inflammatory cytokines linked to neuronal death. Recent research has highlighted lipid dysregulation within astrocytes as a crucial element in neurodegenerative diseases such as Alzheimer's, Parkinson's, and Amyotrophic Lateral Sclerosis (ALS). In this context, we explored lipid metabolic defects in spinal astrocytes derived from ALS induced pluripotent stem cells (iPSCs). Our study advanced existing methods, reducing the differentiation time of hiPSCs into spinal astrocytes to 30 days. Lipidomic analysis uncovered an increase in omega-6 fatty acids, especially arachidonic acid (AA). AA and its pro-inflammatory metabolite, prostaglandin E2 (PGE2), were found to correlate with increased motor neuron (MN) death. These findings elucidate the connections between neuroinflammation and lipid dysregulation in ALS and suggest how astrocyte-driven lipid imbalances may accelerate disease progression. This study enhances our understanding of astrocyte roles in neurodegeneration, presenting potential biomarkers and targets for ALS treatment.

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RESETTING STEM CELLS TO THE EARLY DEVELOPMENT STAGES WITH ENGINEERED TRANSCRIPTION FACTORS

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Human pluripotency with expanded potential can be achieved using chemically defined medium, enabling them to generate both embryonic and extraembryonic tissues, advancing developmental potential and genome engineering. However, currently, human stem cells representing pre-implantation stages face challenges such as genome instability, lack of culture consensus across laboratories, dependency on harsh chemicals and cell batch variations. Blastoid and embryo models based on these cells sometimes fail to capture the true germ layer composition and pause at early developmental stages. To address these challenges, we aim to develop a genetic approach to reset somatic cells or primed pluripotent stem cells to a pre-implantation pluripotent state. We first characterized and further engineered reprogramming transcription factors to boost their efficiency and reduce their genetic payload. We next established a fast and efficient feeder-free system to convert human fibroblasts into induced expanded potential stem cells using engineered transcription factors. Lastly, we tested a panel of factor cocktails to revert primed human pluripotent cells to a naïve pluripotent state. A fast method of generating stem cells with enhanced utility and developmental potential holds great promise for improving embryo modeling, genomic medicine, species conservation, and cell product development.

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INVESTIGATING THE ROLE OF TEASHIRT 3 IN HUMAN PANCREATIC ENDOCRINE SPECIFICATION

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Efforts to generate in-vitro insulin-producing β cells from human pluripotent stem cells (hPSCs) for disease modeling and cell replacement therapy in diabetes have been intensive, building on insights largely derived from studies in mice and other vertebrates. These studies have greatly expanded our understanding of the gene regulatory networks involved in pancreas development. However, fundamental differences between human and mouse pancreatic development have hindered the efficient production of functional human β cells in vitro, due to gaps in our understanding of the human-specific gene regulatory networks governing pancreatic cell specialization. To bridge this gap, we developed a comparative bioinformatics pipeline that integrates human in vitro and in vivo gene expression data, along with ChIP-seq and epigenomic data (H3K27ac at active enhancers), to identify potential effectors of Pancreatic and Duodenal Homeobox 1 (PDX1), a key transcription factor for pancreatic organogenesis. Loss of PDX1 leads to pancreatic agenesis, and heterozygous mutations are linked to β cell dysfunction. Through this pipeline, we identified teashirt zinc finger homeobox 3 (TSHZ3) as a potential downstream target of PDX1 regulation. Using TSHZ3-mutant human embryonic stem cells (hESCs), we explored the role of TSHZ3 in endocrine and pancreatic lineage commitment through directed differentiation studies.

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VHL DEFICIENT HUMAN KIDNEY ORGANOID TO MODEL CLEAR CELL RENAL CELL CARCINOMA

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The inactivation of Von Hippel-Lindau (VHL) tumor suppressor gene, found in approximately 90% of cases, was identified as the primary driver event of clear cell renal cell carcinoma (ccRCC) development. Existing genetic engineered mouse models have failed to fully capture the distinctive features of ccRCC, and understanding VHL mutation in comparable human cells has been limited. Newly developed 3D human kidney organoid comprises of segmentally patterned nephron structures, interstitium and vascular network, serving as a novel research model with higher physiological relevance. In this study, we developed a VHL knockout human kidney organoid model based on CRISPR/Cas9-engineered human embryonic stem cells. The absence of VHL triggered ‘clear cell-like’ phenotype in kidney tubular epithelium, a defining trait of ccRCC in clinics, and recapitulated key metabolism reprogramming observed in patients, including significant lipid and glucose metabolic alterations. Long-term culture of VHL deficient kidney organoids demonstrated increasing proliferation potential. This model offers an innovative platform for ccRCC research and treatment development.

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SPATIOTEMPORAL TRANSCRIPTOMIC ATLAS OF PIG-HUMAN HEART XENOGRAFTS REVEALS MIDKINE-MEDIATED VASCULARIZATION IN CHRONIC MYOCARDIAL INFARCTION MODELS

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Stem cell-based therapy has demonstrated potential for regenerating infarcted heart tissue, but the molecular dynamics and cellular fate of transplanted cells in the myocardium are still largely unknown. Our previous research showed that non-contractile human cardiovascular progenitors (CVPs), derived from pluripotent stem cells, successfully engrafted and notably improved the left ventricular ejection fraction when transplanted into pig hearts with myocardial infarction. In this study, we transplanted human cardiovascular progenitors (CVPs) into pig hearts with chronic myocardial infarction (CMI). We used time-series spatial transcriptomics (ST) to analyze the human CVPs at 1, 4, and 12 weeks post-CMI. The CVPs successfully engrafted and exhibited cardiomyocytes maturation hallmarks over time including increased gene expression related to metabolism, cell cycle regulation, calcium handling, sarcomere development. Furthermore we observed a significant decrease in the expression of fibrosis-related extracellular matrix genes in the transplanted heart tissues. Using ligand-receptor interaction analysis we identified midkine (MDK) protein as a potential chemokine enhancing angiogenesis within the graft. These findings were validated through immunohistochemistry, and by employing lentivirus to produce MDK-overexpressing cardiomyocytes for use in an endothelial cell transwell migration assay. We observed a significant increase in the migration of endothelial cells through transwell assays compared to the control (P-value < 0.05). Our findings identified the MDK signaling pathway as a promising target for boosting angiogenic responses following cellular transplantation. Taken together, we identified Midkine (MDK) signaling through ligand-receptor interaction analysis, suggesting its potential role in regenerative angiogenesis. To enable an in-depth analysis of our transcriptomic data, we created a Shiny application that allows researchers to interactively explore gene expression profiles from our large animal models (bit.ly/human-pig-mi-hearts-st). This study presents, to our knowledge, the first spatial transcriptome atlas of CVP xenografts in MI pig hearts during the critical post-transplantation recovery phase. This study lays the foundation for deeper insights into the mechanisms of cellular therapy, essential for advancing future clinical trials.

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RFX3 IS ESSENTIAL FOR GENERATING FUNCTIONAL HUMAN PANCREATIC ISLETS FROM PLURIPOTENT STEM CELLS

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The transcription factor, Regulatory factor X3 (RFX3), has been recently implicated in islet development in rodents, however, its role in the human pancreas is

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not yet identified. To address this gap, we generated an isogenic human iPSC model carrying loss of function mutations in RFX3 (RFX3 knockout (KO)) and differentiated them to pancreatic lineages. Our study revealed that RFX3 expression begins in pancreatic progenitors (PPs), then co-localizes with NEUROG3 and NKX2.2 in endocrine progenitors (EPs) and was maintained in endocrine cells during islet differentiation and maturation. RFX3 KO PPs had reduced NKX6.1 expression and impaired endocrine gene expression which further impaired their commitment to endocrine lineage. Further differentiation to EPs and islet organoids resulted in abolished islet cell development, including those producing insulin, glucagon, somatostatin, ghrelin, and pancreatic polypeptide, however CHGA levels were not affected. Beta cells derived from RFX3 KO iPSCs had diminished insulin stores and impaired insulin secretion capacity. Transcriptome analysis of RFX3 KO PPs and islets revealed a significant downregulation of pathways related to insulin secretion, potassium and calcium ion transport, response to hypoxia, among others associated with islet development and function. Interestingly, loss of RFX3 resulted in enhanced enterochromaffin lineage specification with an increase in expression of key enterochromaffin regulators during islet differentiation. Furthermore, EPs and islet organoids exhibited a marked increase in apoptosis which resulted in their deteriorated size, correlating with elevated levels of TXNIP in the RFX3 KO cells. These findings indicate that RFX3 is crucial for human pancreatic islet development, and RFX3 deficiency leads to hampered islet commitment, beta cell dysfunction and increased enterochromaffin lineage differentiation.

Funding Source: Qatar Biomedical Research Institute (QBRI) (Grant no. QBRI-HSCI Project 1).

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NOVEL ROLES OF TWO PEPTIDES IN NEURAL STEM CELL REACTIVATION

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Short open reading frame (sORF)-encoded peptides (SEPs) are highly abundant in eukaryotic genomes, but their functions, especially in the nervous system, remain largely unknown. Here, we showed that two previously uncharacterized SEPs, Simba1 and Simba2 encoded by highly conserved sORFs, are new transcriptional regulators in Drosophila neural stem cell (NSC) reactivation. Cell type-specific knockdown reveals that Simba1 and Simba2 are required in both NSCs and Drosophila blood brain barrier (BBB) glial cells (surface glia) to promote NSC reactivation. Overexpression of simba1 or simba2 in the brain is sufficient to trigger premature NSC reactivation. We are performing in vivo protein-DNA interaction profiling assay, DamID-seq, to identify potential targets of Simba1 and Simba2. To embed Simba1 and Simba2 into known cellular protein functional networks, we will perform large scale protein-protein interaction screening of Simba1 and Simba2, using alphafold-multimer against the whole conserved proteome between Drosophila and human. Given the conserveness of Simba1 and Simba2, we aim to study Simba homologs in mammalian CNS system in the future. These findings will contribute to our understanding on the roles of SEPs in NSC reactivation and brain development.

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LIPOSOME MEDIATED LIPID PRELOADING ENHANCES MSC SURVIVAL IN STRESSFUL ENVIRONMENTS

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Mesenchymal stem cells (MSCs) show great promise for use in tissue repair due to their ability to differentiate and modulate the extracellular environment. Despite their promise, clinical applications are hindered by

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substantial cell death following transplantation. This poor cell survival is attributed to a combination of oxidative stress, immune rejection, insufficient nutrient supply, inflammation and lack of matrix support at the transplantation site. Current strategies to improve post-transplantation cell survival include cell preconditioning, which despite being effective, may perturb MSC paracrine secretion and has not yet been evaluated for long-term effects on cells. Here, we propose an innovative approach of preloading MSCs with lipids to improve cell resistance to oxidative stress and starvation. Lipids were specifically chosen for their critical roles in membrane integrity, energy storage, and oxidative protection. To test this approach, preloaded MSCs were exposed to both oxidative stress and starvation for up to 6 days to mimic in-vivo conditions. ATP measurements indicate 150% better survival of liposome loaded cells as compared to unloaded cells under these stressful conditions on Day 4. Moreover, these cells maintained their stemness, as indicated by biomarker expression and differentiation assays. These findings underscore the potential of liposome mediated nutrient preloading as a strategy to enhance MSC survival and efficacy in regenerative medicine applications.

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DIRECT DIFFERENTIATION TO GENERATE FUNCTIONAL STROMA TO ENRICH TISSUE MICROENVIRONMENT IN HUMAN KIDNEY ORGANOID

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Generation of the human kidney in vitro is the ultimate goal for future replacement therapy. Despite significant advancements, current kidney organoids face several limitations. Nephron organoids contain many nephron-like structures, but lack inter-nephron connectivity. Ureteric bud/collecting duct organoids exhibit extensive branching but are devoid of nephrons. Combining these two types of organoids results in limited nephron-collecting duct connectivity, falling short of recapitulating the organotypic kidney structure observed in vivo. In vivo, the kidney exhibits a higher-order structure characterized by multiple branched collecting ducts connected to multiple functional nephrons located at the periphery. To achieve this complex architecture in vitro, the inclusion of the third precursor population, stromal progenitors, is essential. The orchestrated interaction among stromal, nephron, and ureteric epithelium progenitors within the nephrogenic niche is pivotal for kidney development. Although stromal cells are present in current organoids, they may represent off-target cells arising during differentiation. Therefore, this project aims to develop a precise stromal progenitor differentiation protocol and then re-establish a close-to-native nephrogenic niche alongside human PSC-derived metanephric mesenchyme (MM) and ureteric bud (UB). Ultimately, this approach aims to generate an organotypic “higher-order structure” kidney organoid in vitro. To date, several stromal markers indicative of different anatomical locations have been selected by analyzing mouse and human single-cell RNA-seq datasets. These markers have been tested in mouse embryonic kidneys and human PSC-derived stromal cells. A protocol to generate renal stromal progenitor-like cells from human pluripotent stem cells has been initially established. Quantitative PCR results showed gene expression dynamics during differentiation, which aligned with the origin of stromal progenitors. Immunostaining further confirmed the induction of stromal progenitor cells. Further validation and optimization are needed.

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CONSTRUCT SYMPATHETIC NEURONAL NETWORK IN KIDNEY ORGANOID

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Chronic kidney disease (CKD) represents a pathological state that are closely intercorrelated with hypertension. Resistant hypertension can cause kidney damages, while the decline in kidney function can worsen blood pressure dysregulation. Renal sympathetic nerves play important roles in regulating blood pressure. Renal sympathetic nerve activity is commonly increased in pathophysiological conditions, such as hypertension and chronic- and end-stage renal disease. Besides renal denervation, there is no alternative therapeutic approach to manage resistant hypertension caused

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by sympathetic hyperactivity. Little is known about the role of sympathetic hyperactivity in the pathogenesis of CKD. Current kidney organoid platform is not suitable for studying CKD-related hypertension due to the lack of intrinsic sympathetic neurons. Therefore, we aim to establish the sympathetically innervated kidney organoid model to recapitulate disease phenotypes of CKD-related hypertension. To do this, we will on one hand differentiate human pluripotent stem cells (PSCs) into kidney organoids, on the other hand steer human PSCs into functional sympathetic-like neurons, which can release norepinephrine upon nicotine stimulation. Then, the functional sympathetic neurons will be used to innervate kidney organoids to establish a sympathetic neuronal network. The innervated kidney organoids are expected to establish functional synapses between renal cells and sympathetic neurons. We expect that renin release by kidney organoids can be stimulated by sympathetic activation via either pharmacological or optogenetic approach. The establishment of the sympathetically innervated kidney organoids can be used to study inter-organoid and intra-organ crosstalk. Also, this novel organoid platform can help to unveil pathophysiology of CKD-related hypertension and enable candidate drug screen.

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THE INVESTIGATION FOR THE EFFICACY OF ADIPOSE-DERIVED DUAL STEM CELL THERAPY FOR LIMB ISCHEMIA

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Stem cell therapies play a significant role in regenerative medicine, and clinical trials involving adult stem cells have been actively advancing. Among adult stem cells, those residing in adipose tissue exhibit considerable regenerative potential for tissue repair; notably, adipose-derived stem cells (ADSCs) can mitigate vascular damage by secreting angiogenic factors. This study aims to identify novel vasculogenic stem cells within adipose tissue and evaluate efficacy of combination of vasculogenic stem cells and ADSC in treating ischemic disease. Adipose tissue samples were harvested from human donors, and the stromal vascular fraction (SVF) was isolated using enzyme digestion. The SVF was subsequently cultured in endothelial cell growth medium (for vascular multipotent stem cells, VMSC) and in mesenchymal stem cell-specific medium, respectively. Cellular characteristics of each cell type were analyzed at passage 2 using flow cytometry, Western blotting, immunofluorescence, tube formation assays, differentiation asay and enzyme-linked ELISA. In result, VMSCs were positive for CD29, CD44, CD73, CD90, and CD105, and negative for CD34 and CD45. VMSCs demonstrated the ability to differentiate into adipocytes, chondrocytes, and osteoblasts, similar to ADSCs. However, VMSCs could be distinguished from ADSCs by their higher expression levels of CD141, FGFR2 and, Etv2. Moreover, VMSCs were capable of forming tubular structures in Matrigel, while ADSCs tended to aggregate and form pellet-like structures. But the combination of VMSC and ADSC could make compact vascular structure in Matrigel. These findings suggest that VMSCs and ADSCs exhibit distinct characteristics akin to endothelial and smooth muscle cells of the vasculature, respectively. based on in vitro data, when both VMSCs and ADSCs were transplanted into limb ischemia, they successfully created well-formed vascular structures and prevented limb loss in vivo. This study confirmed the presence of both vasculogenic stem cells and mesenchymal cells in adipose tissue. A sufficient number of VMSCs and ADSCs were successfully acquired through ex vivo culture. The transplantation of VMSCs and ADSCs is anticipated to significantly contribute to vascular regeneration in ischemic diseases.

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"Q" ENHANCED PROLIFERATION, SELF-RENEWAL AND ANTI-SENESCENCE OF MSC AND IMPROVES THE EFFICIENCY OF STEM CELL THERAPY IN CARDIOVASCULAR DISEASES

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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 stemness potential. 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, "Q", which is 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 "Q" was mediated by Cyclin E. "Q" 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 "Q" inhibited ageing and enhanced regeneration of mouse heart in myocardial infarction model. These results indicate that "Q" is an effective agent for expanding MSCs with delayed senescence.

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IDENTIFICATION OF VASCULOGENIC PRECURSOR CELLS FROM HUMAN BONE MARROW AND THEIR ENDOTHELIAL ENGAGEMENT IN THE ARTERIOGENESIS BY CO-TRANSPLANTATION WITH MESENCHYMAL STEM CELLS

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Critical limb ischemia (CLI) is a condition characterized by insufficient blood flow to the lower limbs, resulting in severe ischemia and potentially leading to amputation. This study aims to identify novel vasculogenic precursor cells (VPCs) in human bone marrow and evaluate their efficacy in combination with bone marrow-derived mesenchymal stem cells (BM-MSCs) for the treatment of CLI. VPCs and BM-MSCs from bone marrow were cultured ex vivo and comparatively characterized, whose therapeutic effects on neovascularization and long-term tissue regeneration were investigated after singular or a combination transplantation in a mouse CLI model. VPCs, expressing high levels of hepatocyte growth factor and c-MET, were identified from human bone marrow aspirates. These cells exhibited strong vasculogenic capacity in vitro but possessed a cellular phenotype distinct from those of previously reported endothelial precursor cells in circulation or cord blood. They also expressed most surface markers of BM-MSCs and demonstrated multipotent differentiation ability. Screening of 376 surface markers revealed that VPCs uniquely display CD141 (thrombomodulin). CD141+VPCs are present in BM aspirates as a rare population and can be expanded ex vivo with a population doubling time of approximately 20 h, generating an elaborate vascular network even under angiogenic factor-deficient conditions and recruiting BM-MSCs to the network as pericyte-like cells. Intramuscular transplantation of a combination of human CD141+VPCs and BM-MSCs at a ratio of 2:1 resulted in limb salvage, blood flow recovery, and regeneration of large vessels in the femoral artery-removed CLI model, with an efficacy superior to that of singular transplantation. Importantly, large arteries and arterioles in dual cell transplantation expressed human CD31 in the intima and human α-smooth muscle actin in media layer at 4 and 12 weeks, indicating their lineage commitment to endothelial cells and vascular smooth muscle, respectively, in vivo. Dual-cell therapeutics comprising human BM-derived CD141+VPCs and BM-MSCs could be further developed for clinical trials to cure human peripheral artery disease and diabetic ulcers.

Funding Source: Korean Health Technology R&D Project grant (HI18C1492) from the Ministry of Health and Welfare (Sejong, Republic of Korea).

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THURSDAY, 12 DECEMBER 2024

6:00 PM – 6:45 PM
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DYNAMIC REGENERATION PROCESS OF THE OLFACTORY EPITHELIUM FOLLOWING THE LOSS OF LGR5+

Suyeon Lee

Yonsei University, South Korea

Olfactory nerves are exposed to the external environment, making them susceptible to damage. Despite this vulnerability, adult neurogenesis in the olfactory epithelium (OE) continues throughout life, maintaining the function of the olfactory system. The OE is a pseudostratified epithelial structure primarily composed of sustentacular cells, olfactory sensory neurons (OSNs), globose basal cells (GBCs), and horizontal basal cells (HBCs). Among these, HBCs and GBCs are considered stem cells expressing Lgr5. However, under certain conditions, other cells may exhibit plasticity. The role of Lgr5+ stem cells in the OE has been the subject of investigation, particularly after Lgr5+ cell ablation. Administration of diphtheria toxin (DT) in Lgr5DTR-EGFP mice leads to the ablation of Lgr5+ stem cells in the olfactory epithelium, resulting in the loss of Lgr5+ cells, HBCs, GBCs, and sustentacular cells, followed by the degeneration of olfactory sensory neurons (OSNs). However, despite the ablation of Lgr5+ cells, the OE begins to regenerate for six weeks. This indicates that stem cells other than Lgr5+ HBCs and GBCs contribute to regeneration. The regenerative process begins four weeks after DT administration with the recovery of basal cells and sustentacular cells, followed by the differentiation of immature olfactory sensory neurons (iOSNs) and mature olfactory sensory neurons (mOSNs) at six weeks. These observations suggest that alternative stem cell populations play a role in OE regeneration in the absence of Lgr5+ cells. This study demonstrates that although Lgr5+ HBCs and GBCs play a role in the homeostasis of the OE, their ablation does not completely halt the regenerative process. This finding provides valuable insights into the complexity of OE regeneration. It highlights the need for further research to identify and understand the characteristics and roles of these alternative stem cell populations.

Funding Source: This work was supported by the National Research Foundation of Korea (NRF) Grant funded by the Korea Government (MSIP) (2022R1A2B5B03001627 and NRF-2021R1A2C1005506).

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FEATURES OF VASCULAR CELL POPULATION FUNCTIONING AS THE FOUNDATION FOR RESTORING THE NORMAL STRUCTURE OF VASCULAR TISSUE

Alexander Markin, Diana Kiseleva, Ulyana Khovantseva, Vadim Cherednichenko, Deyyara Chakal, Denis Breshenkov, Yuliya Markina, and Eduard Charchyan

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Cardiovascular diseases (CVDs) are the leading cause of death worldwide, with 17.9 million annual deaths, according to WHO. Thoracic aortic aneurysm (TAA) and related conditions like coronary artery disease (CAD), chronic heart failure (CHF), and atherosclerosis (AS) are significant contributors. CAD is present in 38.6% of TAA cases, CHF in 27.6%, and aortic, coronary, and carotid atherosclerosis in 12.9%, 16.6%, and 5.5%, respectively. TAA and AS share similarities at the cellular level, particularly in smooth muscle cells (SMCs) and endothelial cells (ECs). Vascular SMCs are highly plastic, previously categorized into contractile and synthetic phenotypes. However, single-cell RNA sequencing has revealed a greater diversity in dedifferentiated SMCs, with no consensus on the exact number of phenotypes. A central dedifferentiated SMC type is the mesenchymal-like phenotype, capable of transdifferentiating into fibroblast-like, macrophage-like, osteogenic, and adipocyte-like cells, contributing to various CVDs. A key event in the phenotypic switch is the activation of KLF4 and deactivation of the TGF-β signaling pathway, along with the downregulation of miR-143/145, essential for the contractile phenotype. Our study explores SMC and EC behavior in the

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aorta under conditions similar to AS. Phagocytosis is stimulated by native atherogenic LDL particles and latex beads, with ECs treated with polyethylene glycol to form multinucleated cells. We developed lentiviral vectors to enhance dedifferentiation, creating cell lines of dedifferentiated SMCs with increased expression of c-MYC, KLF4, and hTERT. Vectors inducing miR-143/145 and TGF-β components are under testing for their potential in restoring the contractile SMC phenotype. We observed increased cytokine secretion in TAA-derived SMCs exposed to atherogenic LDL compared to controls, suggesting a pre-activated, pro-inflammatory macrophage-like phenotype. Multinucleated ECs accumulated 1.5 times more cholesterol and exhibited elevated cytokine secretion. These findings suggest that atherogenic LDL can influence SMC transdifferentiation, while endothelial dysfunction may accelerate this process.

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

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INTER-DONOR VARIABILITY AND CELLULAR PROLIFERATION: THEIR IMPACT ON THE IMMUNOMODULATORY STRENGTH OF ADIPOSE-DERIVED MESENCHYMAL STEM CELLS IN T CELL-MEDIATED HEPATITIS

Cheryl Chan, Toru Okubo, Shigeyuki Ota, Yui Ueno, Hayato Kurata, Hiromi Kawai, Masatoshi Haga, Masao Hashimoto, and Yoichi Honma

Rohto Pharmaceutical Co., Ltd., Japan

Mesenchymal stem cells (MSCs) are recognized for their numerous therapeutic benefits, notably for their immunomodulatory capabilities. Thus, they are being tested in many clinical trials, including those for GVHD and osteoarthritis. However, MSCs are also known to be highly heterogeneous. In addition to originating from various tissue sources, they also exhibit inter-donor variation within the same tissue, making it hard to standardise MSC therapeutics. Hence, our aim is to improve this by investigating donor variability and the link to their immunomodulatory strength. We tested this using both in-house produced and commercially sold adipose-derived MSCs (AD-MSCs) via the Concanavalin A hepatitis animal model. In both sets of AD-MSCs, we indeed observed varying levels of anti-inflammatory effects across different donors. Microarray analysis on our in-house AD-MSCs revealed donors with stronger effects exhibited activation in cell cycling and proliferation pathways. Single cell transcriptomic analysis on the commercially sold AD-MSCs, however, showed all donors merging into one group regardless of effect strength, indicating a high level of similarity in MSC characteristics. Further examination through cell clustering revealed 12 distinct subclusters that made up different percentages within each donor, suggesting that subtle cell population differences play a crucial role in differentiating AD-MSCs with strong and weak anti-inflammatory effects. Gene ontology analysis on the differentially expressed genes (DEGS) from clusters representing donors with strong effects demonstrated changes in cell proliferation, echoing the results of the in-house AD-MSC microarray analysis. When we assessed the in vitro doubling time (DT) and cell growth in both sets of AD-MSCs, we observed that donors with stronger anti-inflammatory effects indeed exhibited lower DT and higher growth rates. In summary, this study is the first to link the in vivo immunomodulatory effects of different donors to bulk and single cell transcriptomics, with results indicating a positive correlation between cell proliferation and effect strength. Our ongoing research includes further dissecting target subclusters within each donor and identifying common DEGs between both in-house and commercial AD-MSCs that could serve as reliable Critical Quality Attribute (CQA) markers. This will hopefully lead to better standardisation and development of more effective AD-MSC therapeutics.

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ANEURYSM IS RESTRICTED BY CD34+ CELL-FORMED FIBROUS COLLRS THROUGH PDGFRB-PI3K AXIS

Qingzhong Xiao

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Abdominal aortic aneurysm (AAA) development involves an imbalance between connective tissue repair and degradation. CD34+ cells are a heterogeneous population with stem cell properties, but their role in AAAs is unknown. In this study, aortic tissues collected from AAA patients and two mouse AAA models were used to detect CD34+ cells in AAA. Single-cell RNA sequencing and histology analysis were used to identify the cells and cell-cell communications of the vessels. Whole-mount staining and 3D reconstruction depicted the cell distribution within the vessels. Compared with non-dilated aortas, CD34 expression decreased in aneurysmal aortas from both patients and mouse models. Combining Cd34-CreERT2;R26-tdT lineage tracing, bone marrow transplantation, and single-cell sequencing, we found that during the development of AAA, non-bone marrow CD34+ cells were activated to transdifferentiate into Periostin+ myofibroblasts, contributing to the formation of fibrotic collar, while bone marrow derived CD34+ cells gave rise to immune inflammatory cells. Dual recombinase-based lineage tracing using Cd34-Dre;Postn-CreERT2;Dou-tdT-DTR mice further confirmed the existence and involvement of CD34+/Periostin+ myofibroblasts in fibrotic collar formation during AAA development. Functionally, selective depletion of systemic or non-bone marrow CD34+ cells, as well as CD34+/Periostin+ myofibroblasts by diphtheria toxin significantly exacerbated AAA progression and increased disease mortality. Mechanically, cell-cell communication analysis inferred that PDGF signaling might involve in the activation of CD34+ lineage cells. Specific knockout of both Pdgfra and Pdgfrb in CD34+ cells aggravated AAA progression, but PDGFRb rather than PDGFRa was required for the transdifferentiation of Periostin+ myofibroblasts from CD34+ cells. Furthermore, our in-vitro experiments demonstrated that PDGFRb-PI3k axis was essential for CD34+ cell activation and transdifferentiation into Periostin+ myofibroblasts. Therefore, our data showed that non-bone marrow CD34+ cells are one of the major contributors to Periostin+ myofibroblasts forming a fibrous collar to protect the aneurysm from rupture, where PDGF-PDGFRb-PI3K axis is indispensable, offering a novel target for AAA patients at high risk of rupture.

Funding Source: This work was supported by British Heart Foundation (PG/15/11/31279, PG/15/86/31723, PG/20/10458, and PG/23/11371 to Q.X).

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VIMENTIN INHIBITS NEURONAL APOPTOSIS AFTER SPINAL CORD INJURY BY REGULATING AUTOPHAGY

Hongfu Wu[1], Xiaomin Zhang[1], Jie Zhao[1], Liji Chen[1], Yinru Liang[1], Jiajun Zhang[2], Jiahong Chen[2], Manqi Cai[2], and Xiaojun Cui[3]

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The aim of this study was to evaluate the effect of vimentin knockdown on the recovery of neural and motor functions after spinal cord injury and to investigate its specific mechanism of action. The vimentin RNAi adenovirus was constructed and transplanted into T10 rats with total transection injury of the spinal cord, and the recovery of neurological and motor functions after spinal cord injury was evaluated by BBB score, footprint analysis, electrophysiological tests, and immunofluorescence staining. Protein and gene expression were assessed by Western blotting, CO-IP, q-PCR, and immunofluorescence. In addition, neuron-like PC12 cells were infected with adenovirus to further elucidate the effect of vimentin on autophagy and the molecular mechanism of neuronal apoptosis after spinal cord injury. Vimentin expression is upregulated after spinal cord injury, inhibition of vimentin expression after spinal cord injury enhances neuronal autophagy, reduces neuronal apoptosis, and promotes the recovery of motor and neurological functions after spinal cord injury. Notably, this may be related to the formation of Vimentin-14-3-3-Beclin1 complex and PI3K class III complex. These results suggest that inhibition of

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vimentin expression may enhance autophagy and anti-apoptosis in rat neurons after spinal cord injury by affecting the formation of the vimentin -14-3-3-Beclin1 complex, thereby promoting neuronal recovery.

Funding Source: This research was funded by GuangDong Basic and Applied Basic Research Foundation (NO. 2023A1515140184), The National Natural Science Foundation of China (NO.82071374).

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MICRORNAS REGULATING HUMAN TRABECULAR MESHWORK STEM CELL MAINTENANCE AND THEIR DECLINE WITH AGING

Sneha Nair[1], Gowri Chidambaranathan[2], Bharanidharan Devarajan[2], Krishnadas Ramasamy[3], Vanniarajan Ayyasamy[4], and Annalakshmi N.[2]

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The human trabecular meshwork (TM) serves to be the conventional aqueous humor (AH) outflow pathway of the eye which is the primary site of glaucoma pathogenesis. The increased resistance to AH outflow in primary open angle glaucoma (POAG) is associated with the drastic reduction in total TM cell. We recently confirmed that adult stem cells for the TM to be located in the anterior non-filtering region (NF) and a significant reduction of these stem cells (TMSCs) in POAG. Till date, no studies have been carried out to elucidate the molecular basis governing the loss of TMSCs with ageing and in POAG. Hence, this study aims to elucidate the miRNA profile of the F and the NF region of the human TM to identify the molecular signaling pathways/ regulators of TMSCs. The higher expression of ABCG2 and p75 in the NF region and two parameter analysis confirmed the presence of stem cells in NF region, and the proper dissection. Analysis of differentially expressed miRNAs identified 26 significant up regulated miRNAs and three downregulated miRNAs in the non-filtering region with fold change > +1.2 and p-value ≤ 0.05. Based on the previous literature and raw data counts, seven miRNAs were taken for validation. Among seven miRNAs, four miRNAs hsa-miR-184, hsa-miR-107, hsa-miR-34a-5p and hsa-miR-24-3p showed higher expression in NF region (fold change > 1.2) compared to F region and three downregulated miRNAs—hsa-miR-145-5p, hsa-miR-22-3p and hsa-miR-376a-3p had reduced expression in NF region (fold change < 1.2) compared to F region, thus validating the miRNA profiling data. Bioinformatic analysis identified pathways functionally associated with the maintenance of stemness including MAPK, Wnt, PI3K-AKT, and FOXO signalling. Analysis of the expression levels of the NF specific miRNAs in NF region of TM from different age group donors indicated an age-related reduction in the upregulated miRNAs—hsa-miR-107, hsa-miR-184, and hsa-miR-34a-p in the NF region. Further studies are essential to analyse the expression of these differentially expressed miRNAs in glaucomatous condition. This basic study will establish the “Proof of Concept” on whether the molecular regulators of stem cells—miRNAs can be used to reactivate the residual TMSCs/TM cells for TM regeneration.

Funding Source: Science and Engineering Research Board (CRG/2020/005258).

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ENGINEERED NEPHRONS AND URETERIC BUDS CO-EVOLVE TO GENERATE HUMAN KIDNEY ORGANOIDS WITH HIGH-ORDER STRUCTURE

Chao Zhang and Yun Xia

Nanyang Technological University, Singapore

Current kidney organoids can only separately model nephron and collecting duct due to distinct spatiotemporal origins of these two lineages. Moreover, reliance on uncontrolled, spontaneous self-organization during aggregation results in deficiency in organ-level anatomy, which hamper kidney organoids from recapitulating the organ-level function. In this study, we establish a protocol to coordinately differentiate nephrons and ureteric buds (UB) from human pluripotent stem cells in one pot. Nephrogenic niches with UB tip cells in the lower layer while nephron progenitor cells

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(NPCs) in the upper layer were established in the early stage. Intrinsic reciprocal signals from UB tip cells and NPCs instruct each other to develop into collecting ducts and nephrons without any need of exogenous growth factors. Repeated branching of UB gives rise to a collecting duct network. Meanwhile, patterned nephrons are radially aligned along proximal-distal axis and connect to collecting ducts to form contiguous uriniferous tubules. Altogether, we successfully generated highly organized kidney organoids with patterned nephrons connecting to a ureteric bud tree, showing unprecedent structural resemblance to kidney anatomy.

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INVESTIGATING THE SENOTHERAPEUTIC ROLE OF MESENCHYMAL STEM CELL-DERIVED SECRETORY FACTORS DURING NEURO-SENESCENCE

Prakshi Sharma[1], Vidya Rattan[2], and Shalmoli Bhattacharyya[2]

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Aging is an universal phenomenon in which there is deterioration of functional capacity of cells, tissues and organs with time. There are several hallmarks of aging e.g. mitochondrial dysfunction, genomic instability, stem cell exhaustion, telomere attrition, alteration in intercellular communication a and cellular senescence. Studies have shown that accumulation of senescent cells paves the way for the development of neurological damage as these accumulated senescent cells are the main culprits for reducing the cellular functionality by damaging the tissue microenvironment and its restorative potential by spreading the senescence to adjacent normal cells. To circumvent the degenerative role of senescent cells, several senotherapeutics approaches like use of senolytics and senomorphic compounds have been proposed. Recently stem cells have emerged as a potential game changer in field of senotherapeutics. The present study investigated the role of stem cell derived secretory factors or the secretome as a therapeutic approach for countering cellular aging. This will be a cell free approach that can nullify the deleterious effects of direct stem cell transplantation.The neuro-senescence model was established by D-galactose induction in SHSY-5Y cell line and compared to simulatenous treatment with dental pulp stem cell derived secretome. The D-gal induced SHSY-5Y cells shows characteristic senescence features i.e. SA-β-gal expression, mRNA expression of senescence genes like Lamin B,p16, p21 and p53, ROS generation, ɣ H2AX expression.The study revealed that the administration of dental pulp stem cell-derived secretome attenuated key cellular characteristics associated with neural senescence and restores the cellular functionality. It was observed that the dental pulp stem cell derived secretome provided the beneficial factors required for restoring and maintaining the normal neuronal cells functioning. Thus, our study offers a compelleing advancement by highlighting the potential of dental pulp stem cell-derived secretome as a cell-free senotherapeutic strategy in in vitro model of neuro-senescence. Further, in vivo validation is required as a proof of concept of the study.

Funding Source: Prakshi Sharma has received fellowship from CSIR, Government of India. (File no. 09/0141 (12395)/2021-EMR-I).

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THE DC-6-KSP PATHWAY IS A CRUCIAL REGULATOR OF METABOLIC ADAPTATION AND LEUKEMIC PROGRESSION

Hyog Young Kwon[1], Tania Setiawan[2], and Nadya Marcellina[2]

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Acute Myeloid Leukemia (AML) is a highly aggressive cancer originating from myeloid lineage stem cell precursors, affecting both adults and children. It is notorious for drug resistance and high relapse rates. While DC6 has been implicated in the progression of various solid tumors and multiple myeloma, its role in AML has yet to be elucidated. Our study demonstrates that DC6 is pivotal in regulating autophagy and mTOR localization under amino acid deprivation in AML cells. Notably, DC6 expression is elevated in AML cells and

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patient samples, with high levels correlating with poor prognosis. Our findings also show that conditional DC6 deletion impairs leukemogenesis in an MLL-AF9/NRAS mouse model. Further investigation reveals that DC6 interacts with KSP, enhancing its stability by preventing proteasomal degradation. Moreover, DC6 orchestrates the relocation of the KSP-mTOR complex from lysosomes under amino acid scarcity, critically influencing AML cell survival. Collectively, our data illuminate the indispensable role of the DC6-KSP pathway in AML progression, particularly under conditions of amino acid deprivation.

Funding Source: KNRF.

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ROLE OF SHANK2 IN HUMAN NEURON DEVELOPMENT AND FUNCTION

Xin Yi Yeo[1], Suji Hong[2], Han-Gyu Bae[2], Sejin Lee[2], Junho Park[3], Jeongeun Lee[4], MinYoung Kim[5], Dong-Gyu Jo[6], Weiping Han[2], Jiong Tang[2], and Sangyong Jung[7]

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The importance of chemical neurotransmission in human behaviour control cannot be understated, and its formation is dependent on the precise synapse assembly and specification involving the timed recruitment of a myriad of presynaptic and postsynaptic protein molecules into defined synaptic sites. Among the different postsynaptic density (PSD) proteins, the Shank family of proteins have attracted much attention for its role as the central regulator of excitatory synapse development and function. Despite the redundancy and importance of Shank2 in the synaptic assembly, alongside its potential non-neuron function-specific effects, there is a lack of understanding of the fundamental role of Shank2 in synaptic and neuronal functions. Given the inherent differences between rodent models and actual human physiology, a consolidated understanding of Shank2's function in the human neuronal system is lacking. In this study, we sought to elucidate the role of Shank2 in human neuron function in a human embryonic stem cell (hESC)-derived cortical neuron model. An examination of the impact of Shank2 ablation on functional synaptic neurotransmission through standard electrophysiological methods suggests its involvement in the modulation of N-methyl-D-aspartate receptor (NMDAR) response in human neurons. There is a concomitant alteration in the action potential (AP) waveform as a reduction in the AP and the afterhyperpolarization (AHP) duration and an increase in tertiary neurite growth. Bulk RNA sequencing and proteomics suggested that the Shank2-specific changes are due to a global gene expression and cell differentiation dysregulation. Using an alternative chemical-derived human neuron model that recapitulates specific stages of neurogenesis provided further evidence for the impact of Shank2 ablation in neuronal differentiation. The findings in this study highlight the NMDAR-specific impact of Shank2 in the human neuron system and a previously unknown and potential role of Shank2 in neuronal fate determination.

Funding Source: Joint Council Office grant (BMSI/15-800003-SBIC-00E) from A*STAR, Singapore and industry-academic cooperation foundation, CHA University grant (CHA-202300230001).

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MODELLING GENETIC POLYCYSTIC KIDNEY DISEASE USING HUMAN PLURIPOTENT STEM CELL-DERIVED KIDNEY ORGANOIDS

Meng Liu

Nanyang Technological University, Singapore

Polycystic kidney disease (PKD) is an inherited disorder characterized by progressive expansion of fluid-filled cysts in the kidney. Autosomal dominant polycystic

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kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD) represent the most common forms of PKD. Treatment options are limited due to lack of models to faithfully recapitulate PKD pathophysiology. Herein, we generated a collection of kidney organoids from both PKD patient-derived iPSCs and genetically engineered hPSCs, alongside stress paradigm, to emulate PKD cystogenesis. Cyst formation within PKD kidney organoids exhibited a myriad of structural and functional abnormalities that are typically manifested in PKD patients. Patient iPSC-derived kidney organoids developed tubular cysts in vivo upon engraftment into the sub-renal capsule space of immunocompromised mice. We also performed a small-scale drug screening and identified two candidate drugs that can effectively attenuate cyst formation in both ARPKD and ADPKD kidney organoids. Mechanistic studies revealed that autophagy plays critical roles in safeguarding PKD kidney organoid from cyst formation. The PKD kidney organoid model offers a versatile platform for understanding disease mechanism, as well as for shortlisting drugs with clinical potential.

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A COMBINATORIAL TECHNOLOGY PLATFORM FOR RATIONAL REPROGRAMMING OF CELLULAR STATE

Yen Choo[1], Chiara Naddeo[1], Harshyaa Makhija[1], Sachin Luharia[2], and Marina Tarunina[3]

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Cellular plasticity enables reprogramming of cell identity through ectopic expression of transcription factors (TFs) that induce and stabilize gene regulatory networks specifying discrete cellular states. For example, transient expression of the Yamanaka factors reprograms diverse cell types to iPSCs; and these can be programmed into further, more developed, lineages using additional TFs. However, facile identification of TF combinations for cell reprogramming remains elusive, mainly because current screening methods are highly multiplexed and therefore challenging to deconvolute. Even if TF switches and effectors of reprogramming can be identified, the circuitry of these components is obscure since TF expression is not temporally resolved. We have developed a high throughput screening method to test all possible combinations of (2, 3, 4… n) TFs from a predetermined subset to discover functional combinations, and to resolve the order/timing in which they orchestrate reprogramming. We will present screens covering 10,000 combinations of up to 20 TFs involved in hematopoietic and pancreatic development, to discover new combinations of TFs that reprogram iPSCs into medically important cell types such as megakaryocytes and pancreatic beta cells. We believe this platform technology will be widely applicable to cell reprogramming in general, where the ability to rationally map out genetic circuits determining cell identity will enable diverse applications in fields ranging from synthetic biology to regenerative medicine.

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TRANSPLANTATION OF SOMATIC CELL-INDUCED NEURAL PROGENITORS ENHANCES FUNCTIONAL RECOVERY AFTER STROKE

Hung-Chih Kuo

Academia Sinica, Taiwan

Induced pluripotent stem cell-derived neural progenitor cells (iPSC-NPCs) are a promising source of tailor-made cell therapy for neurological diseases. However, major obstacles, such as tumorigenic and spontaneous differentiation of iPSCs remains a concern for linical application. To circumvent complications related to iPSC-associated issues, we have previously established method to directly convert human fibroblast into neural progenitor-like population, namely induced neural progenitors (iNPs). We demonstrated that the iNP cells are able to give rise to various neural subpopulation including various neuronal subtypes, glial cells and oligodendrocytes in vitro. In vivo transplantation of iNP into normal rat brain showed that iNPs can integrate into adult brain tissue and differentiate into major neural cell types in vivo. Furthermore, implantation of iNPs epidurally over the peri-infarct cortex 7 days after permanent middle cerebral artery occlusion in adult rats resulted in improvements in paretic forelimb usage and grip strength from 10 days post-transplantation (dpt) onwards, as well as reductions in lesion volumes. This study demonstrates an alternative method to promote functional recovery after stroke.

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HUMAN STEM CELL-DERIVED NEURAL MODELS REVEAL PREFERENTIAL ENTEROVIRUS-A71 INFECTION AND FERROPTOSIS

Wai Hon Chooi[1], Winanto Ng[1], Yingying Zeng[1], Cheryl Yi-Pin Lee[1], Yuin-Han Loh[1], Sylvie Alonso[2], and Shi-Yan Ng[1]

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Enterovirus A71 (EV-A71), the causative agent of Hand, Foot, and Mouth Disease, is clinically linked to neurological complications. However, its effects on neural tissue maintenance and its neuropathological mechanisms remain unclear due to the limited relevant models available. In this study, human pluripotent stem cells were used to generate motor neuron and 3D spinal cord organoid models to investigate EV-A71 infection. We found that hESC-derived neurons were susceptible to EV-A71 infection, and that infectivity and cytopathic effects increased in response to higher viral loads. Similarly, using the organoid model, we observed that EV-A71 initially infected neural cells at the organoid surface, spreading inward and progressively leading to a loss of structural integrity over time. These observations highlight the virus's capacity to disrupt neural tissue integrity, compromising tissue maintenance in infected organoids. Single-cell transcriptomics of the mixed neuronal population revealed a higher viral RNA load in motor neurons, indicating increased susceptibility to infection and viral replication, which was observed in both 2D and 3D models. Moreover, the elevated viral RNA load in motor neurons was associated with downregulation of ferritin-encoding genes, leading to ferroptosis—characterized by increased levels of labile Fe2+ and lipid peroxidation. Treatment with Fe2+ chelators improved mitochondrial function and increased motor neuron survival post-infection, suggesting a promising therapeutic approach. This study underscores the critical role of stem cell-derived models in advancing the understanding of EV-A71 molecular pathogenesis and highlights potential therapeutic strategies to counter its effects on neural tissue.

Funding Source: This work is supported by the National Research Foundation Singapore (NRF-CRP21-2018-0004) and National Medical Research Council (MOH-001248-01).

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STEM CELL DERIVED MACROPHAGE TAKE RESIDENCE IN KIDNEY ORGANOID

Huamin Wang

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Kidney organoids offer a promising in vitro model for studying kidney development, disease, and regeneration. However, a significant limitation is the absence of immune components, including macrophages, which play critical roles in tissue homeostasis, inflammation, and repair. This study aims to integrate stem cell-derived macrophages into kidney organoids to better mimic the native kidney environment. By co-culturing macrophages with kidney organoids, we investigate their interactions, focusing on macrophage localization, phenotypic differentiation, and functional roles in response to injury or genetic disease. Our approach enables a more comprehensive model for studying kidney diseases such as acute kidney injury, inflammation, and immune responses, and provides insights into the potential for macrophage-mediated repair mechanisms in regenerative medicine. Understanding the crosstalk between stem cell-derived macrophages and kidney organoids could significantly enhance the utility of organoids for drug screening and disease modeling, ultimately improving therapeutic strategies for kidney-related pathologies.

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ON THE EVOLUTION OF THE STEM CELL RELATED FUNCTIONS OF SOX FACTORS AND NATURALLY OCCURING SUPER-SOX

Shi Wing Yeung, Ya Gao, Haoqing Hu, and Ralf Jauch

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The transition to multicellularity in animals is a significant milestone in evolutionary history. The SoxB1 transcription factor family has an essential role in inducing and maintaining pluripotency in animals, yet the origin of these functions has remained an enigma. To elucidate at which point on the evolutionary tree their pluripotency-related functions evolved, we studied a panel of Sox factors from multicellular metazoans and their unicellular relatives in their ability to regulate stemness. Our results show that selected metazoan SoxB factors from Porifera (sponges), Ctenophora (comb jellies) and Arthropoda (e.g. Drosophila) can replace Sox2 in reprogramming mouse somatic cells to induced pluripotent stem cells. SoxB in Mnemiopsis leidyi (mleSox1) is a naturally occurring super-Sox as it matches engineered Sox in their exceptional efficiency in pluripotency induction. Interestingly, unicellular holozoans possess critical SoxB-like HMG box sequences, even though they do not form stem cells. Choanoflagellates Sox have DNA-binding specificity similar to mammalian Sox2 and can induce pluripotency. Our results show that pre-animal Sox is biochemically similar to SoxB and its emergence predates its pluripotency-related functions, which suggests that the evolution of stem cells may have involved the repurposing of pre-existing Sox transcription factor. Beyond shedding light on the origin of animal stem cells, understanding Sox evolution and its sequence-function relationship aids the design and engineering of more potent transcription factors that can advance the field of regenerative medicine.

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MECHANICALLY-STIMULATED HEART ORGANOIDS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS EXPERIENCE EARLIER DEVELOPMENT AND CONTRACTILE ACTIVITY

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Heart organoids derived from human pluripotent stem cells are a powerful biological tool for modeling cardiac development, disease, and drug responses, as they offer a physiologically relevant three-dimensional platform that recapitulates key aspects of the human heart. These in vitro models are increasingly used in developmental biology, drug-toxicology screening, and personalized medicine approaches. However, the maturation and spatiotemporal specification of heart organoids remain a challenge. We investigated the effects of mechanical stimulus on the development and function of heart organoids that were treated with 5 µM or without retinoic acid (RA) to promote epicardium development. The human heart organoids were derived from 10,000, 20,000, and 30,000 human induced pluripotent stem cells tagged to a green fluorescent protein (GFP) linked to troponin (TNNT1). To test the potential effect of a mechanical stimulus, organoids from all groups were cultured in static conditions (control) or exposed to rotational movement during the length of the experiment. Our results indicated that beating heart organoids with different degrees of cardiomyocyte distribution were obtained from all groups. RA treatment also accelerates the formation of beating organoids compared to non-RA treatment groups. However, our data indicated that rotational movement promoted a significant shortage in the days to initiate beating activity, in addition to a stronger and major distribution of GFP expression in the organoids compared to all other conditions, enhancing the development of cardiomyocytes and pace-maker cells that originate the beating activity in these heart organoids derived from pluripotent stem cells.

Funding Source: American Heart Association Award 23IAUST1034173, NSF Award 2026049.

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ANALYSIS OF CHROMATIN ULTRASTRUCTURE IN MOUSE EMBRYOS AND HUMAN STEM CELLS

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Cell identity is defined at the level of chromatin, which is fundamentally regulated by its structure. To recapitulate and manipulate diverse cell states, it is thus critical to define the mechanisms that shape chromatin structure. Our current understanding of this regulation is however limited by the lack of methodologies capable of probing chromatin structure at the base unit in which it is remodeled, the nucleosome. To overcome this, we have developed ChromGEM (Chromatin Gold Electron Microscopy), a method which combines a new strategy for endogenous protein labeling with chromatin tomography to enable nucleosome scale chromatin tracing in the context of cell and epigenetic state. We apply our technique to quantify changes in chromatin structure as cells differentiate after fertilisation in the mouse embryo. We show that states of developmental potential and epigenetic environments exhibit distinct ultrastructural properties that differ between in vitro and in vivo systems, as well as mouse and human stem cells. We further leverage this approach to investigate the ultrastructural basis for silencing of pluripotency genes in the trophectoderm, and mechanistically link changes in chromatin structure to the upregulation of Lamin A/C. We thus define the ultrastructural properties underlying mouse and human stem cells during differentiation and development and establish an approach that allows chromatin structure to be holistically quantified in situ and related to cell and epigenetic state.

Funding Source: EMBO fellow, ALTF #902-2019 K99/R00 fellow, K99HD112607.

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GENETIC MODULATION OF CELLULAR AGEING REGULATES INTEGRATED STRESS RESPONSE (ISR) SIGNALLING TO CONTROL BLOOD CELL HOMEOSTASIS IN DROSOPHILA

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Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), India

Blood cell homeostasis is tightly regulated by maintaining a fine balance between stemness and differentiation. Drosophila hematopoesis occurs in multiple waves and the lymph gland is the larval hematopoietic organ, the main site of hematopoiesis. The lymph gland consists of a Posterior Signalling Center (PSC) that acts as a stem cell niche and maintains the prohemocytes that are housed in the Medullary Zone (MZ). The differentiated hemocytes namely the plasmatocytes, crystal cells and lamellocytes are housed in the Cortical Zone (CZ). There are intricate signalling networks active in the PSC and MZ that orchestrate and regulate homeostasis in this organ. While ageing is associated with a functional decline in stem cell potency often leading to stem cell exhaustion and a significant deviation in differentiation trajectories, the mechanistic alterations that occur in an aged stem cell—niche micro-environment are not well characterized. In this study, we characterize the effect of genetic perturbation of the molecular circuitry of ageing in a localized and systemic manner and investigate its effect on blood cell homeostasis in the lymph gland. Genetic modulation of cellular ageing affects traditionally known hallmarks of ageing like proteostasis, autophagy, accumulation of ROS, DNA damage etc. in the lymph gland. Our results indicate that inducing accelerated ageing in both localized and systemic manner is associated with a reduction in stem cell niche size, increased DNA damage and a differentiation bias leading to skewed differentiation. We show that the Integrated Stress Response (ISR) pathway is activated in an accelerated ageing scenario possibly to recoup back to normalcy. Lymph glands in ISR pathway mutants or upon perturbation of ISR pathway components in prohemocytes show aberrant blood cell differentiation indicating disruption of homeostasis. Genetic epistasis analysis shows that over-expression of ISR pathway genes in an accelerated ageing scenario can rescue the defects in blood cell homeostasis. Overall, our study explores how modulation of cellular ageing locally or systemically can impact tissue and organ homeostasis. Our research aims to understand the mechanisms underlying an aged versus young stem cell-niche micro-environment and how its abrogation may lead to onset of disease.

Funding Source: Department of Biotechnology (DBT) and Department of Atomic Energy (DAE), Government of India.

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PGE2 RESPONSE IN THE SMALL INTESTINE LEADS TO LINEAGE SKEWING THROUGH NOTUM INDUCTION IN PANETH CELLS

Seung-Yeon Lee and Hyuk-Jin Cha

Seoul National University, South Korea

Intestinal lineage skewing, characterized by a shift towards secretory lineage differentiation, is observed in both aging and inflamed intestines, contributing to compromised regeneration. Notably, prostaglandin E2 (PGE2), produced in response to injury, plays a dual role in inflammation and regeneration, yet the molecular mechanisms underlying these divergent effects remain unclear. In our study, we reveal that prolonged exposure to PGE2 over four days leads to increased secretory lineage differentiation: Paneth, goblet, and tuft cells, indicative of lineage skewing rather than regeneration. Utilizing single-cell RNA sequencing analysis, we identified that Notum, a palmitoleoyl-protein carboxylesterase that acts as a feedback inhibitor of Wnt signaling, is significantly upregulated in Paneth cells following chronic PGE2 exposure. Importantly, chemical inhibition of Notum with ABC99, an N-hydroxy hydantoin carbamate, mitigated the skewing effects of PGE2, facilitating intestinal regeneration instead. Our findings suggest that Notum expression in Paneth cells, particularly under aging or chronic inflammatory conditions, mediates the detrimental impact of sustained PGE2 production on intestinal health.

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ENHANCED LIVER REGENERATION VIA TARGETED MRNA DELIVERY FOR PARTIAL IN VIVO REPROGRAMMING

Beom Ki Jo[1], Young Seok Song[2], Woohyun Song[2], Hee ji Eom[1], Yon Jae Lee[2], Jumee Kim[1], Seunghee Hong[2], Seung-Woo Cho[2], and Hyuk-Jin Cha[1]

[1]Seoul National University, South Korea, [2]Yonsei University, South Korea

Recent studies suggest that injury-induced dedifferentiation, which leads to the formation of 'injury-responsive cells', contributes significantly to tissue repair across various organs, including the liver. Utilizing Yamanaka factors (Oct4, Sox2, Klf4, and c-Myc: OSKM) for in vivo partial reprogramming generates 'injury-responsive cells' in the intestine, mirroring those derived from injury-induced dedifferentiation. Thus, the transgene induction of OSKM or viral delivery of Oct4, Sox2, and Klf4 shows promise in facilitating tissue regeneration in the intestine, liver, skeletal muscle, and retina. Herein, we demonstrated that transient OSKM induction produces two distinct liver progenitor-like cell populations. One of these populations resembles liver progenitor-like cells (LPLCs) generated by acute acetaminophen (APAP) injury without triggering immune responses. To explore in vivo reprogramming as a viable strategy for tissue regeneration, we employed lipid nanoparticles (LNP) carrying OSKM mRNA (OSKM mRNA-LNP) to stimulate LPLCs formation. Notably, the production of Sox9+ LPLCs, and OSKM-induced dedifferentiation, was closely correlated with successful tissue regeneration in the liver post APAP injury. Thus, the OSKM mRNA-LNP approach represents a promising therapeutic intervention for the repair of acute liver injuries.

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