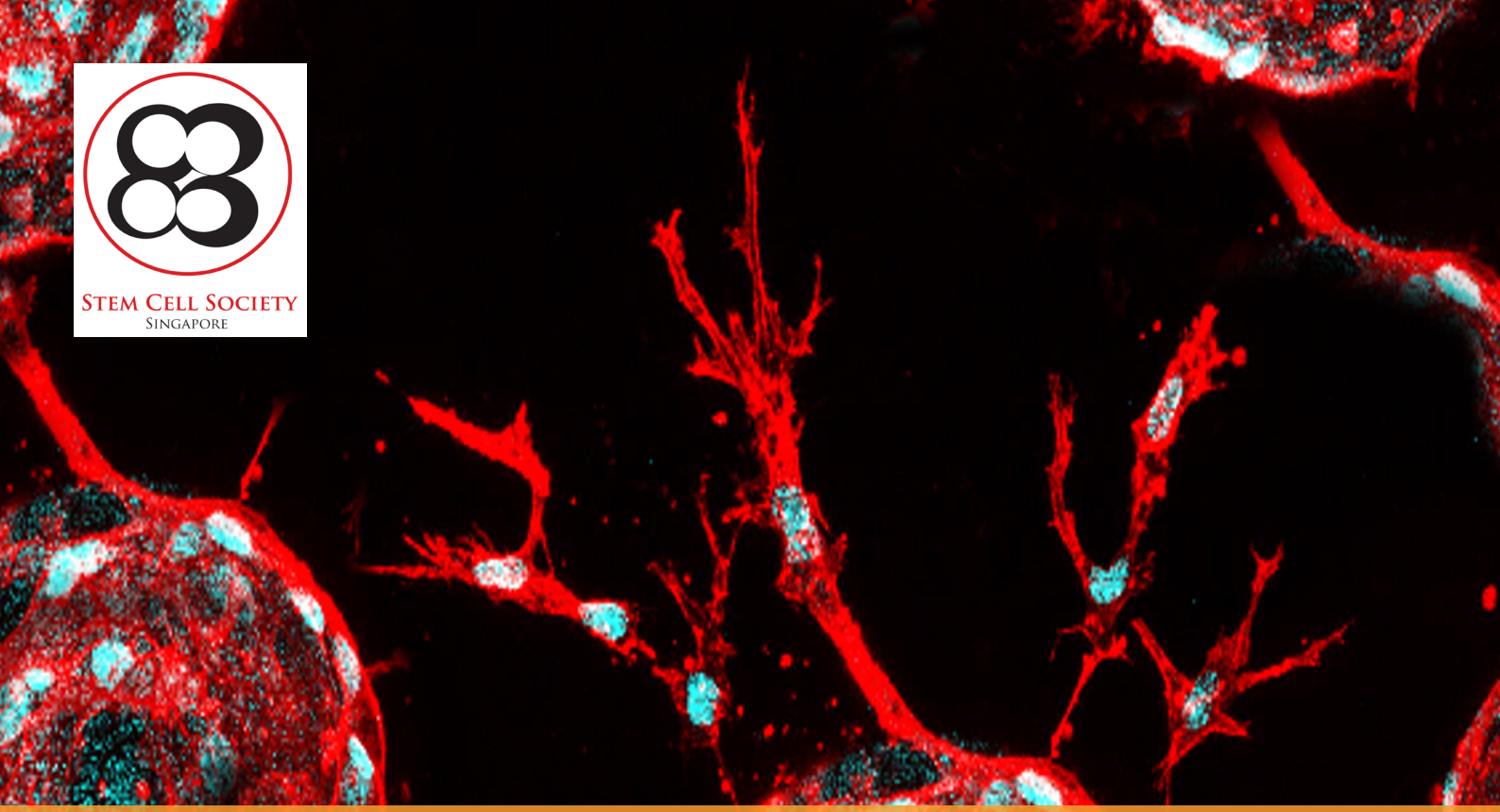


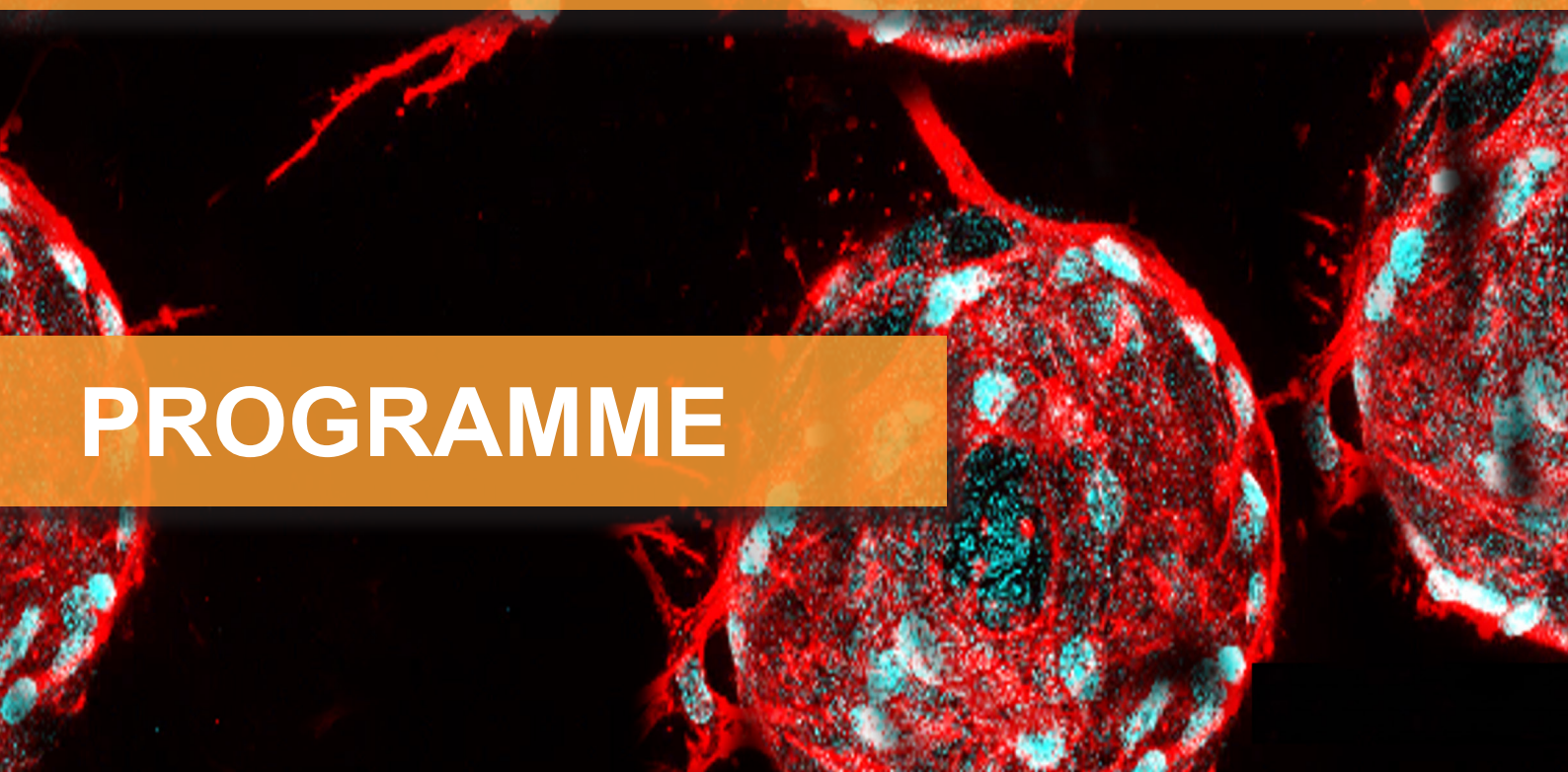
STEM CELL SOCIETY
SINGAPORE

15th Annual Symposium 2023

Stem Cell Society Singapore

29 November - 1 December

Auditorium, Matrix @Biopolis



PROGRAMME

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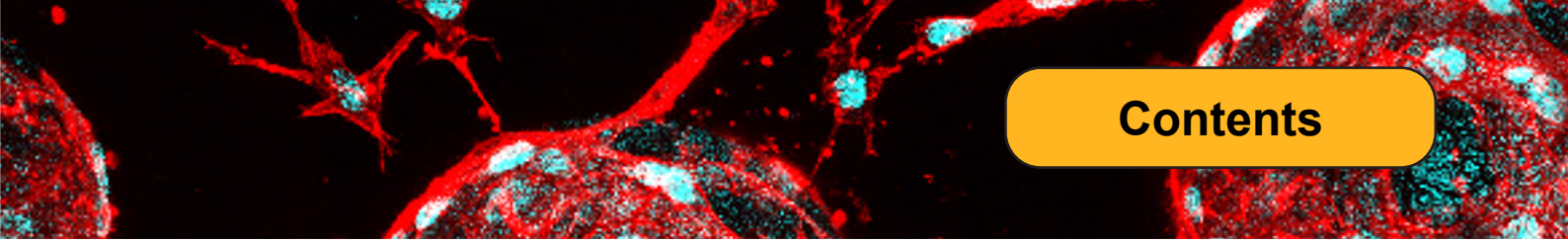

Contents

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Overcome the challenges of genome editing in iPSCs with our latest innovations.

Gene editing — Clonal isolation — Disease model

Design

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- TrueCut gRNAs
- PerfectMatch TALENs
- Cas9 iPSC line

Deliver

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Clone

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Screen

- Ion GeneStudio S5 Systems
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Expand

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- Geltrex™ matrix

Differentiate

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- Dopaminergic Neuron Differentiation Kit
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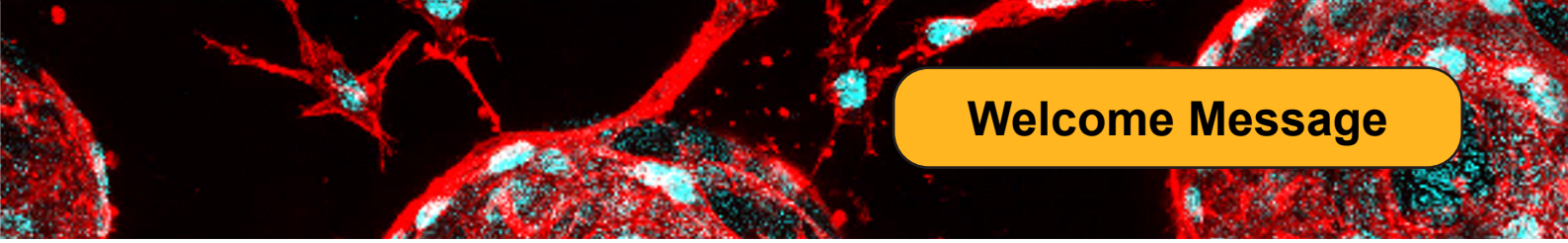


Welcome Message

Dear Colleagues & Friends,

A heartfelt welcome to our ***15th Stem Cell Society Singapore Symposium 2023*** in the vibrant city of Singapore. We are overjoyed to be able to have face-to-face discussions with you that make our symposium truly remarkable.

This annual scientific symposium stands as the flagship event of the Stem Cell Society Singapore, embodying our commitment to fostering collaboration and advancement in stem cell research. Bringing together a diverse array of local and international professionals from various sectors, our symposium facilitates the exchange of ideas, experiences, and groundbreaking discoveries.

In the dynamic landscape of stem cell research, the last few years have witnessed a rapid transition towards applications for treating human diseases. This year's program reflects this evolution, showcasing a spectrum of basic and translational research, alongside insights from industry and clinical endeavours dedicated to bringing the promise of stem cells closer to patients.

Our organizing committee has meticulously curated an exceptional program featuring esteemed speakers from overseas and within our local community. We are particularly thrilled to host two distinguished keynote speakers, *Dr. Shinya Yamanaka* from Kyoto University, Japan, a Nobel Prize laureate, and *Prof. Suchun Zhang* from Duke-NUS, Singapore. Their expertise exemplifies the calibre of discussions we aim to foster.

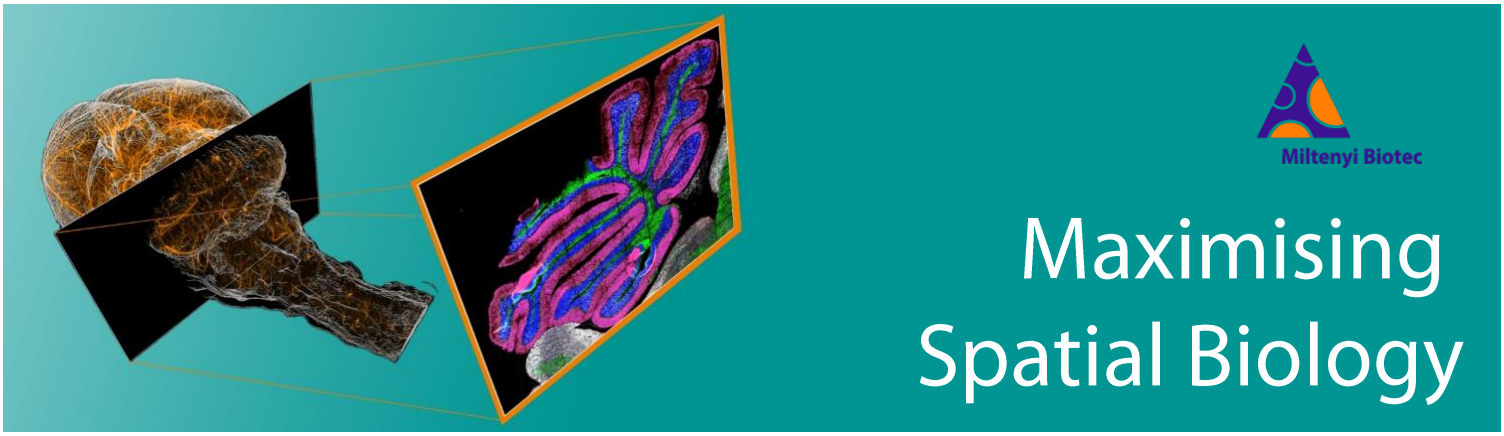
Our symposium spans a diverse range of topics, from modeling development and disease to discussions on adult stem cells, mechanisms of totipotency & pluripotency, advanced organoids, innovations in cell therapy development and assessment, and breakthroughs in cellular agriculture. Moreover, we are proud to provide a platform for emerging young researchers to share their science, culminating in a dedicated poster session to encourage engaging discussions.

A special acknowledgement extends to our dedicated speakers, who have invested time and effort to present their research. We also express gratitude to our academic and industry sponsors, whose invaluable support makes this symposium possible. Dr. Susan Lim deserves a particular mention for her unwavering commitment to the SCSS-Dr. Susan Lim Award for Outstanding Young Investigator. Lastly, we extend our heartfelt thanks to our diligent volunteers, whose contributions ensure the seamless operation of the symposium.

We eagerly anticipate your active participation. Your presence enriches the symposium, and we hope you leave inspired and enlightened by this collaborative experience.

With warm regards,
The Organizing Committee

Christine Cheung (Chair)
Shigeki Sugii (Chair)
Yie Hou Lee
Pentao Liu
Jonathan Loh
Alfred Sun
Gerald Udolph
Hongyan Wang



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Maximising Spatial Biology

A workflow combining 3D imaging of large biological tissues with multiplex spatial analysis

Currently, available methods may only provide information from thin tissue sections, limiting analysis to rather roughly selected areas. Our workflow combines 3D imaging with multiplex spatial analysis to get an overview of complex large samples, identify target structures within them, and to further analyze a carefully selected region in depth with hundreds of markers – all on the same valuable specimen.

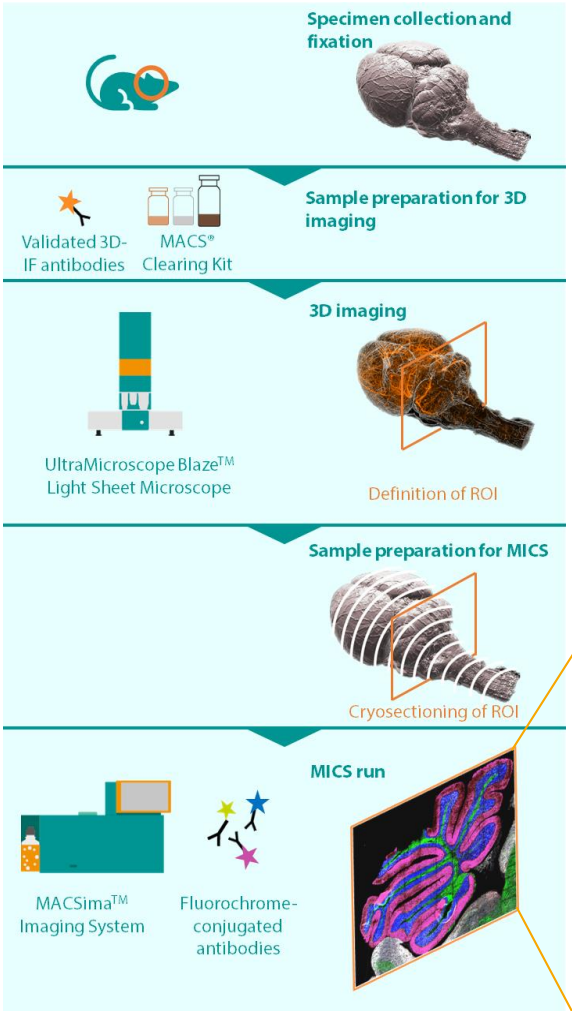


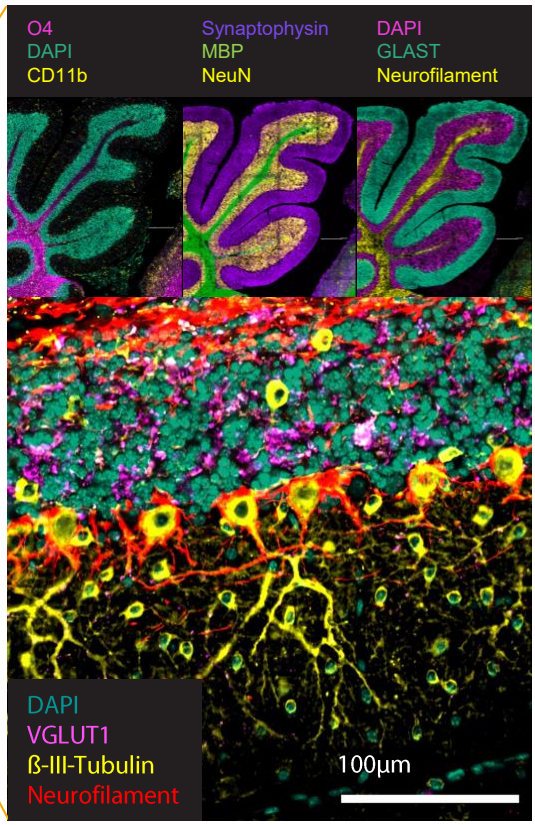
Figure 1: Illustration of a spatial biology workflow that combines MICS technology with prior 3D imaging

Interested to know more?
Download our full application note to learn more about the workflow combining 3D imaging and multiplex analysis

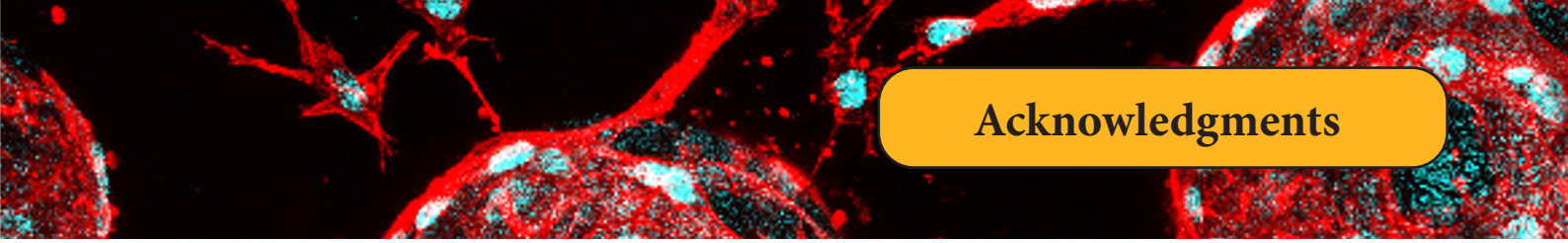
Explore our large-scale 3D imaging light sheet workflow

▶ **miltenyibiotec.com/3D-imaging-workflow**

Get in touch with our experts and explore the full potential of spatial biology through MACSima™ Platform



The pictures are from Luigi Prisco, Sara Rahmati, Marina Vemmer, and Kevin Bigott



Acknowledgments

We sincerely thank our corporate members, exhibitors, and supporters for their generous support. Their participation was crucial in making this symposium possible.

Attend the exhibitors' booths to check out their products, participate in raffles, and engage with their representatives.

The supporters are an integral component of the stem cells and cell therapy ecosystem.

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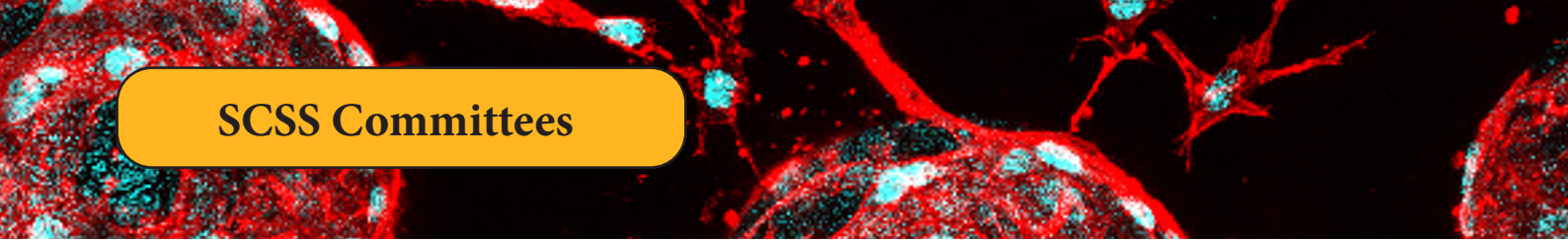

SCSS Committees

Symposium Organizing Committee (2023)

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Shigeki **SUGII** (*Institute of Molecular & Cell Biology, Singapore*)

Committee Members

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Pentao **LIU** (*University of Hongkong, Hongkong*)

Jonathan **LOH** (*Institute of Molecular and Cell Biology, Singapore*)

Alfred **SUN** (*Duke-NUS* Medical School, *Singapore)*

Gerald **UDOLPH** (*Stem Cell Society Singapore*)

Hongyan **WANG** (*Duke-NUS Medical School, Singapore*)

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Hongyan **WANG** (*DUKE-NUS Medical School*), ***Vice-president***

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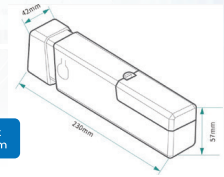
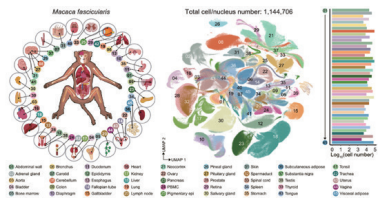


Data Analysis Package | Droplet Generation & Library Prep Reagents | Portable Droplet Generaton System

Application Cases

Case 1 Single-cell atlas of Macaca fascicularis

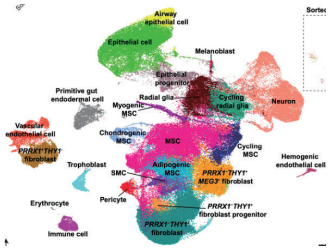


Han, L. et al, Nature 2022

Samples

45 organs or tissues of Macaca fascicularis

Case 2 Human totipotent stem cell

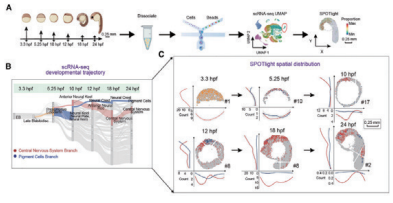


Mazid, MA. et al, Nature 2022

Samples

Human embryonic-like cells at the 8-cell stage (8CLC)

Case 3 Zebrafish development and differentiation

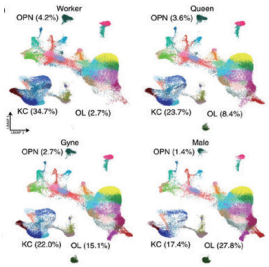


Liu, C. et al, Developmental Cell 2022

Samples

Zebrafish embryo

Case 4 Ant brain nuclei



Li, Q. et al. Nature Ecology & Evolution 2022

Samples

Ant brain

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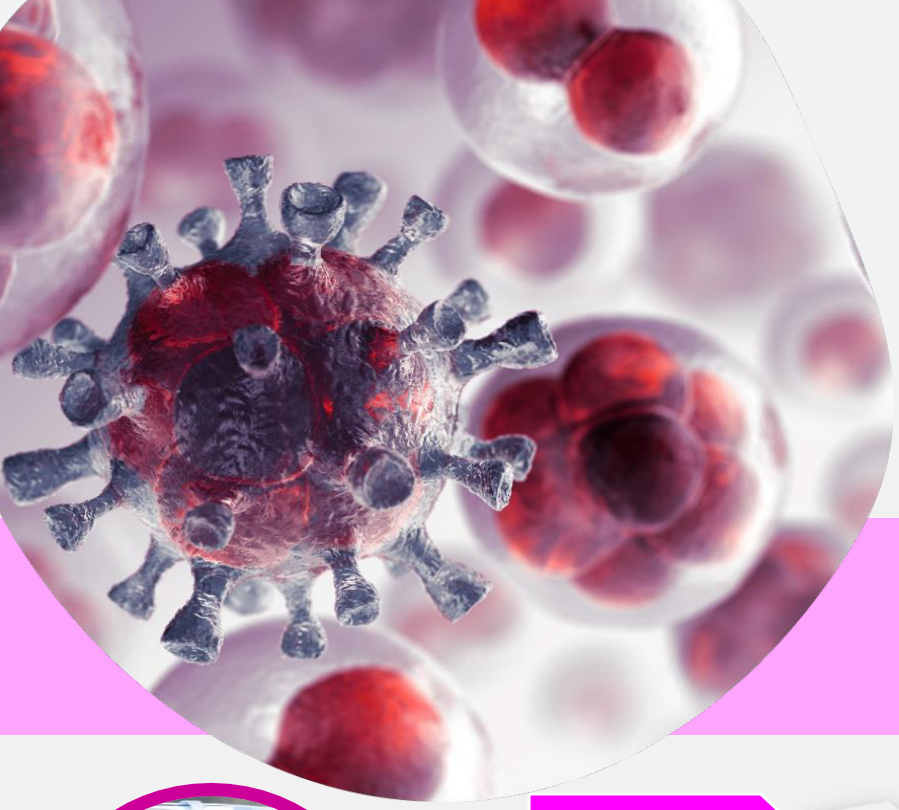

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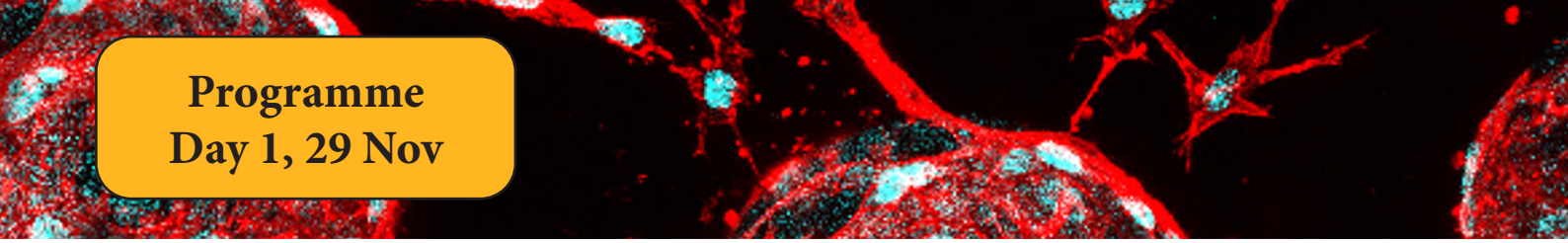
Programme



Programme
Day 1, 29 Nov

9:00 – 9:10 **Welcome & opening of Symposium 2023**
Jonathan Yuin-Han LOH, *Institute of Molecular & Cell Biology, Singapore & President SCSS*
Christine CHEUNG, *Nanyang Technological University, LKCMedicine, Singapore & Co-chair of Organizing Committee*

Session 1. Modelling development and disease

This session is supported by the
LEE KONG CHIAN SCHOOL OF MEDICINE, SINGAPORE
Chair: Christine CHEUNG, *Nanyang Technological University, LKCMedicine, Singapore*

9:10 – 9:35 **Statins inhibit vascular overgrowth in Infantile Hemangioma**
Joyce BISCHOFF, *Boston Children's Hospital, Harvard Medical School, US*

9:35 – 10:00 **Engineering a simple and robust EMULSION liver-chip to recapitulate full NASH functions**
Hanry YU, *National University of Singapore, Singapore*

10:00 – 10:25 **Polystyrene nanoplastics promote neurodegeneration by catalyzing Tau and TDP43 hyperphosphorylation**
Shiyan NG, *Institute of Molecular and Cell Biology, Singapore*

10:25 – 10:50 MORNING TEA BREAK

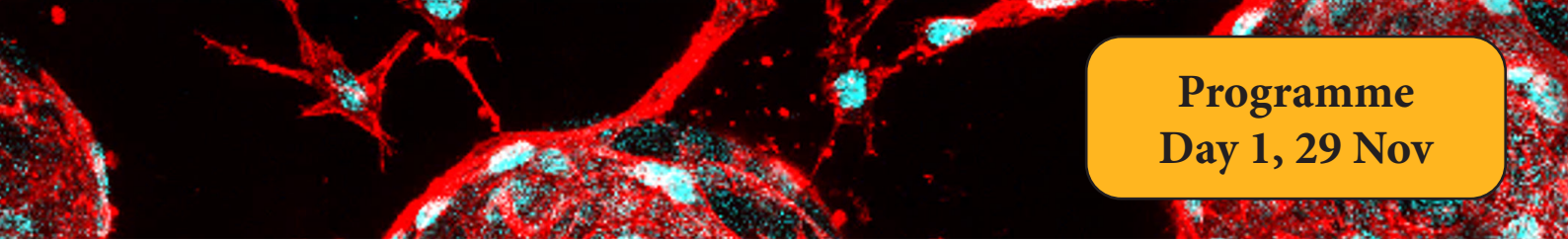
10:50 – 11:15 **Cardiomyocyte differentiation and dedifferentiation: is going up the hill simple a reverse of going down it**
Roger FOO, *National University of Singapore, Singapore*

11:15 – 11:30 Selected Abstract
Novel molecular mechanisms in the reactivation and regeneration of Drosophila quiescent neural stem cells
Mahekta R GUJAR, *Duke-NUS Medical School, Singapore*

11:30 – 11:45 *Industry-themed presentation by Sartorius*
Solutions for the culturing, maintenance and characterization of induced pluripotent stem
Ujwal Kumar JONNALA, *Sartorius India Pvt Ltd, India*

11:45 – 12:10 **Nose formation in a dish**
Shifeng XUE, *National University of Singapore*

12:10 – 13:30 LUNCH BREAK



Session 2. Adult stem cells and human diseases

This session is supported by the
DUKE-NUS MEDICAL SCHOOL, SINGAPORE
Chair: Hongyan WANG, *Duke-NUS Medical School, Singapore*

13:30 – 13:55 **Common mechanisms underlying the expansion of brain size and complexity in gyrencephalic mammals**
Fumio MATSUZAKI, *Kyoto University, Japan*

13:55 – 14:20 **Stem cell diversity and competition during brain development**
Qingfeng WU, Institute of Genetics and Developmental Biology, China

14:20 – 14:45 **Elucidating the novel functional role of astrocytes in Alzheimer's disease**
Li ZENG, *National Neuroscience Institute, Singapore*

14:45– 15:15 AFTERNOON TEA BREAK

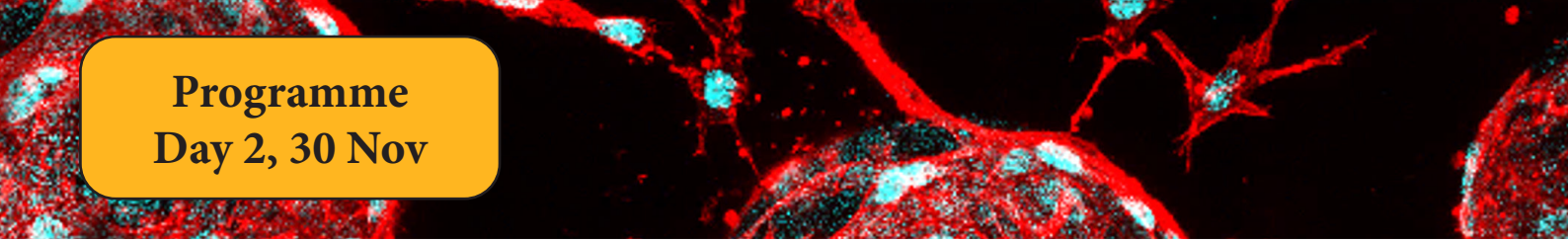
15:15– 15:40 **Uncovering novel glioblastoma dependencies for mechanism-guided therapies**
Derrick ONG, *National University of Singapore, Singapore*

15:40– 15:55 *Selected Abstract*
FOXO-regulated Deaf1 controls muscle regeneration through autophagy
Kah Yong GOH, *Duke-NUS Medical School, Singapore*

15:55– 16:20 **Deciphering stem cell roles in driving onset and progression of gastric cancer**
Nick BARKER, *Institute of Molecular & Cell Biology, Singapore*

16:20 – 18:30 POSTER SESSION WITH WINE & CHEESE

END OF DAY 1



Programme
Day 2, 30 Nov

9:00 – 10:00 **PRESIDENTIAL KEYNOTE LECTURE**
Chair: Jonathan Yuin-Han LOH, *Institute of Molecular and Cell Biology, Singapore*

Recent progress in iPSC research and application
Shinya YAMANAKA, CIRA, *Kyoto University, Japan*

Session 3. Totipotency & pluripotency

This session is supported by the
INSTITUTE OF MOLECULAR AND CELL BIOLOGY (IMCB), SINGAPORE
Chairs: Pentao LIU, *University of Hongkong, Hongkong* & Jonathan Yuin-Han LOH, *IMCB, Singapore*

10:00 – 10:25 **Recapitulating early mammalian development with totipotent-like stem cells**
Jichang WANG, *Sun Yat-Sen University, China*

10:25 – 10:50 **Expanded potential stem cells: A new tool for basic and translational research**
Pentao LIU, *The University of Hong Kong, Hongkong*

10:50 – 11:20 MORNING TEA BREAK

11:20 – 11:45 **Exploring molecular pathways associated with autism using cell-based models and genomics approaches**
Larry STANTON, *Qatar Biomedical Research Institute, Qatar*

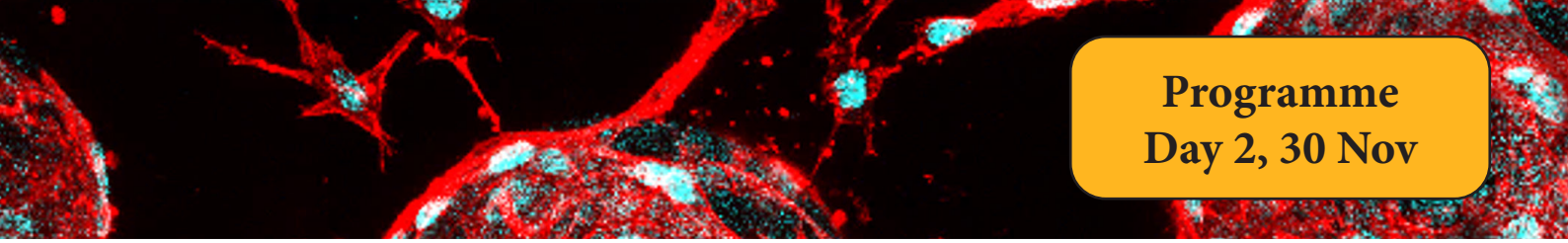
11:45 – 12:10 **Investigating the role of NELFA in the induction of totipotency**
Wee-Wei TEE, *Institute of Molecular & Cell Biology, Singapore*

12:10 – 12:25 *Selected Abstract*
Identification of novel transcription factor HOX regulating MSC stemness as an upstream factor of Twist1
Tong Ming LIU, *Institute of Molecular & Cell Biology, Singapore*

12:25 – 12:40 *Industry-themed presentation by MGI*
From decay to renewal: Harnessing the potential of human totipotent and pluripotent cells for rejuvenation
Md. Abdul MAZID, *Chinese Academy of Sciences, China*

12:40 – 13:05 **Ribosomal proteins regulate 2-cell-stage transcriptome in mouse embryonic stem cells**
Jonathan Yuin-Han LOH, *Institute of Molecular & Cell Biology, Singapore*

13:05 – 14:30 LUNCH BREAK



Programme Day 2, 30 Nov

Session 4. Maintaining cell quality of gene edited human pluripotent stem cells

Chair: Kimberly A. SNYDER, *STEMCELL Technologies Inc, Canada*

14:30 – 14:50 **Enhanced genetic stability of human pluripotent stem cells maintained as single cells under optimized culture conditions**
Kimberly A. SNYDER, *STEMCELL Technologies Inc, Canada*

14:50 –15:10 **PAX4 loss of function increases diabetes risk by altering human pancreatic endocrine cell development**
Hwee Hui LAU, *Institute of Molecular & Cell Biology, Singapore*

15:10 –15:30 **Genome-wide CRISPR screen and gene-editing for early regulators of cardiac lineage**
Mick LEE, *Genome Institute of Singapore*

Session 5. Advanced Organoids

Chair: Alfred SUN, *Duke-NUS, Singapore*

15:30 – 15:55 **Development and application of human neural organoids**
Yangfei XIANG, *ShanghaiTech University, China*

15:55 – 16:10 *Industry-themed presentation by Thermo Fischer Scientific*
Case studies for rapid scale up and directed differentiation of pluripotent stem cells in 3D culture
Wei Ching LOW, *Thermo Fisher Scientific, Singapore*

16:10 – 16:40 AFTERNOON TEA BREAK

16:40 – 17:05 **Morphing bioelectronics for developing organoids and animals**
Yuxin LIU, *National University of Singapore, Singapore*

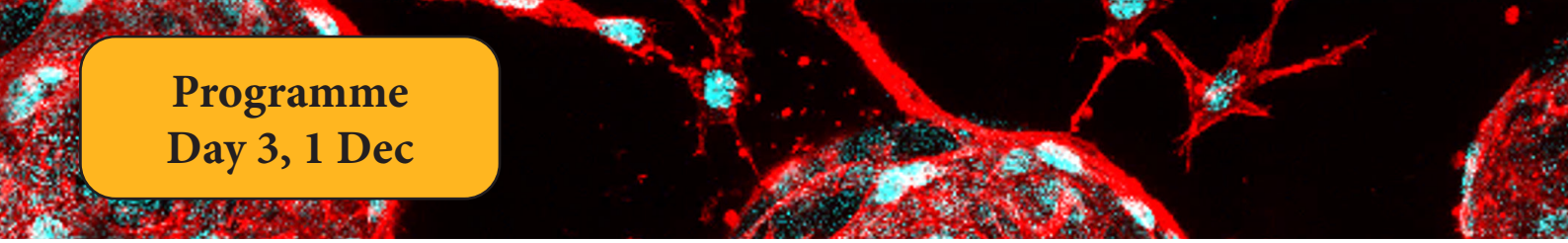
17:05 – 17:30 **Advancing therapeutic modalities discovery for multi-organ regeneration with tissue-derived organoid platform**
Winston CHAN, *Guangzhou National Laboratory, China*

17:30 – 17:55 **Interrogating kidney diseases using hPSC-derived kidney organoids**
Yun XIA, *Nanyang Technological University, LKCM, Singapore*

17:55 – 18:10 *Selected Abstract*
Uncovering the complexities of alveolar capillary development with organoid models
Nicole PEK, *Cincinnati Children's Hospital Medical Center, USA*

18:10 – 18:35 **Shaping neuronal networks by FEZ1-mediated trafficking**
John CHUA, *National University of Singapore, Singapore*

18:35 END OF DAY 2



Programme
Day 3, 1 Dec

9:00 – 10:00 **KEYNOTE LECTURE**
Chair: Hongyan WANG, Duke-NUS Medical School, Singapore

Translating stem cell technology to therapeutics
Suchun ZHANG, *Duke-NUS Medical School, Singapore*

Session 6. Innovations in cell therapy development and assessment
Chair: Wei Xiang SIN, *SMART-CAMP, Singapore*

10:00 – 10:25 **Decoding and rewiring Immunity**
Michael BIRNBAUM, *Massachusetts Institute of Technology, US*

10:25 – 10:50 **Integrative single cell transcriptomics and functional profiling with TRAPS-seq and PAINTKiller-seq**
Lih Feng CHEOW, *National University of Singapore*

10:50 – 11:15 **Challenges in manufacturing Natural Killer (NK) cells as allogeneic therapies targeting solid tumors**
Andy TAN, *Bioprocessing Technology Institute, Singapore*

11:15 – 11:45 MORNING TEA BREAK

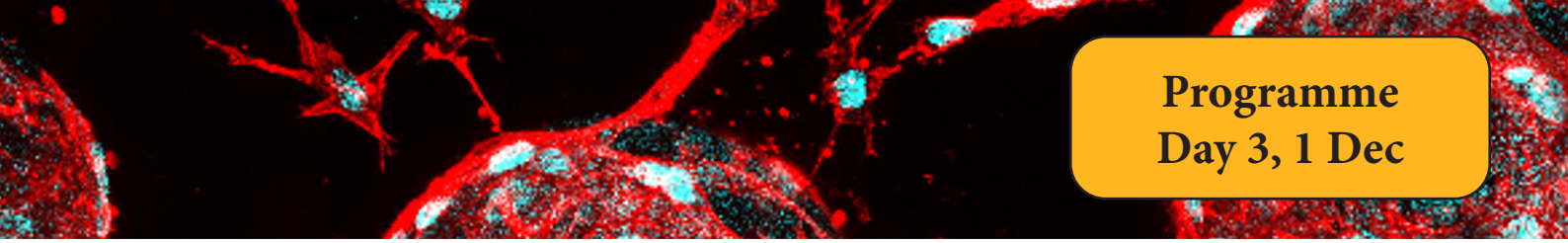
11:45 – 12:10 **3-dimensional human pancreatic microtumors to advance Immunotherapy**
Giulia ADRIANI, *Singapore Immunology Network*

12:10 – 12:25 *Selected Abstract*
COVID-19 antigenic peptides show immune response activity in allogeneic dendritic cells for possible surveillance-based vaccine
Francisco M. HERALDE III, *University of the Philippines Manila, Philippines*

12:25 – 12:40 *Industry-themed presentation by Miltenyi*
Advancing pluripotent stem cell research and manufacturing with Miltenyi Biotec
Parivash NOURI & Michaela DIAKATOU, *Miltenyi Biotec, Germany*

12:40 – 13:05 **A platform technology for rational reprogramming of cellular state**
Yen CHOO, *Nanyang Technological University, Singapore*

13:05 – 14:30 LUNCH BREAK



Programme Day 3, 1 Dec

Session 7. Innovations in cellular agriculture

Chair: Shigeki SUGII, *Institute of Molecular & Cell Biology, Singapore*

14:30 – 14:55 **Construction of structured Wagyu meat by 3D stem cell printing**
Michiya MATSUSAKI, *Osaka University, Japan*

14:55 – 15:20 **Surveillance of allergens in cultivated meat with AllerCatPro 2.0**
Sebastian MAURER-STROH, *Bioinformatics Institute, Singapore*

15:20 – 15:45 **The state of global cultivated meat research and development of edible plant-based scaffolds**
Deepak CHOUDHURY, *Bioprocessing Technology Institute, Singapore*

15:45 – 16:15 AFTERNOON TEA BREAK

16:15 – 16:40 **Cultivated meat as a climate solution: State of the science and the road ahead**
Maanasa RAVIKUMAR, *Good Food Institute - Asia Pacific, Singapore*

16:40 – 16:55 *Selected Abstract*
Accelerating cell therapy safety: rapid and accurate absolute quantification of adventitious agents using digital CRISPR approaches and beyond
Xiaolin WU, *Singapore-MIT Alliance for Research and Technology Centre, Singapore*

16:55 – 17:20 **Continuous flow cellular processing to scale complementary proteins**
XL LIN, *ESCO ASTER, Singapore*

17:20 – 17:55 **SCSS-Dr Susan Lim Award for Outstanding Young Investigator Presentation**
Chair: Xinyi SU, *Institute of Molecular & Cell Biology, Singapore*

Using human midbrain organoids to understand human dopamine neuron formation
Alfred SUN, *Duke-NUS Medical School, Singapore*

17:55 – 18:05 **AWARD PRESENTATION & CLOSING REMARKS**
Chair: Shigeki SUGII, *Institute of Molecular & Cell Biology, Singapore*

END OF DAY 3 - FAREWELL

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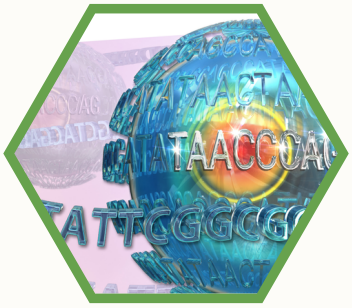

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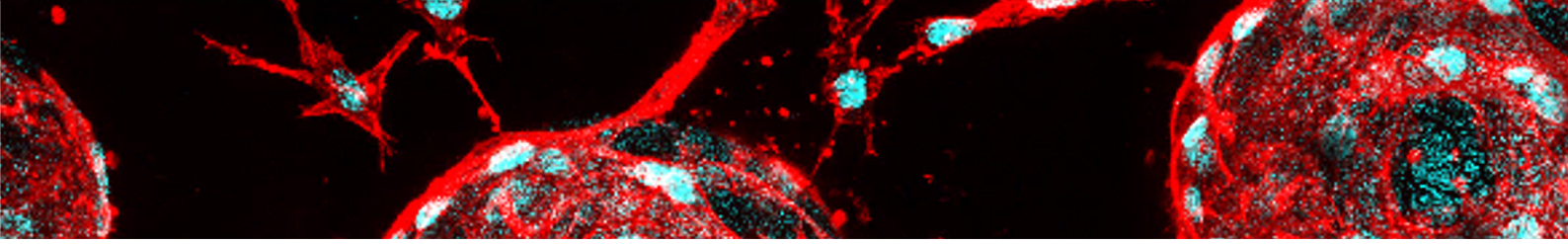


SPEAKER ABSTRACTS

Day 1

29 Nov 2023

Statins inhibit vascular overgrowth in Infantile Hemangioma

Joyce BISCHOFF

Vascular Biology Program, Boston Children's Hospital and Department of Surgery, Harvard Medical School, USA

ABSTRACT

Infantile Hemangioma (IH) is an extraordinary example of vascular overgrowth wherein vessels form rapidly over a year, then undergo a slow spontaneous involution. Propranolol was discovered serendipitously to be effective therapy for IH. We showed the non-beta blocker R+ enantiomers of propranolol and atenolol prevent hemangioma endothelial differentiation by directly interfering with the activity of the transcription factor SOX18 in hemangioma stem cells (HemSC). Further we showed that R+ propranolol and R+ atenolol, along with the small molecule SOX18 inhibitor Sm4, block hemangioma vessel formation in vivo in a pre-clinical model. By transcriptional profiling of R+ propranolol treated HemSC, we discovered the mevalonate pathway is an etiological component of IH, and that statins block HemSC endothelial differentiation and blood vessel formation. We propose a novel SOX18-mevalonate pathway axis as a central regulatory process that underpins IH-vascular overgrowth and further that mevalonate pathway blockers could be repurposed to prevent vascular overgrowth

BIO

Joyce Bischoff is Professor of Surgery at Harvard Medical School with a primary appointment in the Vascular Biology Program at Boston Children's Hospital. She received an A.B. in Chemistry from Duke University, a Ph.D. in Biochemistry and Molecular Biology from Washington University School of Medicine in St. Louis and post-doctoral training at the Whitehead Institute in Cambridge, MA. Her current research is focused on stem cells that drive vascular overgrowth in infantile hemangioma and on capillary malformations in Sturge-Weber Syndrome. Dr. Bischoff was co-Editor-in-Chief of Angiogenesis from 2005-2020, and currently serves on the editorial boards of Journal of Clinical Investigation, Circulation Research, ATVB and the Journal of Vascular Anomalies. She has served on numerous NIH study sections including as a member of the Cardiovascular Differentiation and Development Study Section from 2004-2008. She is the 2022 recipient of the Earl P. Benditt Award from the North American Vascular Biology Organization.

Engineering a simple and robust EMULSION liver-chip to recapitulate full NASH functions

Gowri BALACHANDER[1], Vishnu Goutham KOTA[1], NG Inn Chuan[1], Farah TASNIM[5], Roopesh PAI[1], LIM Yeesiang[1], Yoohyun SONG[5], Jacky ZHAO Junzhe[1], Kartik MITRA[1], Wahyunia SEPTIANA[2], Kexiao ZHENG[5], YAN xu[1], TENG Yao[1], LIM Sei Hien[4], Clarissa Bernice QUAH[4], Royston KWOK Peng Seng[5], Ng Huck Hui[5], Hanry Yu[1,2,3,5]

*[1]Department of Physiology & the Institute for Digital Medicine (WisDM), Yong Loo Lin School of Medicine & NUS College, National University of Singapore; [2]Mechanobiology Institute, National University of Singapore; [3]Critical Analytics for Manufacturing Personalized Medicine Interdisciplinary Research Group, Singapore-MIT Alliance for Research and Technology, Singapore; [4]AIM Biotech, Singapore; [5]A*STAR, Singapore*

ABSTRACT

Designing an in vitro model to recapitulate disease and progression involves identifying the simplest cell types and culture configurations to minimize the complexity and heterogeneity caused variability or inconsistency, yet sufficient to recapitulate all the functional features, thus the concept of function modules and complex functions. We have cultured organoids using human adult liver stem cells, and co-cultured iPSC-derived Kupffer cells and iPSC-derived Hepatic Stellate cells in adjacent compartments separated by micropillars allowing diffusion-based communications. We demonstrate that all cell types exhibit desirable phenotypes and together with induction factors all features of NASH progression such as macrosteatosis, microsteatosis and true microsteatosis, sustained inflammation linked to early and late ER stress and megamitochondria, hepatocellular ballooning and fibrosis. We have shown the robustness of the model in testing drug candidates used in various clinical trials and high degrees of concurrence with clinical and pre-clinical in vivo findings. Time permits, I will elaborate on methodology of how to define function module.

BIO

Hanry Yu is Professor of Physiology (NUSMed), NUS-College, and Mechanobiology (MBI) at the National University of Singapore; and co-leads a cell therapy manufacturing programme (CAMP) at the MIT research entity (SMART) in Singapore. He integrates biomaterials, tissue mechanobiology and engineering, biomedical optics and AI data analytics into solutions for pharmaceutical, environmental, and recently food industries. He has trained many students and staffs in leading universities in the US and Asia; built several institutions and companies, published >250 papers, delivered >250 invited talks, and consulted for international organisations and agencies.

Polystyrene nanoplastics promote neurodegeneration by catalyzing Tau and TDP43 hyperphosphorylation

Winanto[1,2], Li-Yi Tan1, Yong Shan Lim[3], Jolie Wan-Yun Ho[3], Boon Seng Soh[1], Yih-Cherng Liou[2], Cathy Chia-Yu Huang[4], Shuo-Chien Ling[3], Shi-Yan NG[1,3,5]

*[1]Institute of Molecular and Cell Biology (Cell and Biological Therapies Division), A*STAR Research Entities, Singapore; [2]National University of Singapore, Faculty of Science (Department of Biological Science), Singapore; [3]National University of Singapore, Yong Loo Lin School of Medicine (Department of Physiology), Singapore; [4]Department of Life Sciences, National Central University, Taoyuan, Taiwan; [5]National Neuroscience Institute, Singapore*

ABSTRACT

Polystyrene is a major environmental pollutant which eventually erodes into microplastics and nanoplastics, which leech into the environment and enter food that humans eventually ingest. Polystyrene nanoplastics (PS-NPs) cross the blood-brain barrier, and are taken up by cells in the central nervous system. Yet, it is still unclear if PS-NPs result in neurodegeneration in humans. If it does, the mechanisms by which PS-NPs potentiate neurodegeneration still remain elusive. In this study, we combined proteomics tools and human induced pluripotent stem cell-based modeling and demonstrated that PS-NP exposure led to neurodegenerative phenotypes that resemble Alzheimer's disease (AD) and Amyotrophic Lateral Sclerosis (ALS). We found that PS-NPs enter neurons readily and behave as catalytic scaffolds that co-bind Tau, Tar-DNA binding protein 43-kDa (TDP43) and their kinases, resulting in hyperphosphorylation of these aggregation-prone proteins. Our data therefore provides evidence that PS-NPs accelerate the onset of neurodegenerative diseases in humans.

BIO

Shi Yan NG is a Principal Investigator at the Institute of Molecular and Cell Biology, A*STAR and holds a concurrent appointment as the Director of Graduate Affairs (BMRC). She is also Adjunct Assistant Professor at the National University of Singapore (Yong Loo Lin School of Medicine, Department of Physiology) and a Visiting Scientist at the National Neuroscience Institute. Research in her lab centres around metabolic dysfunction in human neurological diseases and using patient iPSCs to evaluate potential therapeutics. Shi Yan is also a recipient of a number of scientific awards, such as the National Research Foundation Fellowship (2018), the L'Oréal For Women In Science National Fellowship (2019) and the SCSS Dr Susan Lim Award for Outstanding Young Investigator (2021). Prior to her current position, Shi Yan was a postdoctoral fellow in Lee Rubin's lab at Harvard University (2012-2015) and a PhD student at Larry Stanton's lab at the Genome Institute of Singapore (2008-2012).

Cardiomyocyte differentiation and dedifferentiation: is going up the hill simple a reverse of going down it

Roger FOO

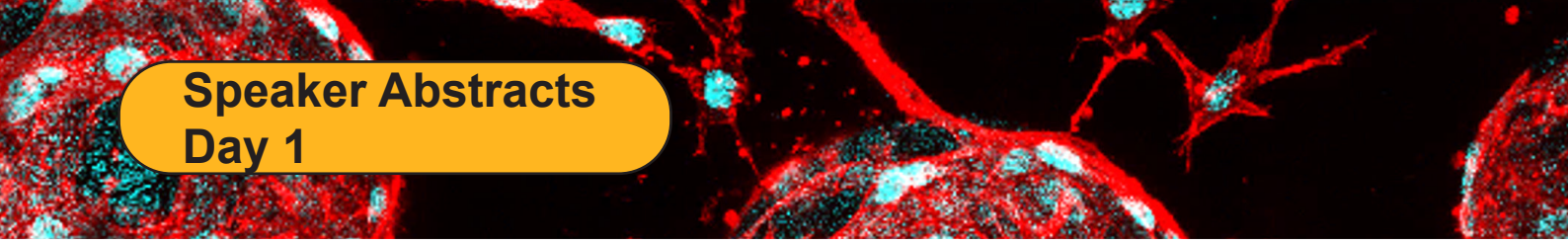
National University of Singapore, Singapore

ABSTRACT

The Foo lab makes use of the ES cell, iPS cell and animal cell models for the purpose of gene candidate discoveries. Gene expression regulation underpins cardiac cell state transitions, and the recognition of gene control switches points to potential new therapeutic or disease biomarker options. Here, we have used the genome-wide CRISPR approach and uncovered a molecular candidate, which mediates a mechanism as part of the cell adherens junction complex, that is necessary for cardiomyocyte differentiation. Conversely, a separate genome-wide screen identifies a marker gene for cardiomyocyte dedifferentiation which we find a gateway to precede cardiomyocyte regeneration. Both projects raise the interesting question of whether cardiomyocytes journey back up the hill of the epigenetic landscape (dedifferentiation) is simply the reverse of its journey downhill (differentiation).

BIO

Roger FOO is Zayed bin Sultan Al Nahyan Professor at the NUS School of Medicine, Director NUHS Cardiovascular Metabolic Disease Translational Research Programme, Cardiovascular Research Institute, Assistant Dean, Head NUHS Clinician Scientist Academy, and Senior Consultant, National University Heart Centre. He is an NUS graduate, spending 20 years abroad before returning to Singapore in 2013. His training was undertaken at Kings College Hospital, London, and Addenbrooke's Hospital, Cambridge. He was Wellcome Trust Fellow at Albert Einstein College of Medicine, New York, and returned to Cambridge to start a group as British Heart Foundation Fellow and Consultant Physician, before eventually returning to Singapore. His lab was the first to publish an epigenomic map of the failing human heart. More recently, he has published an in-depth analysis of the human cardiac chromatin 3D organisation, elucidating its changes during the heart disease response. The lab deep dives into the heart epigenome in continuing aspirations to discover mechanisms of disease for new therapies or biomarkers.



Speaker Abstracts Day 1

Abstract selected talk

Novel molecular mechanisms in the reactivation and regeneration of Drosophila quiescent neural stem cells

Mahekta R GUJAR[1], Yang Gao[1], Xiang Teng[2], Qiannan Deng[1], Kun-Yang Lin[1], Wei Yung Ding[1], Jiaen Lin[1], Ye Sing Tan[1], Liang Yuh Chew[1], Yusuke Toyama[2, 3], Hongyan Wang[1, 4, 5]

[1]Neuroscience and Behavioral Disorders Programme, Duke-NUS Medical School, Singapore, Singapore; [2]Mechanobiology Institute, Singapore, Singapore; [3]Department of Biological Sciences, National University of Singapore, Singapore, Singapore; [4]Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore; [5]Integrative Sciences and Engineering Programme, National University of Singapore, Singapore, Singapore

ABSTRACT

The ability of stem cells to switch between quiescent and proliferative states is crucial for maintaining tissue homeostasis and regeneration. In Drosophila, quiescent neural stem cells (qNSCs) extend a primary protrusion, which is a hallmark of qNSCs. Here, 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. A Golgi-resident GTPase Arf1 and its guanine-nucleotide exchange factor Sec71 promote NSC reactivation and regeneration via the regulation of microtubule growth. However, how Arf1 is regulated in qNSCs remains elusive. Here, we show that the microtubule minus-end binding protein Patronin/CAMSAP promotes acentrosomal microtubule growth and quiescent NSC reactivation. Patronin is important for the localization of Arf1 at Golgi and physically associates with Arf1, preferentially with its GDP-bound form. Patronin is also required for the regeneration of qNSC protrusion, likely via the regulation of microtubule growth. Finally, Patronin functions upstream of Arf1 and its effector Msps/XMAP215 to target the cell adhesion molecule E-cadherin to NSC-neuropil contact sites during NSC reactivation. Our findings have established Drosophila qNSCs as a new regeneration model and identified a novel Patronin-Arf1/Sec71-Msps pathway in the regulation of microtubule growth and NSC reactivation.

Industry-themed presentation by Sartorius

Solutions for the culturing, maintenance and characterization of induced pluripotent stem cells

Ujwal Kumar JONNALA

BioAnalytics, LPS, Sartorius India Pvt Ltd., India

ABSTRACT

From drug discovery to organoid modeling of disease, stem cells are increasingly being used in research as a vital tool for scientific investigation. The current trend away from animal models and the push to more relevant systems for simulating the human body require flexible and specific tools to achieve this goal. Induced Pluripotent Stem Cells (iPSCs) are produced from normal tissue, through the forced expression of key transcription factors, providing a limitless supply of these precious cells for research and development. Due to the specialized nature of these cells, their maintenance and culture is more intensive than most cell lines. Characterization of stem cells can be difficult and unreliable, depending on the methodology used, which is why it is important to develop robust techniques for monitoring stem cells throughout culture and experimental testing. If conditions are not optimal during the maintenance of iPSCs, their pluripotency can be lost. The presentation showcases the advantages of using a streamlined workflow combining multiple Sartorius systems for the culture, monitoring and characterization of iPSCs for several applications from drug development, disease modeling and clinical therapy research.

BIO

Ujwal Kumar JONNALA, a Field Application Scientist at Sartorius India, oversees Live Cell Imaging and Flow Cytometry. With 16+ years of experience, he's skilled in CRISPR Gene Editing and Cancer Cell Biology, and has a patent and 8 publications to his credit. He earned his undergraduate degree in Biochemistry, Genetics, and Biotechnology from SBMJC, Bangalore University, and diplomas in Genetic Engineering and Plant Tissue Culture. He further specialized in Biotechnology at Sri Ramachandra University, affiliated with Harvard Medical International. Ujwal began his career at Navya Biologicals, focusing on recombinant protein expression. He has since worked at the Centre for Cellular and Molecular Biology, India, Vimta Labs, Syngene-Biocon Bristol Myers Squibb R&D Centre, and Dr. Reddy's Laboratories. Additionally, he holds an MBA with a specialization in Operations Management and Pharmaceutical Business Management.

Nose formation in a dish

Shifeng XUE

Department of Biological Sciences, National University of Singapore, Singapore

ABSTRACT

Bosma arhinia microphthalmia syndrome (BAMS) is a rare genetic disorder where patients are born without a nose. Developmentally, the nose is made of cells from two embryonic origins, neural crest and cranial placode. To study the defects in nose formation in these patients, we differentiated iPSCs into neural crest and cranial placode cells. We find no defects in proliferation or apoptosis between control and BAMS cells. Instead, BAMS neural crest cells showed a reduced ability to migrate. There was also a reduced differentiation into the cranial placode lineage. Differential gene expression analysis detected cell adhesion as a major process that was misregulated in BAMS. In conclusion, BAMS is caused by reduced formation of the nasal placode and reduced migration of the neural crest cells. This work increases our understanding of the cellular processes involved in nose formation. Our ongoing work will examine the interactions between these two cell types.

BIO

Shifeng XUE obtained her PhD in developmental biology from University of California, San Francisco. She did a postdoctoral training at A*STAR, Singapore, in human genetics. She is currently an assistant professor at the National University of Singapore. She was awarded the Harold Weintraub Graduate Student Award in 2015 and the Young Scientist Award by the Singapore National Academy of Sciences in 2018. Her lab studies epigenetic regulation in development and disease, with a particular focus in epigenetic regulators involved in rare genetic diseases.

Common mechanisms underlying the expansion of brain size and complexity in gyrencephalic mammals

Quan Wu[1], Taeko Suetsugu[1], Yuji Tsunekawa[2], Hiroshi Kiyonari[3] and Fumio Matsuzaki[1]

[1]Department of Aging Science and Medicine, Graduate School of Medicine, Kyoto University; [2]Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy, The University of Tokyo. [3]Laboratory for Animal Resources and Genetic Engineering, Riken Center for Biosystems Dynamics Research, Japan

ABSTRACT

A rapid expansion of the cerebral cortex in size and complexity is a hallmark of mammalian evolution, leading to a folded brain. However, cortical size and complexity are highly variable across species, while all of them share a basic 6-layered structure. We have been investigating mechanisms underlying these features, using ferrets, as a gyrencephalic model that is experimentally manipulatable, with comparing with mice and humans. We found that ferret neural stem cell subtypes and their temporal pattern are similar to those in humans while the time scale is very different (3 weeks vs. 3months). This is more evident between mice and humans (a week vs. 3 months). Furthermore, ferret stem cell lineages turned out to be highly variable in contrast to the mouse stereotyped pattern. We discuss mechanisms for each of lineage variation and temporal scaling, and a possible unifying mechanism.

BIO

Fumio MATSUZAKI got his Ph.D. from the University of Tokyo. After a postdoctoral fellowship at the Tokyo Metropolitan Institute, then at the Rockefeller University, he began genetic research on *Drosophila* neurogenesis in 1989 as a section chief at the National Institute of Neuroscience in Tokyo. In 1998, he was appointed professor at Tohoku University, and then as a founding member of the RIKEN Center for Developmental Biology in 2002. He has been interested in the genetic programs and plastic mechanisms working for brain development. He established a principal mode in asymmetric division of neural stem cells based on Drosophila genetics. He is currently using mice and ferrets as model systems to explore (1) the common mechanisms for neural development as well as (2) specific mechanisms for brain expansion and complexity formation during mammalian evolution. His lab moved to Kyoto University Medical School in July 2023.

Stem cell diversity and competition during brain development

Yu-Hong ZHANG[1], Xue-Lian SUN[1], Zhenhua CHEN[1], Xize GUO[1], Qing-Feng WU[1,2]

Institute of Genetics and Developmental Biology, China

ABSTRACT

Brain develops in an intricately orchestrated sequences of stages, including neural patterning, neurogenesis, synaptogenesis and gliogenesis. Throughout the neurogenic process, the types and numbers of neurons produced by diverse neural stem cells contribute to the emergence of brain structure and function. However, the cellular mechanisms controlling the generation of either neuronal diversity or neuronal number have not been thoroughly studied. While fate predetermined model has been proposed to underlie the production of different neuronal types in the neocortex, we combined single-cell RNA-seq, population and single-cell lineage tracing to reveal a cascade diversifying model for the generation of extraordinary neuronal diversity in the hypothalamus. In parallel, we found that endogenous cell competition occurs between neural stem cells and intrinsically correlates with the activity of Axin2-p53 axis during neurodevelopment, which regulates neuronal number output and thereby brain size. Taken together, our studies offer novel insights into the cellular logic governing neurodevelopment.

BIO

Qing-Feng WU received an M.D. degree from Fudan University, obtained a Ph.D. degree from the Institute of Neuroscience, Chinese Academy of Sciences, and subsequently pursued his postdoctoral training at the Johns Hopkins University. After establishing his own lab in 2017 at the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, his research interests has been focusing on 1) hypothalamus development: from neurogenesis to circuit assembly; 2) hypothalamus function, hormones and disorders; and 3) homeostatic regulation of neural stem cells. He has garnered several accolades including Human Frontier Science Program (HFSP) postdoctoral fellowship, Maryland Stem Cell Research Fund (MSCRF) award and Hundred-Talent Program award. His recent impactful work has been featured in esteemed journals such as Cell, Cell Stem Cell, Developmental Cell, Science Advance and Nature Communications.

Elucidating the novel functional role of astrocytes in Alzheimer's disease

Li ZENG

National Neuroscience Institute, Singapore

ABSTRACT

Alzheimer's disease (AD) is the most common neurodegenerative disease worldwide. Due to the complexity of the brain and the disease, as well as the limitations with animal models, the understanding of AD pathophysiology is still rudimentary. Cerebral organoids (COs) are self-organizing and offer an unprecedented model with better structural and functional complexity that resembling the human brain. Here, we differentiated COs from patient PBMC-derived iPSCs that harbors PS1 mutation (MT) and its isogenic mutant-corrected controls (CRT). To decipher the function and regulation of different cell types in PS1 mutant COs, we conducted single-cell RNA sequencing (scRNA-seq) on PS1 MT and CRT COs and reveals the genes implicated in mitochondria function and lipid metabolism were dysregulated in PS1 MT astrocytes. We hypothesis that dysregulation of mitochondria and lipid metabolism in astrocyte at an early stage might play an important role in the pathogenesis of familial AD.

BIO

Li ZENG, a senior research scientist leading the Neural Stem Cells Research Lab and an Assistant Director, Research (Research Program) at the National Neuroscience Institute (NNI), Singapore. By closely working with NNI clinicians and clinician scientists, her laboratory focuses on translational research in neurodegenerative diseases, conducting research to unravel the neuro-pathophysiology crosstalk between Alzheimer's disease and Parkinson's disease. In addition, in order to circumvent the caveat in recapitulating the complexity and delicacy of the human brain, her lab generate 3D models of the human brain through cerebral organoids using human induced pluripotent stem cells (iPSCs) from NNI dementia patients who carry genetic mutations. Her study would value-add to the development of drug screening applications and personalized medicine strategy that targets dementia patients in early phase diagnosis.

Uncovering novel glioblastoma dependencies for mechanism-guided therapies

Derrick ONG

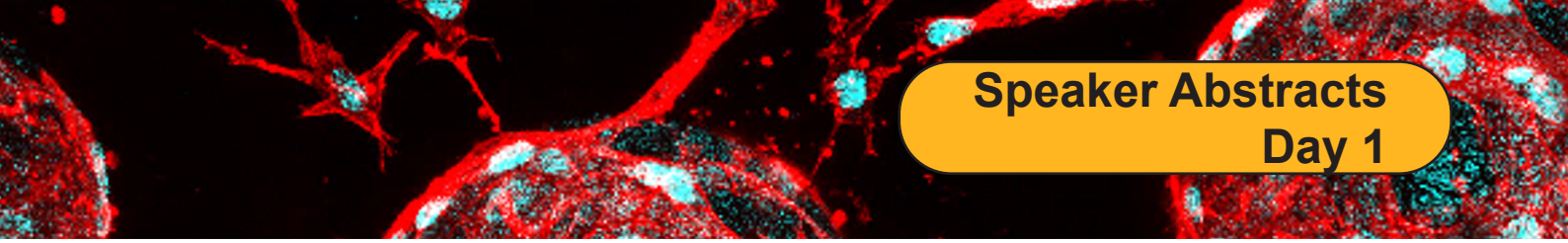
Department of Physiology, National University of Singapore, Singapore

ABSTRACT

Glioblastoma (GBM) is the most common and malignant adult brain tumor with an abysmal patient prognosis. The current standard of care for GBM remains to be aggressive surgery followed by radiotherapy, in combination with adjuvant temozolomide treatment. Tumor recurrence is almost inevitable due to the presence of glioma stem cells (GSCs), which exhibit stem cell–like traits, robust proliferation, invasiveness, therapy resistance and extensive cellular plasticity. We employ patient-derived GSCs as an experimental model to uncover new GBM dependencies that contribute to GBM clinical hallmarks. Here, I will outline some of our efforts towards a better molecular understanding of GBM pathogenesis, and how some of our basic science findings may be translated into actionable clinical modalities.

BIO

Derrick ONG is the President's Assistant Professor at the Department of Physiology, National University of Singapore. He obtained his PhD (Chemical Biology) at The Scripps Research Institute (CA) under Dr Jeffery Kelly, a pioneer in proteostasis. For his postdoctoral work, Derrick was mentored by Dr Ronald DePinho, an expert in aging and cancer, first at Dana Farber Cancer Institute/ Harvard Medical School, and then University of Texas MD Anderson Cancer Center. His major research interest lies in the identification of novel molecular mechanisms that contribute to glioblastoma pathogenesis, and to develop new therapeutic options for this deadly disease.



Abstract selected talk

FOXO-regulated Deaf1 controls muscle regeneration through autophagy

Kah Yong GOH[1,#], Wen Xing Lee[1,#], Sze Mun Choy[1], Gopal Krishnan Priyadarshini[1], Kenon Chua[1,2,3], Qian Hui Tan[4], Shin Yi Low[1], Chee Seng Wong[5], Jun Nishiyama[5], Nathan Harmston[1,4], and Hong-Wen Tang[1,6]

[1]Program in Cancer and Stem Cell Biology, Duke-NUS Medical School, Singapore; [2]Department of Orthopaedic Surgery, Singapore General Hospital, Singapore; [3]Programme in Musculoskeletal Sciences Academic Clinical Program, SingHealth/Duke-NUS, Singapore; [4]Division of Science, Yale-NUS College, Singapore; [5]Program in Neuroscience and Behavioral Disorders, Duke-NUS Medical School, Singapore; [6]Division of Cellular & Molecular Research, Humphrey Oei Institute of Cancer Research, National Cancer Centre Singapore, Singapore; [#]These authors contributed equally to this work

ABSTRACT

The commonality between various muscle diseases is the loss of muscle mass, function, and regeneration. Targeting muscle stem cells (MuSCs) regulators have been shown to be a promising therapeutic in repairing damaged muscles. However, the underlying molecular mechanisms driving myogenesis are poorly understood. We have identified a new regulator of muscle regeneration, Deformed epidermal autoregulatory factor 1 (Deaf1) - a transcriptional factor downstream of FOXO signaling. We showed that Deaf1 is transcriptionally repressed by FOXOs and that Deaf1 targets to PI3KC3 and Atg16l1 promoter regions and suppresses their expressions, thus inhibiting autophagy. Deaf1 depletion therefore induces autophagy which blocks MuSC survival and differentiation. In contrast, Deaf1 overexpression inactivates autophagy in MuSCs, leading to increased protein aggregation and cell death. Deaf1 depletion and overexpression both lead to defects in muscle regeneration, highlighting the importance of fine tuning Deaf1-regulated autophagy during myogenesis. Significantly, we further showed that Deaf1 expression is altered in sarcopenic and cachectic MuSCs. Manipulation of Deaf1 expression can attenuate muscle atrophy and restore muscle regeneration in the mouse models of sarcopenia and cancer cachexia. Together, our findings unveil an evolutionarily conserved role for Deaf1 in muscle regeneration, providing insights into the development of uncovering new therapies against muscle atrophy.

Deciphering stem cell roles in driving onset and progression of gastric cancer

Nick BARKER

*Research Director, A*STAR Institute of Molecular and Cell Biology, Singapore & Adjunct Professor, YLL School of Medicine, NUS*

ABSTRACT

Through comparative expression profiling of mouse Lgr5+ adult stem cells along the gastrointestinal tract, we identified novel pylorus-specific stem cell markers, including the membrane protein Aqp5. AQP5+ populations isolated from healthy human pylorus enriched for functional stem cells in organoid assays. Using new Aqp5-driven CreERT2 mouse models to selectively target conditional mutations to the pyloric stem cell compartment in vivo, we established that pyloric stem cells are a source of Wnt-driven, invasive gastric cancer. Furthermore, tumour-resident Aqp5+ cells can selectively initiate cancer organoid growth in vitro, identifying them as potential cancer stem cells. Ongoing characterization of the Aqp5+ cancer stem cells, their niche and the role of Aqp5 in promoting gastric cancer progression will be discussed. Using novel colon stem cell-specific Cre-ert2 drivers we additionally characterize the role of colon stem cells in driving colon cancer and generate accurate new mouse models of advanced colon cancer for mechanistic studies.

BIO

Nick BARKER obtained his PhD from Reading University, UK in 1996, before moving to the Netherlands to join Professor Hans Clevers research group as a Postdoc. In 2001, he joined Semaia Pharmaceuticals to develop colon cancer therapeutics, then returned to Hans Clevers' group as a Senior Staff Scientist in 2006. In 2010, he joined A*STAR's Institute of Medical Biology (IMB) as a Senior Principal Investigator and now holds the position of Research Director at the A*STAR Institute of Molecular and Cell Biology (IMCB). He has been a Web of Science Highly Cited Researcher since 2019 with over 36,000 citations. In 2022, he was elected to EMBO as an Associate Member and was awarded the Japanese Cancer Association International prize for cancer research. He is also an Adjunct Professor at Yin Long Loon School of Medicine, NUS Singapore.

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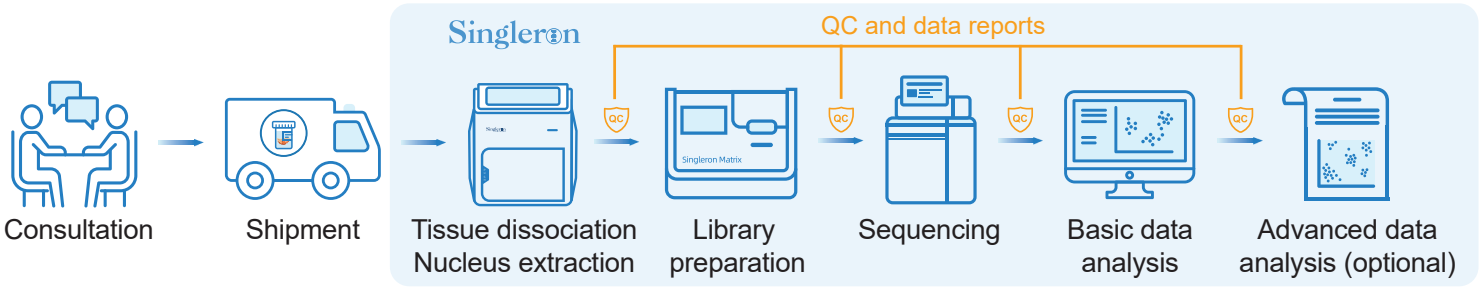

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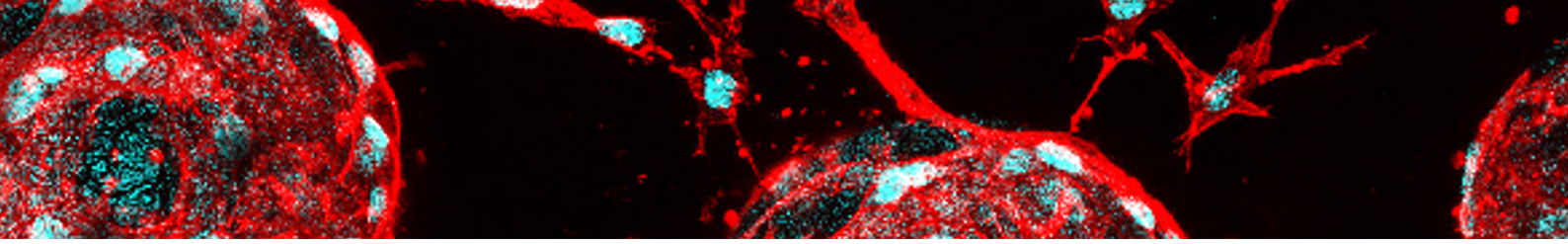
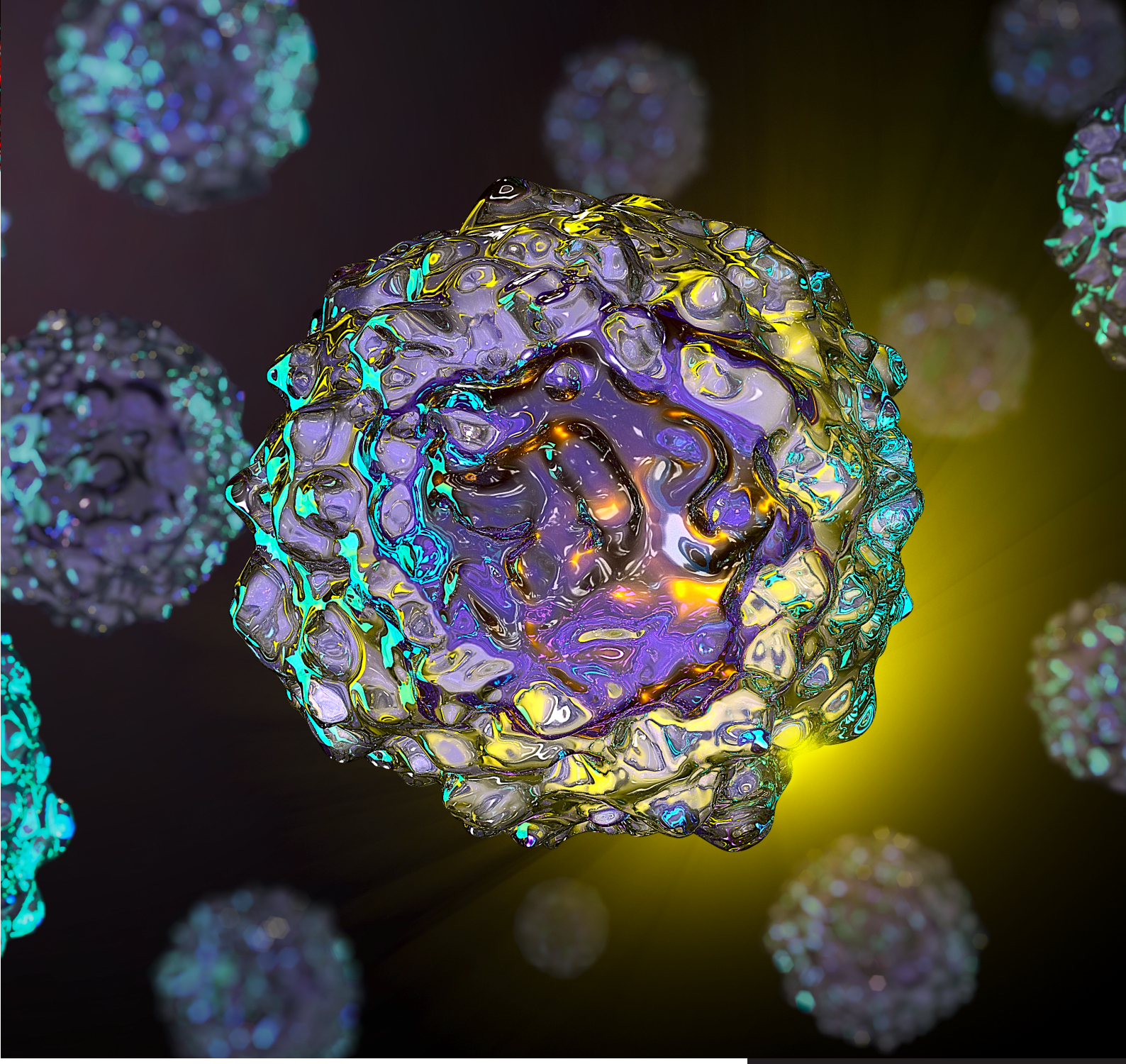


SPEAKER ABSTRACTS

Day 2

30 Nov 2023



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PRESIDENTIAL KEYNOTE LECTURE

Recent progress in iPSC research and application

Shinya YAMANKA[1,2,3]

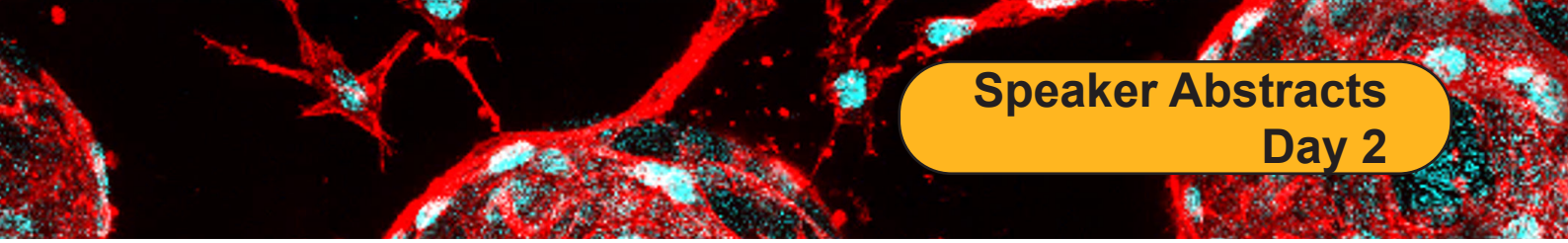
[1]CiRA, Kyoto University, Japan; [2]CiRA Foundation, Japan; [3]Gladstone Institutes, U.S.A.

ABSTRACT

Induced pluripotent stem cells (iPSCs) possess the remarkable ability to proliferate almost indefinitely and differentiate into multiple lineages, granting them a wide array of medical applications. The discovery of iPSCs has ushered in a new era of cell therapy. In Japan, clinical trials using iPSC-derived target cells and tissues for various diseases are already in progress. Moreover, the development of disease models using patient iPSCs has created an entirely new drug discovery process, particularly in the realm of intractable diseases. Furthermore, iPSC technology is significantly increasing the feasibility of personalized medicine, allowing us to treat patients in ways never before possible and promising to be a pivotal cornerstone of future medical practices. In the presentation, I would like to showcase recent progress and future prospects of medical applications using iPSCs in Japan, as well as the challenges we currently face and the initiatives we are undertaking to realize this vision.

BIO

Shinya YAMANAKA is the Director Emeritus of the Center for iPS Cell Research and Application (CiRA), Kyoto University and a Senior Investigator and the L.K. Whittier Foundation Investigator in Stem Cell Biology at the Gladstone Institute of Cardiovascular Disease (GICD). He is also a President of Public Interest Incorporated Foundation, CiRA Foundation. He is most recognized for his original research on induced pluripotent stem (iPS) cells. Since his breakthrough finding, he has been the recipient of many prestigious awards, including the Nobel Prize in Physiology or Medicine jointly with Dr. John Gurdon (2012). Human iPS cells and their derivatives offer a new model for disease modeling, drug discovery, and regenerative medicine. His primary vision is to overcome diseases by delivering iPS cell-based innovative therapeutic options.



Recapitulating early mammalian development with totipotent-like stem cells

Jichang WANG

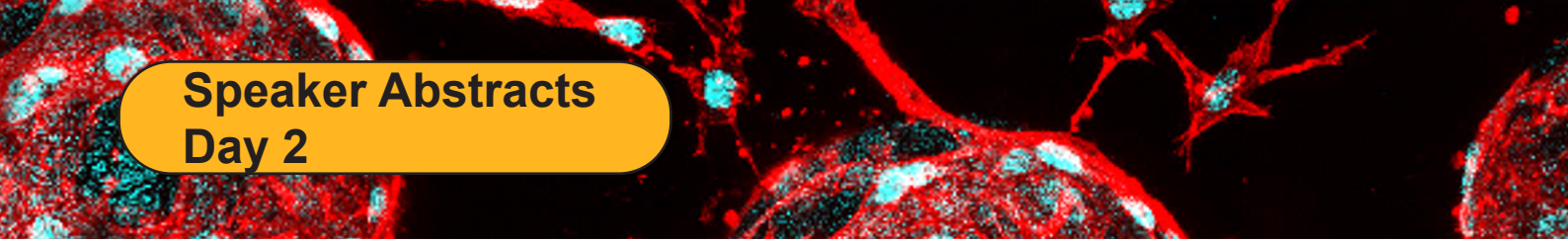
Zhongshan School of Medicine, Sun Yat-sen University, China

ABSTRACT

Abnormal zygotic genome activation (ZGA) leads to developmental defects and even contributes to the failure of mammalian blastocyst formation or implantation. An in vitro cell model mimicking early blastomeres would be invaluable to understanding the mechanisms regulating key biological events during early mammalian development. By multi-omics analysis and targeted chemical screening with totipotent-specific reporters, we successfully established totipotent-like stem cells from mouse and human embryonic stem cells. These novel totipotent-like stem cells express a panel of ZGA genes and have a unique transcriptome resembling that of the mouse 2C and human 8C embryo, respectively. Functionally, these early embryonic-like cells can self-organize to form blastocyst-like structures. Therefore, these totipotent-like stem cells represent a highly valuable cell type for studying totipotency and early mammalian development.

BIO

Jichang WANG (王继厂), is currently a Professor of Histology and Embryology in Sun Yat-sen University (SYSU), Guangzhou, P.R. China. My main research interest is to elucidate the function of retrotransposons (e.g., endogenous retrovirus, LINE-1) in mammalian early development and neurodevelopmental diseases by using stem cells and interspecies chimeras as models and by integrating genome editing and multi-omics technologies. I am supported by the National Science Fund for Distinguished Young Scholars, the National Recruitment Program for Young Scholars, the National Key Research and Development Program of China, the National Science Foundation of China and the Start-up Fund of SYSU. I was a winner of the Chinese Government Award for Outstanding Self-financed Students Abroad (2014) and the “Publication of the Year” award of the German Stem Cell Network (2015). I have published many papers in Nature, Cell Stem Cell, Nature Protocols, Cell Reports, Nucleic Acids Research, Nature Communications, etc.



Speaker Abstracts Day 2

Expanded potential stem cells: A new tool for basic and translational research

Pentao LIU

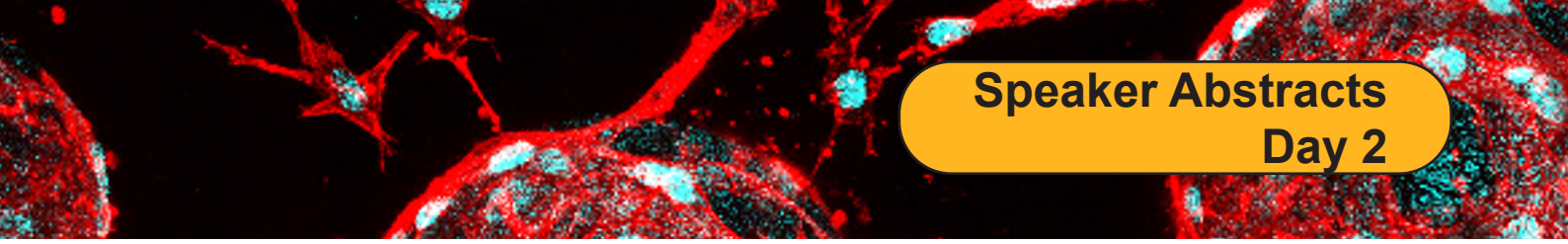
School of Biomedical Sciences, Li Ka Shing Faculty of Medicine, The University of Hong Kong; Centre for Translational Stem Cell Biology Health@InnoHK

ABSTRACT

By inhibiting signals implicated in the earliest embryo development, we established mouse expanded potential stem cells (EPSCs) from 4-cell and 8-cell blastomeres. A single EPSC can contribute to both the embryo proper and the TE lineages in chimera assay. Molecular analyses of the epigenome and single-cell transcriptome reveal that EPSCs possess enriched features of cleavage stage embryos. The EPSCs concept has enabled the establishment of EPSC lines of multiple mammalian species. EPSCs share similar molecular features and developmental potential cross the species, are genetically and epigenetically stable in homogenous long-term cultures, and permit efficient precision genome editing. EPSCs thus provide a new tool for studying development and open up new avenues for translational research in biotechnology and agriculture. For example, early syncytiotrophoblasts generated from human EPSCs are highly susceptible to coronavirus infection and are sensitive to antiviral treatment, which may facilitate stem cell-based antiviral drug discovery.

BIO

Pentao LIU is a biologist and a geneticist with a long-standing interest in stem cells, development, genomics and immunity. He is recognized for his work on developing genetic tools for engineering mouse stem cells, discovery of functions of Bcl11a and Bcl11b genes in lymphocyte development, and establishment of expanded potential stem cells. Pentao graduated from Henan Normal University in China with a BS degree. He received MPhil from Chinese Academy of Sciences and Ph.D. from Baylor College of Medicine. Following a postdoc training at National Cancer Institute USA, he joined the faculty of the Wellcome Trust Sanger Institute in Cambridge, U.K. He is currently a professor at the HKU and heads Centre for Translational Stem Cell Biology, aiming to develop new stem cell technologies and to screen and produce clinically relevant products.



Speaker Abstracts
Day 2

Exploring molecular pathways associated with autism using cell-based models and genomics approaches

Lawrence W. STANTON[1,2]

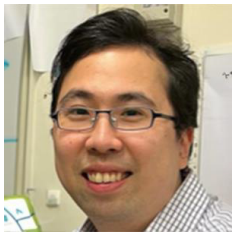
[1]Qatar Biomedical Research Institute; [2]College of Health and Life Sciences Hamad bin Khalifa University, Qatar

ABSTRACT

Whole genome sequencing of a Qatari cohort of autism spectrum disorder (ASD) subjects and non-affected family members has revealed hundreds of rare genetic variations arising de novo or recessively inherited. The study of the genetic basis of complex human diseases, such as autism, is hampered by lack of access to patient-derived tissues. Disease modeling has recently made a tremendous leap forward with the ability to make patient specific and gene-edited induced pluripotent stem cells. By directed in vitro differentiation, the human iPSC can be converted to human cell types of interest, including neurons, that are endowed with the underlying genetic variation. We have generated isogenic iPSC lines that are CRISPR-edited to express a candidate ASD genetic variant of the TRPC6 gene. We have also generated iPSC lines from monozygotic ASD triplets and genetically corrected isogenic lines to investigate the role of NAPB in autism etiology. We have compared wild-type vs mutant-expressing neuronal cells using functional assays and genomics approaches to reveal the molecular mechanisms underlying the disease pathology.

BIO

Larry STANTON joined the Qatar Biomedical Research Institute in 2019 as Director of the Neurological Disorders Research Center. QBRI is a government-funded research institute, affiliated with the Hamad bin Khalifa University, focused on translating basic research into treatments for diabetes, neurological disorders, and cancer. His lab is generating induced pluripotent stem cells (iPSC) from patients with neurodegenerative/neurodevelopmental disorders with the aim to direct the growth, differentiation, and reprogramming of these cells into clinically relevant tissues and models of human diseases. Converting these patient-specific iPSC into various neural cell types provides the opportunity to study in vitro the molecular and cellular bases of neurodegeneration and impaired neurodevelopment. Dr. Stanton served (2017-2019) as Executive Director of Cell and Molecular Biology Research at Humacyte Inc [USA] where he led a tissue engineering group developing transplantable, human blood vessels. Previously (2002-2016), he was a Principal Scientist and served as Deputy Director at the Genome Institute of Singapore. Prior to relocating to Singapore, he spent 14 years at several biotechnology companies in the San Francisco Bay Area.



Investigating the role of NELFA in the induction of totipotency

Dennis TAN Eng Kiat[1], Zhenhua HU[1], Ying DING[1], Hwei Fen LEONG[1] and Wee-Wei TEE[1,2]

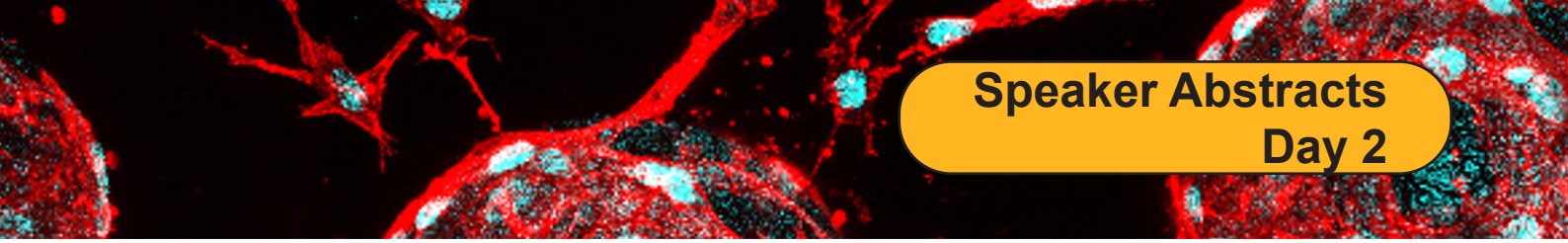
*[1]Chromatin Dynamics and Disease Epigenetics Group, Institute of Molecular and Cell Biology, Agency for Science, Technology and Research (A*STAR), Singapore; [2]Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore.*

ABSTRACT

Totipotency represents the pinnacle of the cellular hierarchy of developmental potency. Extensive epigenetic reprogramming occurs around fertilization that is important for the re-establishment of totipotency in the embryo. Recently, we identified a novel maternal factor, NELFA, that is able to induce an experimental totipotent-like state in pluripotent embryonic stem cells (Nat Cell Biol. 2020 Feb;22(2):175-186). However, the mechanistic underpinnings of how NELFA achieves this remarkable feat remain unclear. In this talk, I will present our latest findings how NELFA catalyses distinctive epigenetic reprogramming events during the pluripotent-to-totipotent transition, including selective deployment of histone variants and heterochromatin remodelling. Furthermore, through a systematic deletion analysis, we have uncovered crucial regions of NELFA that play a pivotal role in driving the expression of totipotent genes, independent of the conventional NELF complex.

BIO

Wee-Wei TEE previously trained in the laboratories of Prof. Azim Surani, an eminent stem cell biologist, and Prof. Danny Reinberg, a leading expert in epigenetics. He is currently a Principal Investigator at A*STAR IMCB, and an Assistant Professor at National University Singapore. In his independent laboratory, he is dedicated to investigating the epigenetic mechanisms that govern cell fate transitions during early mammalian development, as well as in the context of cancer cell phenotypic plasticity. He has received several scientific awards and fellowships, including the highly competitive NIH Pathway to Independence (K99/R00) Award, New York Stem Cell Foundation Druckenmiller Fellowship, the Singapore National Research Foundation Fellowship, and more recently, selected as an EMBO Global Investigator.



Abstract selected talk

Identification of novel transcription factor HOX regulating MSC stemness as an upstream factor of Twist1

Tong Ming LIU[1], Dennis Eng Kiat1, Yingying Zeng[1], Autio Matias Ilmari[2], Yating Michelle Eio[1], Xiaohua LU[1], Wei Lam[1], Eng Hin Lee[3], James H Hui[3], Bing Lim[2], Wee Wei Tee[1], Jonathan Loh Yuin Han[1], Simon Cool[1]

*[1]Institute of Molecular and Cell Biology (IMCB), Agency for Science, Technology and Research (A*STAR), Singapore; [2]Genome Institute of Singapore, Agency for Science, Technology and Research (A*STAR), Singapore [3]Department of Orthopaedic Surgery, National University of Singapore, Singapore*

ABSTRACT

Human mesenchymal stem cells (MSCs) represent one of the most used stem cells for clinical application. However, the molecular basis of MSC stemness still remains poorly understood, which greatly affects their clinical applications. Using our established step-wise iPSC-MSC platform, we screened one novel transcription factor HOX regulating MSC stemness. Knockdown of HOX gene abolished MSC proliferation and greatly decreased colony formation (CFU-F). Moreover, accelerated MSC senescence and a decrease in the expression of cell surface antigens linked to the MSC phenotype was observed, multi-linage differentiation was greatly impaired. Notably, overexpression resulted in improved multi-lineage differentiation of MSCs. HOX expression decreases with MSC important genes during in vitro expansion. ChIP-seq data showed that HOX binding sites at early passage of MSCs were associated with H3K4me3 binding sites. HOX lose majority of binding sites at late passage of MSCs. Most importantly, HOX directly regulated Twist1. Twist1 overexpression partially rescued decreased MSC proliferation by HOX knockdown. In addition, HOX reporter can be used to enrich good quality of MSCs. These data showed HOX regulates MSC stemness as an upstream factor of Twist1. The identification of the novel transcription factor HOX provides insights into MSC stemness and novel strategy for gene therapy of MSCs.

Industry-themed presentation by MGI

From decay to renewal: Harnessing the potential of human totipotent and pluripotent cells for rejuvenation

Md. Abdul MAZID1 , Wenjuan LI1, Longqi LIU2,3 and Miguel A. ESTEBAN1,3

[1]Laboratory of Integrative Biology, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou China; [2]BGI-Hangzhou, China; [3]BGI-Shenzhen, China

ABSTRACT

Understanding early human embryonic development is crucial for fertility-related disorders, regenerative medicine and in vitro studies. The zygote, formed by the fusion of oocyte and sperm, remains transcriptionally quiescent until the totipotent 8-cell embryo stage when zygotic genome activation occurs. However, limited ethical access, scarce embryos and lack of in vitro models have impeded our understanding of early human embryogenesis and totipotency. Recently, we have established novel methodologies for producing human 8-cell embryo-like cells (8CLCs) from pluripotent stem cells (PSCs), closely resembling the 8-cell embryo both transcriptionally and epigenetically. Importantly, 8CLCs efficiently differentiate into embryonic and extraembryonic tissues, both in vitro and in vivo. Remarkably, using donor naïve PSCs induced by our method, we have generated live-born chimeric monkeys with germline contributions and produced human kidneys in pigs. Therefore, our approach facilitates studying human early embryogenesis and brings us closer to harnessing the potential of totipotency and pluripotency for rejuvenation.

BIO

I am **Md Abdul MAZID**, originally from Bangladesh, currently serving as an Associate Professor at the Guangzhou Institutes of Biomedicine and Health, China. My research focuses on investigating human early embryogenesis, particularly zygotic genome activation at the totipotent 8-cell stage, as well as functional cells and organ generation for rejuvenation purposes. I have contributed as a first, co-first and corresponding author to publications in prestigious journals such as “Nature”, “Cell”, “Stem Cell Research” and in “Cell Stem Cell”, “Nature Communications”, “Nucleic Acids Research”, “Science Advances”, “Nature Cell Biology” as co-author. Additionally, I have four patents, two international and two domestic in China. I have secured several grants, including the NSFC, CAS-PIFI and the Foreign Young Talent Project. Throughout my scientific career, I have been recognized with various awards, including the Huangpu District High-Level Talent Award, the Foreign Young Talent Award, the CAS-PIFI Foreign Expert Award, the CAS-TWAS President’s PhD Fellowship.

Ribosomal proteins regulate 2-cell-stage transcriptome in mouse embryonic stem cells

Jonathan Yuin-Han LOH

*Cell Fate Engineering and Therapeutic Laboratory, A*STAR Institute of Molecular and Cell Biology (IMCB), Singapore*

ABSTRACT

A rare sub-population of mouse embryonic stem cells (mESCs), the 2-cell-like cell, is defined by the expression of MERVL and 2-cell-stage-specific transcript (2C transcript). Here, we report that the ribosomal proteins (RPs) RPL14, RPL18, and RPL23 maintain the identity of mESCs and regulate the expression of 2C transcripts. Disregulation of the RPs induces DUX-dependent expression of 2C transcripts and alters the chromatin landscape. Mechanistically, knockdown (KD) of RPs triggers the binding of RPL11 to MDM2, an interaction known to prevent P53 protein degradation. Increased P53 protein upon RP KD further activates its downstream pathways, including DUX. Our study delineates the critical roles of RPs in 2C transcript activation, ascribing a novel function to these essential proteins.

BIO

Yuin-Han Jonathan LOH is the Deputy Executive Director and Research Director at the A*STAR Institute of Molecular and Cell Biology (IMCB), where he also serves as the Director for the Cell Biology and Therapies Research Division. Concurrently, he is a Professor (Adjunct) at the National University Singapore (NUS) Yong Loo Lin School of Medicine and a Faculty member of the NUS Graduate School of Integrative Sciences and Engineering. His current research focuses on dissecting the mechanisms regulating cell fate changes, including how 1) epigenetic factors work with endogenous retro-element to coordinate gene expression programme (2015 Cell), 2) transcription factors direct transdifferentiation and somatic cell reprogramming (2016 Nat Commun.; 2020 Science Adv.), and 3) epitranscriptome regulation of cell states (2023 Molecular Cell). His research work has earned him several prestigious national and international accolades including the MIT TR35 Asia Pacific Award, Singapore Young Scientist Award. World Technology Network Fellowship, Stem Cell Society Singapore Outstanding Investigator Award, Entrepreneurship World Cup. He serves as the President of Stem Cell Society Singapore and the Executive council member of the Singapore Association for the Advancement of Science.

Enhanced genetic stability of human pluripotent stem cells maintained as single cells under optimized culture conditions

Kimberly A. SNYDER[1], Adam J. HIRST[2], Vicky J. WANG[1], Darielle J. LIM[1], Helen VO[1], Thuy T. HOANG[1], Allen C. EAVES[1,3], Sharon A. LOUIS[1], and Arwen L. HUNTER[1]

[1]STEMCELL Technologies Inc., Vancouver, Canada, [2]STEMCELL Technologies UK Ltd., Cambridge, United Kingdom, [3]Terry Fox Laboratory, BC Cancer, Vancouver, Canada

ABSTRACT

Genetic instability is a significant challenge for stem cell researchers as cytogenetic abnormalities in human pluripotent stem cells (hPSCs) can confer strong selective advantages and altered phenotypes. While aggregate passaging is the gold standard method for maintaining hPSCs, routine single-cell passaging can simplify some workflows. Increased selective pressure associated with single-cell passaging is linked with genetic instability. To address this, we developed eTeSR™, a novel medium formulated to support routine, single-cell maintenance of hPSCs. To assess genomic stability, 48 independent clones from two hPSC lines were single-cell passaged for 20 weeks in eTeSR™ or two control media. Clones were screened for recurrent abnormalities using a qPCR-based method, as well as in-depth SNP microarray analysis. 53% (25/47) and 46% (22/48) of the hPSC clones developed recurrent abnormalities after maintenance in Control Medium 1 and 2, respectively. In contrast, the incidence of abnormalities was remarkably lower for hPSCs maintained in eTeSR™, with abnormalities detected in only 7% (3/46) of clones 20 weeks of single-cell maintenance. hPSCs cultured as single cells in eTeSR™ displayed characteristic hPSC morphology, undifferentiated cell marker expression, tri-lineage differentiation, and displayed improved cellular expansion and cloning efficiencies. In summary, eTeSR™ supports high-quality single-cell hPSCs suitable for downstream applications.

BIO

Kimberly Snyder obtained her Master of Science in Experimental Medicine at the University of British Columbia in 2014. She completed her studies under the supervision of Dr. Kelly McNagny studying the role of the CD34-related sialomucin, podocalyxin in metastatic breast cancer. In 2014, Kim was recruited to STEMCELL Technologies Inc. and is a Senior Scientist in R&D working in the Pluripotent Stem Cell Biology Team. In the past, Kim has worked on novel pluripotent stem cell media development for the maintenance of human pluripotent stem cells (hPSCs) and has recently developed a new cGMP animal origin-free hPSC medium, TeSR™-AOF. Currently, Kim is leading a team working on a variety of hPSC projects from novel hPSC medium formulation and development to evaluation and commercialization of new hPSC technologies.

PAX4 loss of function increases diabetes risk by altering human pancreatic endocrine cell development

HH Lau[1,2,#], N Krentz[3,4,5,#], F Abaitua[4], M Perez-Alcantara[4], JW Chan[1,2], J Ajeian[6], S Ghosh[7], Y Lee[3], J Yang[3], S Thaman[3], B Champon[4], H Sun[3], A Jha[3], S Hoon[8], NS Tan[2,9], D Gardner[10], SL Kao[11,12], ES Tai[11,12,13], AL Gloyn[3,4,6,14,*], AKK Teo[1,12,15,*]

*[1]Stem Cells & Diabetes Laboratory, IMCB, A*STAR; [2]School of Biological Sciences, NTU, SG; [3]Division of Endocrinology, Stanford University School of Medicine, USA; [4]Wellcome Centre for Human Genetics, University of Oxford, UK; [5]Faculty of Pharmaceutical Sciences, University of British Columbia, Canada; [6]Oxford Centre for Diabetes Endocrinology & Metabolism, University of Oxford, UK; [7]Cancer Science Institute of Singapore, National University of Singapore; [8]Molecular Engineering Laboratory, A*STAR; [9]Lee Kong Chian School of Medicine, SG; [10]Department of Endocrinology, Singapore General Hospital; [11]Department of Medicine, National University Health System, SG; [12]Department of Medicine, Yong Loo Lin School of Medicine, SG; [13]Saw Swee Hock School of Public Health, SG; [14]Stanford Diabetes Research Center, Stanford University, USA; [15]Department of Biochemistry, Yong Loo Lin School of Medicine, SG; [#]These authors jointly contributed to this work. *These authors jointly supervised this work*

ABSTRACT

The coding variant (p.Arg192His) in the transcription factor *PAX4* is associated with an altered risk for type 2 diabetes (T2D) in East Asian populations. In mice, *Pax4* is essential for beta cell formation but its role on human beta cell development and/or function is unknown. We demonstrate that individuals without diabetes that carry either the *PAX4* p.Arg192His or a newly identified p.Tyr186X allele exhibit decreased pancreatic beta cell function. In the human beta cell model, EndoC-βH1, *PAX4* knockdown led to impaired insulin secretion, reduced total insulin content, and altered hormone gene expression. Deletion of *PAX4* in isogenic human induced pluripotent stem cell (hiPSC)-derived beta-like cells resulted in derepression of alpha cell gene expression. *In vitro* differentiation of hiPSCs from carriers of *PAX4* p.His192 and p.X186 risk alleles exhibited increased polyhormonal endocrine cell formation and reduced insulin content. Correction of the diabetes associated *PAX4* alleles reversed these phenotypic changes. Together, we demonstrate the role of *PAX4* in human endocrine cell development, beta cell function, and its contribution to T2D-risk.

BIO

Hwee Hui LAU received the A*STAR Scientific Staff Development Award and obtained her PhD from Nanyang Technological University Singapore. Her PhD theses focused on using donor-derived stem cells to model Asian-specific diabetes development and demonstrated that genetic defects have immense consequences on pancreatic beta cell function. She is currently a Research Scientist and a Laboratory Manager in Stem Cells and Diabetes Laboratory led by Dr. Adrian Teo (IMCB, A*STAR). Her research focuses on the use of human in vitro models to study mechanisms underlying various metabolic disorders. Her research focuses on the use of of human in vitro models such as induced pluripotent stem cells (hiPSCs), skin fibroblasts, pancreatic islet cells to study mechanisms underlying various metabolic disorders.

Genome-wide CRISPR screen and gene-editing for early regulators of cardiac lineage

Chang Jie Mick LEE[1,2], Matias I. AUTIO[1,2], Wen Hao ZHENG[1,2], Xi YEI[1,2], Yoohyun SONG[3,5], Shyi Chyi WANG[3,5], Darren Chen Pei WONG[3,4], Wan Kee CHOCK[1], Boon Chuan LOW[3,4,7], Marius SUDOL[6], *Roger S-Y FOO[1,2]

*[1]Genome Institute of Singapore, Singapore; [2]Cardiovascular Disease Translational Research Programme, National University Health System, Centre for Translational Medicine, Singapore; [3]Mechanobiology Institute Singapore, National University of Singapore, Singapore; [4]Department of Biological Sciences, National University of Singapore, Singapore; [5]Institute of Bioengineering and Bioimaging, A*STAR, Singapore; 7Icahn School of [6]Medicine at Mount Sinai, Department of Medicine, One Gustave Levy Place, USA; [7]University Scholars Programme, National University of Singapore, Singapore*

ABSTRACT

Cardiac differentiation involves a stepwise clearance of repressors and fate-restricting regulators through the modulation of BMP/Wnt-signaling pathways. However, the mechanisms and how regulatory roadblocks are removed with specific developmental signaling pathways remain unclear. Here, we performed a genome-wide CRISPR screen to uncover essential regulators of cardiomyocyte specification in human embryonic stem cells (hESCs) and identify NF2, a Moesin-Ezrin-Radixin Like (MERLIN) Tumor Suppressor, as an upstream driver of early cardiomyocyte specification. Transcriptional regulation and trajectory inference from NF2-null cells reveal the loss of cardiomyocyte identity and the acquisition of non-mesodermal identity. Sustained elevation of early mesoderm lineage repressor SOX2 and upregulation of late anti-cardiac regulators CDX2, MSX1 in NF2 knockout cells reflect a necessary role for NF2 in removing regulatory roadblocks. Since YAP is a known repressor of mesendoderm genes, we found that NF2 and AMOT cooperatively bind to YAP during mesendoderm formation, thereby preventing YAP activation independent on canonical MST-LATS kinase activity. Mechanistically, through site-directed mutagenesis, we show that the critical FERM domain-dependent formation of the AMOT-NF2-YAP scaffold complex at the adherens junction is required for early cardiomyocyte lineage differentiation. These results provide mechanistic insight into the essential role of NF2 for cardiomyocyte lineage specification by sequestering the repressive effect of YAP and relieving regulatory roadblocks en route to cardiomyocytes.

BIO

Mick LEE is a Research Fellow in the Cardiovascular Research Institute, at NUS Yong Loo Lin School of Medicine. He completed his Ph.D. training with Professor Roger Foo's Group in 2022, and he has continued to expand his research focus on functional genomics in cardiac development, disease, and regeneration using CRISPR on human induced pluripotent stem cells (iPSCs). Being trained in both dry and wet laboratory work, he has contributed to many of the laboratory's seminal works on cardiac enhancers and epigenetics in heart failure (Tan et al., 2019. Circulation Research; Anene-Nzelu et al., 2020 circulation; Anene-Nzelu et al., 2021. Nature Cardiology Review), including the use of CRISPR tools to validate the role of prioritized heart disease-relevant enhancers. He played a leading role in establishing the iPSC work and preclinical models in the laboratory, as well as extending support to other research groups.

Development and application of human neural organoids

Xangfei XIANG

School of Life Science and Technology, ShanghaiTech University, Shanghai, China

ABSTRACT

Neural organoids are in vitro three-dimensional models that mimic the human brain or other structures of the nervous system. Beginning with stem cells, neural organoids are formed through unguided or guided neural differentiation under three-dimensional suspension culture conditions, relying on cell self-organization. In the past decade of research, we have focused on guided differentiation to construct various human brain region-specific organoids. Furthermore, by integrating multiple brain regions or cell lineages, we have explored the development of more complex human brain organoid technologies, providing new models for studying brain development, function, diseases, and drug effects in the context of human genetic backgrounds in vitro. As a cutting-edge technology, neural organoids still face various technical challenges that need to be overcome. This talk will introduce our efforts in the refined construction of human neural organoids, including how to build organoids that possess characteristics of human brain nuclei.

BIO

Xangfei XIANG received his Ph.D. degree in 2013 from Jinan University, China. He worked as a postdoc at Yale Stem Cell Center and the Department of Genetics at Yale University from 2013 to 2019 and associate research scientist from 2019 to 2020. Dr. Xiang joined the School of Life Science and Technology at ShanghaiTech University as a tenure-track assistant professor in March 2020. His main research topic in the past decade includes the development and application of brain region-specific organoids and complex brain organoid models based on human pluripotent stem cells. Dr. Xiang developed some of the early models of cross-brain-region neural circuits and organoid vascularization, by incorporating distinct brain region-specific organoids and cell fate programming. His work has been highlighted in a line of articles and organoid-themed collections of prestigious journals, including introduced on the topic Methods to Watch 2021 by Nature Methods.

Industry-themed presentation by Thermo Fisher Scientific

Case studies for rapid scale up and directed differentiation of pluripotent stem cells in 3D culture

Wei Ching LOW

Biosciences Division, Life Sciences Solutions Group, Thermo Fisher Scientific, Singapore

ABSTRACT

Large-scale generation of pluripotent stem cells (PSC) or PSC-derived cell types through conventional 2D cultures is often challenged by laborious hands-on steps and contamination risks. Suspension culture in the form of self-aggregating spheroids simplifies handling and scale-up of PSC growth and differentiation workflows. Here, we present case studies showing robust expansion of PSC using a novel 3D suspension culture medium (Gibco™ StemScale™ PSC Suspension Medium) that scales easily from small-scale orbital shaker platform cultures such as well plates and shake flasks to bioreactor cultures. Efficient cell passaging strategies for PSC spheroid dissociation within a closed-system environment will also be discussed. Finally, we demonstrate that these spheroids can then be utilized for a variety of downstream applications, such as PSC differentiation, and showcase protocols that successfully differentiates 3D PSC spheroids into various lineages.

BIO

Wei Ching LOW, is the Regional Market Development Manager for the cell & gene therapy and advanced cell model portfolio at Thermo Fisher Scientific. She has a decade of commercial experience serving the needs of laboratory professionals, clinicians, and researchers from both the healthcare and life science industry in the Asia Pacific region. Wei Ching earned her doctoral degree in Bioengineering from the Nanyang Technological University (Singapore) where she published multiple peer-reviewed articles in the field of neural stem cell differentiation and scaffold-mediated controlled release of biomolecules.

Morphing bioelectronics for developing organoids and animals

Yuxin LIU

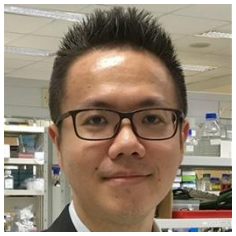
National University of Singapore, Singapore

ABSTRACT

Neural interface establishes bidirectional communication between the nervous system and bioelectronics and provides unprecedented opportunities for neuroscience research and treatments of medical conditions such as Parkinson's disease and autoimmune diseases. Despite the advancement of neurotechnology, the electrical and mechanical mismatch between the soft neural tissue and existing rigid electronics causes adverse immune response, data inaccuracy, tissue constraint, and motion artifacts. This talk will feature our recent works on soft neurochemical and electrophysiological interfaces that can accommodate biomechanical motion in dynamically moving tissues and morphing electronic that is shape-shifting and self-adapting to cellular growth in growing organoid in vitro and during the adolescent developmental in vivo.

BIO

Yuxin Liu is an assistant professor of biomedical engineering and principal investigator of N.1 Institute for health, and institute of health technology (iHealthTech) at National University of Singapore. He obtained M. S and Ph.D. in Bioengineering at Stanford University in 2016 and 2019 respectively. He was awarded presidential young professorship and MIT Technology Review Innovators Under 35 (Asia Pacific) in 2022. His research interests include tissue-mimicking brain-machine interface and translation research on precision electronic medicine.



Advancing therapeutic modalities discovery for multi-organ regeneration with tissue-derived organoid platform

Ling Yan YANG[1], Shi Xiang WANG[1], Yue LIU[1,3], Ling Ling ZHOU[1,4], Winston Yun Shen CHAN[1]

[1]Guangzhou National Laboratory, China; [2]Department of stem Cell Biology and Tissue Engineering,Zhongshan School of Medicine, Sun Yat-Sen University, China; [3]College of Life Science, Yunnan University, China

ABSTRACT

The human body degenerates as an individual ages or suffers from injuries and diseases. While many organs and tissues exhibit regenerative ability, the capacity decreases over time. To date, various strategies have been explored, including cell and gene therapy, to replenish cellular and tissue loss. In parallel, our understanding of how these tissue progenitors and stem cells are regulated and invoked during injury continues to expand. This knowledge enables the devising of treatment strategies that can augment or initiate the tissue regenerative process through the activation of specific signaling pathways and cellular processes. Human organoids that resemble the in vivo counterpart and are enriched with tissue progenitor cells are excellent in vitro models for rapid evaluation of such therapeutic modalities. In this talk, I will share our ongoing efforts to utilize tissue-derived organoids to evaluate engineered exosomes for inducing tissue-specific regeneration.

BIO

Winston CHAN graduated from the National University of Singapore and conducted his research training in stem cell biology at the Genome Institute of Singapore. He is also a Gilead Sciences Research Scholar in Liver Disease. His contribution to the stem cell field includes the identification of novel determinants of pluripotency with the first Genome-wide siRNA screen and the development of novel culture methods to induce naïve human pluripotent stem cells (PSCs). His team was also one of the first to generate human liver organoids with functional bile canaliculi system. Dr. Chan currently runs a lab in the Guangzhou laboratory, China, and continues to pursue his interest in stem cell research. His lab research focus includes understanding the role of tissue stem/progenitor cells in the initiation and progression of diseases, and the development of novel therapeutic strategies to induce tissue regeneration in vivo.

Interrogating kidney diseases using hPSC-derived kidney organoids

Meng Liu[1], Chao Zhang[1], Ximing Gong[1], Tian Zhang[1], Huamin Wang[1], Yixuan Wang[1], Jia Nee Foo[1,2], Yun Xia[1]

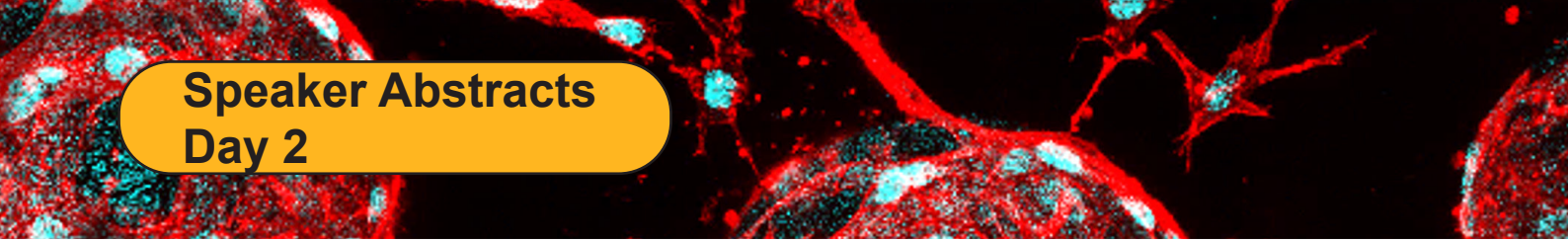
[1]Lee Kong Chain School of Medicine, Nanyang Technological University, Singapore; [2]Genome Institute of Singapore, Singapore

ABSTRACT

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 of the in vivo kidney. One significant limitation associated with the current generation of organoids is that generic cell types, including vasculature, neurons, and immune cells, are underrepresented. Our laboratory is interested in developing methodologies for the generation of kidney organoids that harbor a comprehensive repertoire of generic cell types in an effort to better understand and interrogate the collective behavior of this multicellular system. We wish to reconstruct the close-to-native tissue microenvironment and intercellular crosstalk that are critical for structural sophistication, functional maturation, and pathogenesis. To accomplish this, we are employing multidisciplinary experimental frameworks, including multicellular self-organization, genetic perturbation, high-resolution imaging, and single cell analysis.

BIO

Yun Xia's research is focused on the establishment of human stem cell-based models for studying kidney development and diseases. Her lab employs direct differentiation and multicellular self-organization to construct kidney organoids that present both architecture and functionality reminiscent of their cognate organ. Experimental tools, including genetic perturbation, single cell analysis, and high-resolution imaging are utilised to reveal the mechanistic underpinning of kidney organogenesis and disease pathogenesis. Dr. Xia received her PhD from National University of Singapore in Molecular/Cell Biology and Biochemistry in 2010. During her postdoctoral training with Dr. Juan Carlos Izpisua Belmonte at the Salk Institute for Biological Studies, she developed a strong interest in establishing differentiation protocols for in vitro lineage specification with a special focus on mesodermal lineages. Since 2015, she has been a faculty member in Lee Kong Chian School of Medicine at Nanyang Technological University Singapore. In 2021, Dr. Xia joined EMBO Global Investigator Network in recognition of her scientific work on kidney organoids.



Speaker Abstracts Day 2

Abstract selected talk

Uncovering the complexities of alveolar capillary development with organoid models

Nicole PEK[1,2,3], Yifei MIAO[1,2], Cheng TAN[1,2], Darrell KOTTON[4], Robert ROTTIER[5], Ya-Wen CHEN[6], Minzhe GUO[1,2,3], Mingxia GU[1,2,3]

[1]Division of Pulmonary Biology, Perinatal Institute, Cincinnati Children's Hospital Medical Center (CCHMC), USA, [2]Center for Stem Cell and Organoid Medicine, CCHMC, USA, [3]College of Medicine, University of Cincinnati, USA, [4]Center for Regenerative Medicine, Boston University, USA, [5]Erasmus University Medical Center, Netherlands, [6]Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, USA

ABSTRACT

Recent studies revealed that alveolar capillaries consist of two specialized endothelial subtypes – aerocytes (aCap) and general capillary (gCap) cells. However, the developmental origins of aCap in mammalian lungs remain ambiguous. The goal of the project is to uncover the exact cellular and molecular mechanisms underlying aCap development and specification. One strategy is to scrutinize the microenvironment of developing lungs to identify aCap-specifying signals. Here, we describe efforts to interrogate ligand-receptor interactions found to be enriched in the mammalian alveolar niché using induced pluripotent stem cell (iPSC)-derived blood vessel organoids (VOs) to decipher signals governing aCap specification. We found that exogenous Endothelin-1 (EDN1) and VEGFA-189 were able to effectively induce aCap marker gene expression in the iPSC-VOs. The ligand-treated VOs displayed significant expansion in vessel area, akin to the drastic morphological changes undergone by aCap during development. Accompany vessel expansion was evidence of cytoskeletal remodeling and activation of aCap transcriptional program. Thereafter, we determined that mechano-transduction Rho/ROCK pathway is involved in ligand-mediated aCap specification. In summary, our results suggest that ligands such as EDN1 and VEGFA-189 are critical aCap specifiers; both ligands induce drastic morphogenetic changes to the endothelium in VOs perhaps by inducing cytoskeletal remodeling in a Rho/ROCK-dependent manner.

Shaping neuronal networks by FEZ1-mediated trafficking

Yinghua Qu[1,2], Jonathan Lim[1], Omer An[3], Henry Yang[3], Yi-Chin Toh[2,4,5] and John Jia En Chua[1,6-8]

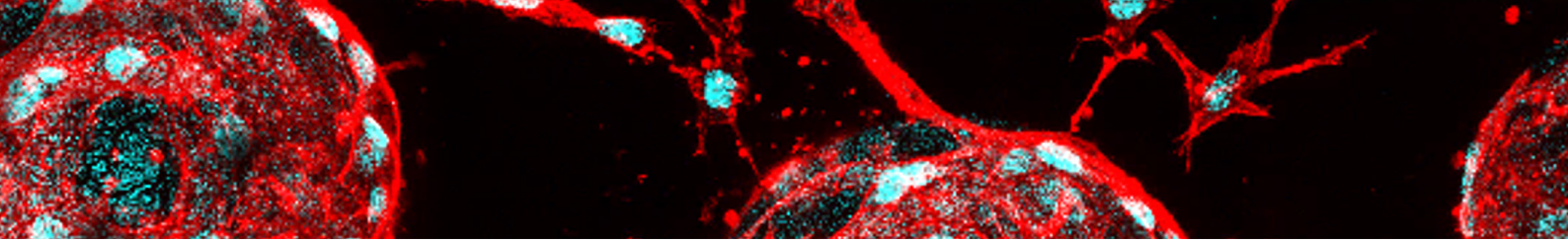
*[1]Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore. [2]Department of Biomedical Engineering, National University of Singapore, Singapore. [3]Cancer Science Institute of Singapore, National University of Singapore, Singapore. [4]School of Mechanical, Medical and Process Engineering, Queensland University of Technology, Australia. [5]Centre for Biomedical Technologies, Queensland University of Technology, Australia. [6]Healthy Longevity Translational Research Program, Yong Loo Lin School of Medicine, National University of Singapore. [7]LSI Neurobiology Programme, National University of Singapore, Singapore. [8]Institute for Molecular and Cell Biology, A*STAR, Singapore.*

ABSTRACT

The myriad functions of the human brain is endowed by its networks of neurons. During brain development, neuronal projections sent out by billions of neurons navigate, with help from guidance cues, to target neurons and form trillions of synaptic connections to establish neuronal networks. Molecular motors of the Kinesin superfamily and their adapters play critical roles in supporting the growth and maturation of these networks by delivering essential biological materials to extending neuronal projections and developing synapses. We previously uncovered FEZ1 as a Kinesin-1 adapter involved in the delivery of synaptic cargoes. I will share how our recent findings have shed further light on the adapter's involvement in shaping the development of neurons and how perturbation of its function contributes to neuropsychiatric and neurodegenerative disorders.

BIO

John Jia En CHUA is an Assistant Professor in the Department of Physiology, Yong Loo Lin School of Medicine at the National University of Singapore (NUS). He is also a Principal Investigator in the Life Science Institute Neurobiology Programme, NUS and Joint Principal Investigator at the Institute for Molecular and Cell Biology. His research focuses on mechanisms involved in neural circuit formation and dysfunction. His group uncovered the role of FEZ1 in a major synaptic transport pathway that plays critical roles in neuronal development and is of relevance to human disorders such as Jacobsen syndrome. Disruption of this transport pathway is also implicated in Alzheimer's disease. John received his B.Sc. (Hons 2nd Upper) and M.Sc from NUS, and Ph.D. from the University of Hamburg. He completed his postdoctoral training with Prof Reinhard Jahn at the Max Planck Institute for Biophysical Chemistry and became a Research Group Leader at the Institute.



SPEAKER ABSTRACTS

Day 3

1 Dec 2023

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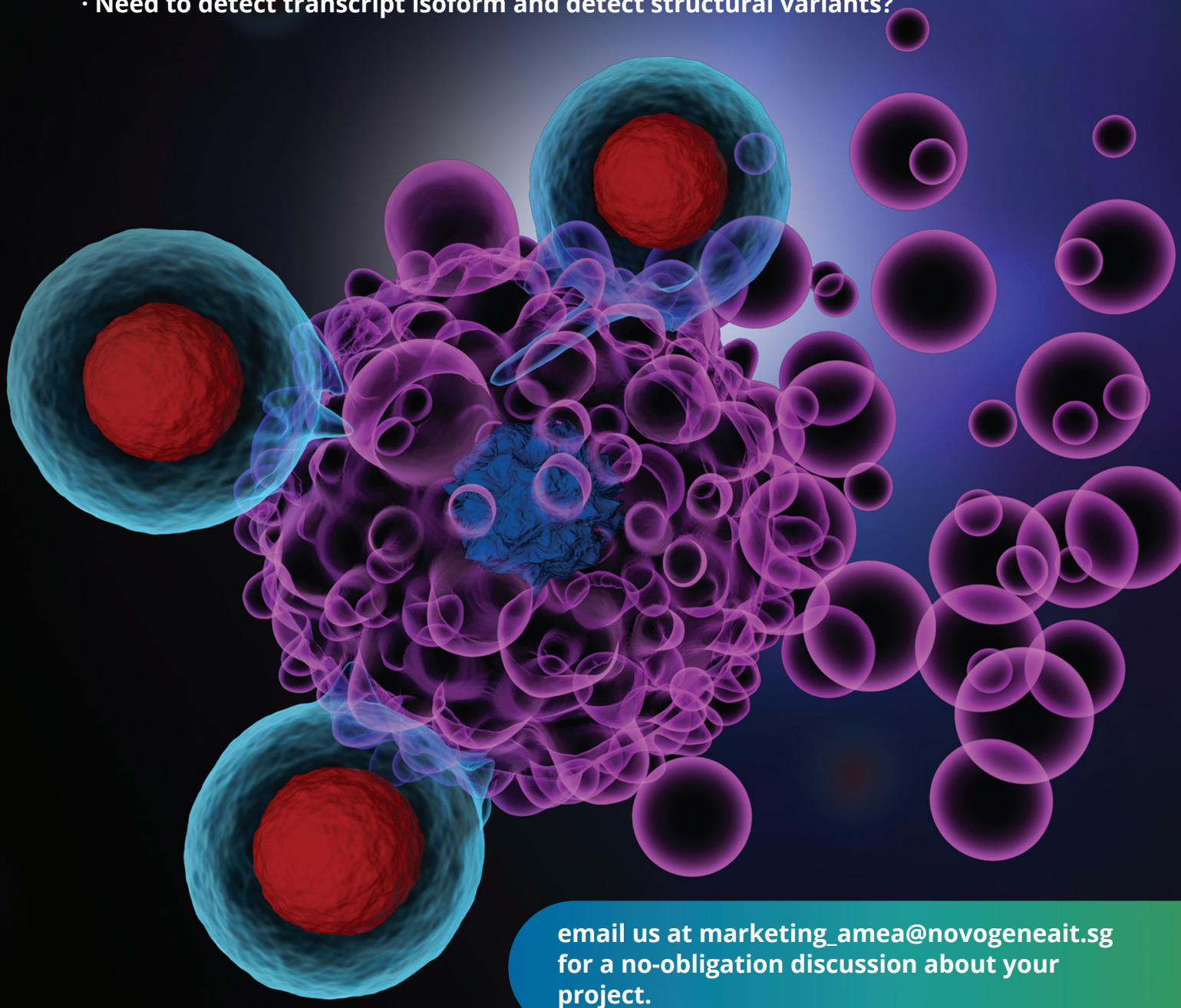

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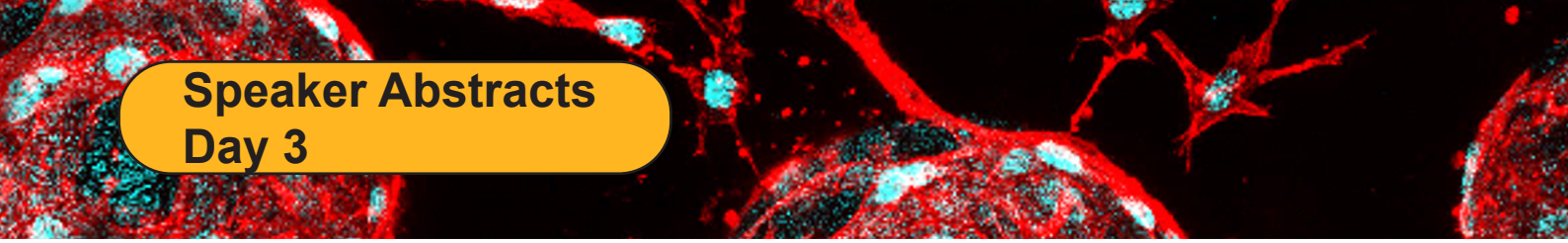


Speaker Abstracts Day 3

KEYNOTE LECTURE

Translating stem cell technology to therapeutics

Su-Chun ZHANG

Duke-NUS Medical School, Singapore & University of Wisconsin-Madison

ABSTRACT

Human pluripotent stem cells (hPSCs) can differentiate to defined cell and tissue types of the body, offering a platform for identifying and testing therapeutic drugs as well as a source for cell therapy. Following transplantation into animal models of neurological disorders, we found that the human neurons not only project axons in a long distance and find their targets but also receive appropriate inputs in the mature brain, thus reconstructing the functional neural circuit. Strikingly, the pathfinding and circuit reconstruction are largely dependent upon the grafted neuronal type, highlighting the therapeutic potential of hPSC-derived neural cells and the necessity for guiding hPSCs to enriched and functionally specialized subtype-specific neural cells.

BIO

Su-Chun ZHANG, MD, PhD, is professor and director of the Signature Program in Neuroscience & Behavioral Disorders at Duke-NUS Medical School, Singapore and professor of Neuroscience and Neurology at the University of Wisconsin-Madison. Dr. Zhang has developed technology to guide human stem cells to functionally specialized nerve cell types with 23 awarded patents and several pending applications. He has developed stem cell-based platforms for studying neural degeneration and testing drugs for neurological diseases. In parallel, he is developing cell therapy for neurological diseases like Parkinson's disease, spinal cord injury and stroke. Dr. Zhang was a founding member of the WiCell Institute and co-founder of BrainXell, Inc and BrainXell Therapeutics, Inc.

Decoding and rewiring Immunity

Michael BIRNBAUM[1, 2]

[1]Dept. of Biological Engineering, Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, USA; [2]Singapore-MIT Alliance for Research and Technology Centre, Singapore, Singapore

ABSTRACT

Immune recognition and signaling are breathtakingly complex. In order to specifically recognize antigens derived from pathogens or tumors, the adaptive immune system relies upon immense molecular diversity in the T and B cell receptor repertoires. These collections of immune receptors in turn must balance specificity, sensitivity, and cross-reactivity to ensure effective immune protection. Upon antigen recognition, immune cells rely on precise signaling cues to ensure correct cellular activation and regulation. Our lab aims to better understand natural immune recognition and signaling with the intent of developing novel immunotherapies. Projects often require technology development to enable work at scales that would otherwise not be feasible. Since our research often results in the creation of large datasets, we work at the interface between data generation and computational analyses/predictions.

BIO

Michael is an Associate Professor of Biological Engineering at MIT. He received his PhD in Immunology from Stanford University. At Stanford, he worked under Chris Garcia to study the molecular mechanisms of T cell receptor recognition, cross-reactivity, and activation. For the past seven years, his lab in the Koch Institute has been developing ways to engineer immune recognition and signaling.

Integrative single cell transcriptomics and functional profiling with TRAPS-seq and PAINTKiller-seq

Lih Feng CHEOW

[1]Department of Biomedical Engineering, National University of Singapore, Singapore; [2]Institute for Health Innovation and Technology, National University of Singapore, Singapore; [3]Critical Analytics for Manufacturing Personalized Medicine IRG, Singapore-MIT Alliance for Research and Technology Singapore

ABSTRACT

Single-cell transcriptomics have significantly advanced our understanding of molecular cell subtypes. However, the correlation between cell types and their intrinsic functions remains elusive. We present two novel methods that can allow simultaneous functional and molecular profiling of single cells compatible with high throughput single-cell analysis platforms. Firstly, TRAPS-seq utilize a cell-surface affinity matrix to immobilize secreted proteins onto the source cell surface. Proteins secreted by cells over consecutive time windows are quantified using different feature barcoding antibodies, to establish the correlation between protein secretion dynamics, surface protein expression and transcriptome from single cells. Secondly, PAINTKiller-seq utilize a cell-surface affinity matrix to immobilize intracellular proteins. Cytotoxic cells that are “painted” by intracellular proteins from lysed target cells at close proximity and identified with feature barcoding antibodies. These approaches that combines functional readout with single cell transcriptomic measurements will be an important toolkit for understanding the molecular determinants of cellular functions.

BIO

Lih Feng CHEOW is an Assistant Professor in the NUS Department of Biomedical Engineering and Principal Investigator at the Institute of Health Innovation and Technology (iHealthtech). He received his B.Sc. (Electrical and Computer Engineering) from Cornell University and PhD (Electrical Engineering and Computer Science) from Massachusetts Institute of Technology. His research interest is in developing technology platforms to perform precision measurements of multiple modalities (e.g. genetic, epigenetic, transcriptomics) in individual cells, and inventing innovative technologies for bio-sample preparation and disease diagnosis to meet the evolving healthcare needs of society.

Challenges in manufacturing Natural Killer (NK) cells as allogeneic therapies targeting solid tumors

Andy TAN, Chelsia WANG, Arleen SANNY

*Bioprocessing Technology Institute (BTI), Agency for Science, Technology and Research (A*STAR), Singapore*

ABSTRACT

Conventional T cells genetically modified to express chimeric antigen receptors (CARs) that specifically recognize tumor cells have been remarkably successful in treating hematological malignancies. However, CAR-T cells are confined to autologous use since they provoke life-threatening graft-versus-host disease (GvHD) if administered to patients with mismatched human leukocyte antigen (HLA). To circumvent this, allogeneic NK cells have been increasingly adopted as an “off-the-shelf” therapy due to their superior safety profile characterized by negligible GvHD and low incidence of toxicities compared with T cells. Despite these advantages, challenges exist in using NK cells, including their poor CAR transduction efficiency, suboptimal anti-tumor cytotoxic function compounded by heterogenous tumor-associated expression of CAR antigens and lack of functional persistence in vivo. In addition, manufacturing bottlenecks and regulatory issues related to employing feeder cells for NK expansion remain. In this talk, I will discuss some of these challenges and their proposed solutions such as selection of tumor antigens exhibiting disrupted cellular compartmentalization or ectopic expression in solid tumors for specific CAR targeting, media optimization and engineering of novel feeder cell lines to support production of potent NK cells.

BIO

Andy TAN leads the Immune Cell Manufacturing group at BTI, A*STAR which focuses on developing processes and media optimization to support the lab- and bioreactor-scale production of unmodified or chimeric antigen receptor (CAR)-engineered T and NK cells for tumor immunotherapy. He was the lead investigator in a joint laboratory with a local contract development and manufacturing organization (CDMO) to establish large scale expansion of CAR virus-producing cells and CAR-T cells. Andy also co-directs the Centre of Innovation for Sustainable Banking and Production of Cultivated Meats (CRISP Meats) which develops innovative technologies to enable manufacturing of cultivated meat. His group also optimizes nucleic acid- and immune-based analytics for biologics characterization. Andy received his BSc and MSc in Physics from National University of Singapore (NUS) and PhD in Immunology from NUS Graduate School for Integrative Sciences and Engineering. He then completed postdoctoral training in Immunogenomics at John Curtin School of Medical Research, Australian National University before joining BTI.

3-dimensional human pancreatic microtumors to advance Immunotherapy

Giulio GIUSTARINI[1], Ermes CANDIELLO[2], Jiaming BI[1], Andrea PAVESI[3,4], Paola CAPPELLO[2], Giulia ADRIANI[1,5]

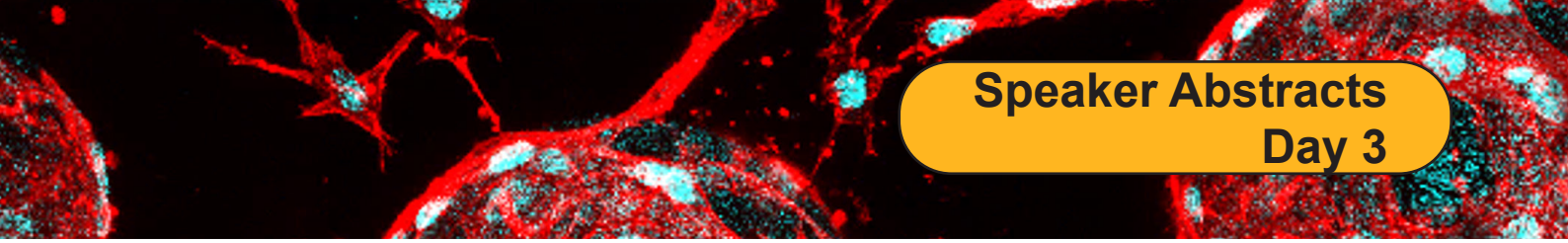
[1]Singapore Immunology Network (SIgN), Singapore; [2]Department of Molecular Biotechnology and Health Sciences, University of Turin, Italy; [3]Institute of Molecular and Cell Biology (IMCB), Singapore; [4]Mechanobiology Institute, National University of Singapore, Singapore; [5]Department of Biomedical Engineering, National University of Singapore, Singapore

ABSTRACT

To replicate the complexity of the tumor microenvironment (TME) we generated a 3D multicellular model of pancreatic ductal adenocarcinoma (PDAC). This model incorporates multiple cell types, including immune, endothelial, and stromal cells, alongside cancer cells to advance the understanding of treatment response. These multicellular microtumors displayed increased expression of collagen-I, HIF-1α, tumor-associated macrophages markers, and cytokine patterns that matched patients' ones. Single cell RNA sequencing data collected from our multicellular microtumors revealed the modulation of genes which correlate with unfavorable prognosis in patients, suggesting that our microtumors could accurately represent response to therapy. These microtumors are vascularized within microfluidic devices to evaluate immune cell infiltration and study the cellular crosstalk in absence of interleukin (IL)-17A. Indeed, preliminary in vivo studies suggested that IL-17A re-shapes the stromal compartment by improving the recruitment of CD8+ T cells. Overall, our results will aid the development of effective immunotherapeutic strategies to target the TME.

BIO

Giulia ADRIANI is a Principal Scientist at the Singapore Immunology Network (SIgN), established by the Agency for Science, Technology and Research (A*STAR), and an Adjunct Assistant Professor at the Department of Bioengineering of the National University of Singapore (NUS). She completed her bachelor's and master's degrees with honors in Mechanical Engineering at the Polytechnic of Bari in Italy. She was awarded the Interpolytechnic Doctoral School Fellowship and spent part of her Ph.D. at The Methodist Hospital Research Institute in Houston, Texas (USA) at the Department of Nanomedicine. After receiving her Ph.D. in Biomedical and Biomechanical Engineering, she moved to Singapore to work at NUS and MIT's research center in Singapore (SMART Program). Dr. Adriani is now leading her research group in SIgN, working on 3-dimensional vascularized immuno-competent tumor models to study the interactions of cancer cells with their microenvironment and develop better anti-tumor therapies.



Abstract selected talk

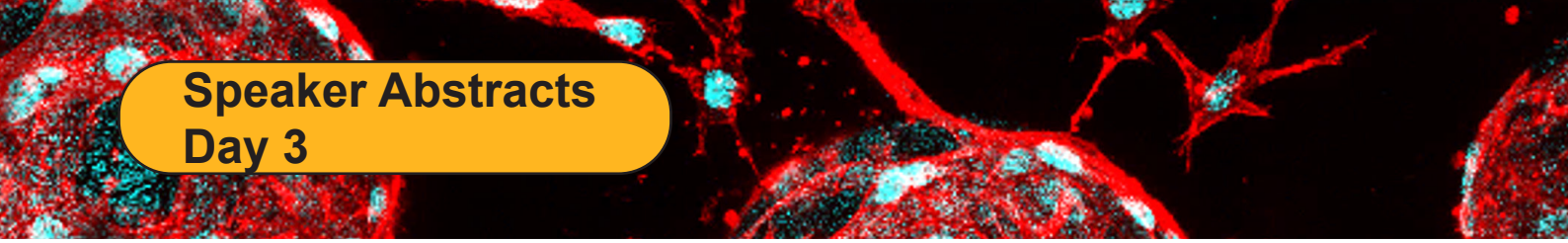
COVID-19 antigenic peptides show immune response activity in allogeneic dendritic cells for possible surveillance-based vaccine

Francisco M. HERALDE III[1,2,3], Kim Claudette J. FERNANDEZ[1], Nelia, TAN-LIU[2,3], Salvador C. CAOILI[1], Janice CAOILI[3], Joey BORROMEO[3], Romulo DE CASTRO[4], Ma. Teresa A. BARZAGA[2,3,5]

[1]Department of Biochemistry and Molecular Biology, College of Medicine, University of the Philippines Manila; [2]Molecular Diagnostics and Cellular Therapeutics Laboratory, Lung Center of the Philippines, Diliman, Quezon City, Philippines; [3]Infectious Disease; Center for Regenerative Medicine, Makati Medical Center, Philippines; [4]University of San Agustin, Iloilo City, Philippines; [5]College of Medicine, De La Salle Medical and Health Sciences Institute, Cavite, Philippines

ABSTRACT

Global efforts for COVID-19 control through vaccines remain a challenge despite WHO's pandemic lift order. The highly mutating nature drives the variant-emergence making vaccination more relevant. This study explores the utility of a locally existing cellular-based vaccine for COVID-19. DC-based vaccines have been shown as safe in cancer and other infectious agents including viruses. This study evaluated an allogeneic approach for DC-based vaccination in vitro. Peptides based on the SARS-COV-2 genome were in-silico screened. Nine peptides (i.e., 7 MHC I and 2 MHC II binding) were shortlisted based on frequently occurring HLA alleles in Filipinos, and binding affinities of generated complexes to TCRs followed by T-cell activation experiments. Results showed that the majority of the peptide-loaded DCs were able to elicit IFN-gamma, with spike-based peptides being the most active. Control-patient profiles also suggest that allogeneic DCs are immunogenic and can elicit responses higher than the autologous DCs for some of the peptides. Case-patient-derived DCs have low to none IFN-gamma release suggesting immunocompromised patients to be poor DC sources. The study was able to provide preliminary evidence for the "proof-of-concept" experiments suggesting the potential utility of allogeneic DC vaccination for surveillance-based COVID-19 vaccine that can be evaluated in a clinical trial.



Speaker Abstracts Day 3

Industry-themed presentation by Miltenyi Biotec

Advancing pluripotent stem cell research and manufacturing with Miltenyi Biotec

Parivash NOURI[1], Michaela DIAKATOU[1]

Global Marketing, Miltenyi Biotec, Germany

ABSTRACT

This presentation provides an overview of Miltenyi Biotec's dedication to advancing regenerative medicine through Pluripotent Stem Cell (PSC) workflows, encompassing the entire process. We delve into the reprogramming of fibroblasts into iPSCs using our efficient StemMACS™ iPSC mRNA reprogramming kit, showcasing its remarkable efficacy. Additionally, we explore the vital role of culture media in preserving PSC quality and pluripotency, introducing the StemMACS™ PSC Brew with its advanced FGF-2 formulation, designed for convenient weekend-free schedules. During the second part we present the automated workflows for clinical applications. We show how CliniMACS Prodigy® Adherent Cell Culture Process offers automated and GMP-compliant PSC expansion and banking. We also show an example of how MACS Quant® Tyto®, a microvalve sorter, can gently sort RPE cells. The scientific significance of our products in driving cutting-edge research and clinical applications in regenerative medicine is a central theme of this presentation.

BIOS

Parivash NOURI: During her PhD, Parivash conducted research on iPSCs and their differentiation into midbrain dopaminergic neurons. Currently, she holds the position of Application Product Manager for Pluripotent Stem Cells in research within the Global Marketing department.

Michaela DIAKATOU: Michaela holds a PhD in modelling retinal diseases through iPSC differentiation to retinal organoids and CRISPR genetic engineering. Currently, she is the Global Product Manager for PSCs in clinical applications.

A platform technology for rational reprogramming of cellular state

Yen CHOO

Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore

ABSTRACT

Cellular plasticity allows for reprogramming of cell states through ectopic expression of transcription factors (TFs) which activate gene regulatory networks (GRNs) that in turn determine cell identity. For example, transient expression of the Yamanaka TFs (Oct3/4, Sox2, Klf4 and c-Myc) activates a stable GRN that reprograms diverse cell types into induced pluripotent stem cells (iPSCs). These iPSCs can be themselves reprogrammed using specific TF cocktails that induce new GRNs to specify different somatic cell types. Recently, several bioinformatics pipelines have been developed to propose shortlists of candidate TFs for rational reprogramming of cell states. Nevertheless, identifying productive TF cocktails for cell reprogramming remains challenging, because the current theoretical (algorithmic) approaches have limited predictive power, while empirical (screening) approaches are laborious and limited in their coverage of experimental space. This talk will set out an integrated (theoretical + empirical) strategy to develop a versatile platform for reprograming of cellular fate, with broad-ranging applications in basic and biomedical science.

BIO

Yen CHOO is Associate Professor of Stem Cell Science and Regenerative Medicine at Lee Kong Chian School of Medicine (LKC Medicine). His research centers around innovative platform technologies to manipulate cellular identity through precise programming of cell genotype and/or phenotype. He is a serial entrepreneur with 25 years of industry experience in biotech companies developing advanced therapies. He was the founding Executive Director of LKC Medicine's Office of Innovation and Entrepreneurship, Co-Executive Director of NTU's Institute for Health Technologies (HealthTech NTU) and is currently Executive Director of co11ab@Novena, a BioMedtech incubator jointly established by NTU, NHG and A*STAR. He holds a PhD in Molecular Biology from the University of Cambridge and the MRC Laboratory of Molecular Biology.

Construction of structured Wagyu meat by 3D stem cell printing

Michiya MATSUSAKI

Department of Applied Chemistry, Graduate School of Engineering, Osaka University, Japan

ABSTRACT

With the current interest in cell-based meat, mammalian cell-based meats are largely unstructured. Thus, demand for high-quality steak-like meat remains high. We demonstrate in vitro construction of engineered steak-like tissue assembled of three types of bovine cell fibers (muscle, fat, and vessel). Since actual meat is an aligned assembly of the fibers connected to tendons for the contraction and relaxation actions, we developed tendon-gel integrated bioprinting to stably construct each fiber tissue. A total of 100 fibers were constructed by tendon-gel integrated bioprinting and manually assembled to produce a steak-like meat of 1.5 cm x 1.5 cm in size and 2.0 cm in length. After cooking, secretion of flavor molecules of wagyu beef specific was detected. Construction of a fully automated system based on our technology is currently underway. Our method could be a promising technology for the fabrication of the desired types of structured steak-like meats.

BIO

Michiya MATSUSAKI was born in Kagoshima, Japan in 1976. He received his Ph.D. degree in 2003 from Kagoshima University. He started his academic career as a Postdoctoral fellow at Osaka University in 2003. He was a visiting scientist at Lund University in 2004. In 2006, he joined the Department of Applied Chemistry in the Graduate School of Engineering at Osaka University as an Assistant Professor. He was promoted to Associate Professor in 2015 and to full Professor in 2019. He was a JST-PRESTO researcher (Concurrent position) from 2008 to 2011 and from 2015 to 2019. He was awarded 20 awards including the Young Scientist's Prize by the Minister of Education, Culture, Sports, Science, and Technology. His research interest is biomaterials and tissue engineering for regenerative medicine and pharmaceutical applications.

Surveillance of allergens in cultivated meat with AllerCatPro 2.0

Vachiranee LIMVIPHUVADH, Minh NGUYEN, Sebastian MAURER-STROH

*A*STAR Bioinformatics Institute, Singapore*

ABSTRACT

Proteins in novel food including cultured meat can pose a risk for an immediate immunoglobulin E (IgE)-mediated allergic response. Bioinformatic tools can assist to predict and investigate the allergenic potential of proteins. This presentation will describe AllerCatPro 2.0, a web server that can be used to predict protein allergenicity potential with better accuracy than other computational methods and new features that help assessors making informed decisions. We predict the similarity between input proteins using both their amino acid sequences and predicted 3D structures towards the most comprehensive datasets of reliable proteins associated with allergenicity. We extend the sensitivity of the tool for food species from plants to seafood and animal meat through family-specific thresholds derived from clinical validations. The web server is freely accessible at https://allercatpro.bii.a-star.edu.sg.

BIO

Sebastian MAURER-STROH studied theoretical biochemistry at the University of Vienna and wrote his master and PhD thesis at the Institute of Molecular Pathology (IMP). After FEBS and Marie Curie fellowships at the VIB-SWITCH lab in Brussels, he has been leading the sequence analytics portfolio in the A*STAR Bioinformatics Institute (BII) since 2007 and Infectious Disease Programme since 2010. He is the Executive Director of BII since January 2021. His computational team is well known for successes at the public-private interface in Singapore from Precision Medicine to Consumer Product and Food Safety and of course for his critical contributions to national and global viral pathogen surveillance through the GISAID data science initiative that has become the single most important source for virus outbreak data sharing and analysis in this pandemic powering public health responses globally.

The state of global cultivated meat research and development of edible plant-based scaffolds

Deepak CHOUDHURY[1], Priyatharshini MURGAN[1], Wee Swan YAP[1], Ratima SUNTORNNOND[1], Hariharan EZHILARASU[1], Pei Leng TAN[2], Jasmine Si Han SEAH[2], Lay Poh TAN[2]

*[1]Biomanufacturing Technology, Bioprocessing Technology Institute (BTI), Agency for Science, Technology and Research (A*STAR), Singapore; [2]School of Materials Science and Engineering, Nanyang Technological University, Singapore*

ABSTRACT

Cultivated Meat is gaining popularity as a substitute for animal agriculture. However, the creation of animal-free scaffolds is crucial to fabricating a structured meat product that mimics conventional meat, which should be edible, dense, nutritious, and possess suitable mechanical properties for cell growth. We have created highly macro-porous plant-based scaffolds that provide an advantage in producing textured meat. These scaffolds promote cell alignment and orientation, which is essential for muscle cell differentiation. We used our proprietary scaffolds with C2C12 myoblasts to develop a model system for a cultured meat prototype. To provide further evidence of the viability of our method, we conducted experiments where we cultured porcine adipose-derived mesenchymal stem cells (pADMSCs) on these scaffolds and guided their transformation into muscle tissue. This process was carried out alongside the co-culture of matured fat cells.

BIO

Deepak CHOUDHURY is currently a Principal Scientist and Group Leader of Biomanufacturing Technology at Bioprocessing Technology Institute (BTI) A*STAR. His research interests include biomaterials/ biofabrication processes development for cellular agriculture, regenerative medicine, cell therapy and tissue engineering. Dr. Choudhury with his team have published several unique insights in the CM field – starting with cover story on “The business of cultured meat”, which catalogued CM companies/meat focus/funding landscape, etc. The team wrote two more analyses in 2020 on the nomenclature and impact of 3D printing in CM. Then in 2021-2022, followed up with state of edible scaffolds, developing CM ecosystem, fermentation effect as well as the first patentometric analysis in the CM. Dr Choudhury was invited as an expert for consultation on “Scientific advice on cell-based food products and food safety considerations” in 2022. This 3-day consultation session was organised by FAO & WHO in Singapore. Dr Choudhury is recipient of the 1st & 2nd Alternative Protein Seed Challenges under Singapore Food Story (SFS) R&D Programme, as well as a Co-Investigator of the CentRe of Innovation for Sustainable banking and production of cultivated Meats (CRISP Meats). He has secured > S$4 million in competitive funding and has five patent applications and more than 35 publications.

Cultivated meat as a climate solution: State of the science and the road ahead

Maanasa RAVIKUMAR

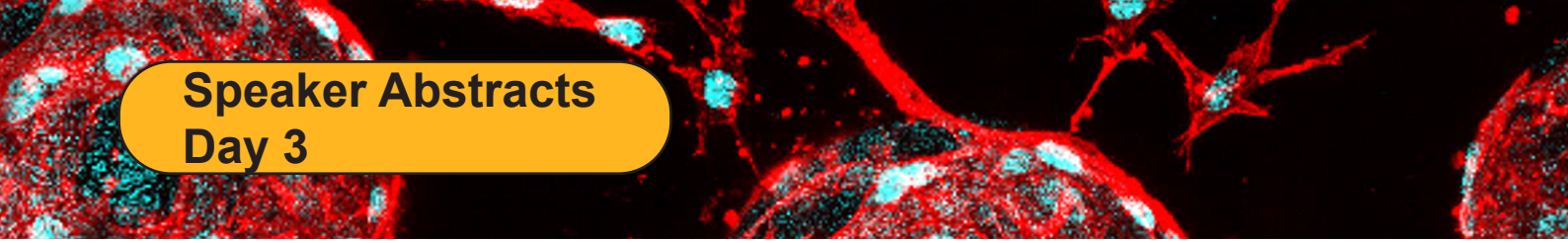
The Good Food Institute, APAC, Singapore

ABSTRACT

With every year that passes, we are seeing new advancements in the alternative protein industry that are opening the door for a global shift to a more sustainable, secure, and just food system. Across the cultivated meat ecosystem too, scientific and technological progress has accelerated. However, as with all newly emerging technologies, many critical technical challenges and fundamental research gaps are posing industry bottlenecks towards driving innovation and manufacturing at a commercially relevant scale. This talk will discuss the current state of the cultivated meat industry and underscore areas of opportunity to apply stem cell and molecular biology, tissue engineering, and bioprocess advances to active areas of cultivated meat research. The pressing need for more multidisciplinary collaboration between academia and industry as well as open-access research will also be highlighted.

BIO

Maanasa RAVIKUMAR is the Science & Technology Specialist for cultivated meat at The Good Food Institute APAC, a non-profit think tank working to accelerate alternative protein innovation. In her role, she works closely with academic research groups, industry stakeholders and government agencies across the region to identify fundamental research whitespaces, strengthen public-private research collaboration, boost funding opportunities, and support talent building efforts to catalyze cultivated meat R&D. Prior to joining GFI, Maanasa was awarded A*STAR's SINGA scholarship to pursue her graduate research in stem cell glycobiology at IMCB and obtained a Ph.D. from the Yong Loo Lin School of Medicine, NUS. She then went on to work as a scientist at a cultivated meat startup in Oxford, UK, where she spearheaded research into multi-species cultivated meat products.



Speaker Abstracts Day 3

Abstract selected talk

Accelerating cell therapy safety: rapid and accurate absolute quantification of adventitious agents using digital CRISPR approaches and beyond

Xiaolin WU[1], Joshua RAYMOND[1], Cheryl CHAN[1], Yaoping LIU[1], Yie Hou LEE[1], Timothy LU[1,2], Stacy SPRINGS[1,3], Hanry YU[1,4,5]

[1]Critical Analytics for Manufacturing Personalized Medicine Interdisciplinary Research Group, Singapore-MIT Alliance for Research and Technology Centre (SMART-CAMP), Singapore; [2]Department of Biological Engineering, Massachusetts Institute of Technology (MIT), USA; [3]Center for Biomedical Innovation, Massachusetts Institute of Technology (MIT), USA; [4]Department of Physiology & the Institute for Digital Medicine (WisDM), Yong Loo Lin School of Medicine, National University of Singapore (NUS), Singapore; [5]Mechanobiology Institute, National University of Singapore (NUS), Singapore

ABSTRACT

Cell therapy production relies on cell culture, susceptible to contamination by bacteria and viruses. Rapid, accurate adventitious agent detection in real-time and release testing is crucial. Current culture-based detection methods are slow, and DNA/RNA-based techniques, like qPCR, suffer from variability and false positives. Recent advancements in CRISPR/Cas systems enable rapid diagnostics by detecting specific DNA/RNA targets but lack quantification abilities. To address this, we introduce the development of RApid DIgital Crispr Approaches (RADICA and warm-start RADICA). These methods combine CRISPR/Cas-based diagnosis with digital sample partitioning, enabling absolute quantification of nucleic acids within just 60 minutes. In our validation tests against common viral contaminants in biomanufacturing, including SARS-CoV-2, Epstein-Barr virus (EBV), human adenovirus, and herpes simplex virus, RADICA/WS-RADICA demonstrated remarkable sensitivity (detecting as low as 1 copy/μL), no cross-reactivity to similar targets, and a high tolerance to human background DNA and PCR inhibitors. Furthermore, we have developed a digital LAMP-based method for detecting live bacterial and fungi contaminations in CAR-T cell manufacturing, achieving detection down to 2 CFU/ml. Our methods offer rapid, sensitive absolute quantification applicable in cell manufacturing safety testing. They hold the potential to significantly enhance the efficiency and product safety in biopharmaceutical and cell manufacturing processes.

Continuous flow cellular processing to scale complementary proteins

Xiangliang LIN

ESCO ASTER, Singapore

ABSTRACT

Esco Aster, a leader in continuous flow processes, is spearheading a revolution in the cultivated meat industry by leveraging its expertise to scale up production efficiently and sustainably. Xiangliang will explain how, when implementing continuous flow processes in cultivated meat complementary protein production, Esco Aster enables manufacturers to significantly increase output while maintaining stringent quality standards. Continuous flow eliminates the limitations and complexities associated with batch production, allowing for a seamless, uninterrupted flow of cell cultures and biofabrication processes. The company's solutions integrate state-of-the-art bioreactor technology, automation, and advanced process control to optimize cell growth, differentiation, and tissue formation. Real-time monitoring and data analytics provide vital insights, enabling producers to make informed decisions and ensure consistent product quality throughout the production line.

BIO

XL LIN, graduated with a Bachelor of Sciences from the University of Sydney, joining Esco Lifescience Group upon graduating. He started the Esco Group of Companies' Healthcare business unit from beginning to end as a "Discovery to Delivery" platform. He conceptualized and commercialized Esco Patented Tide Motion adherent cell and tissue engineering processing platforms which are used in 9 commercial human/animal health vaccines. Its presence expands from Singapore as its headquarters to opening factories in USA, UK and Taiwan as well as opening various Esco Offices within Asean, Oceania, East Asia, South Asia, South Africa.

SCSS-Dr Susan Lim Award for Outstanding Young Investigator Presentation

Using human midbrain organoids to understand human dopamine neuron formation

Alfred SUN

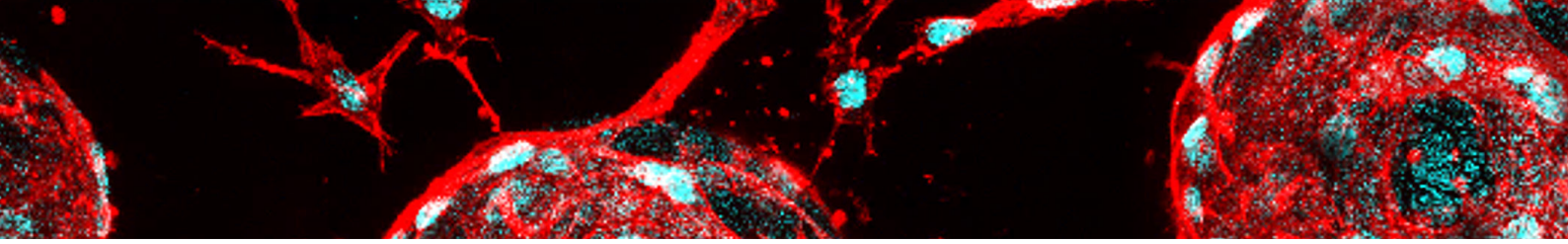
Neuroscience & Behavioral Disorders, Duke-NUS Medical School, Singapore; National Neuroscience Institute, Singapore

ABSTRACT

Human brain organoids offer a unique opportunity to unveil human-specific physiological as well as pathophysiological features. We have previously developed a midbrain organoid model that contained neuromelanin. Disruption of Parkinson-linked genes in the organoids result in Lewy-body like aggregations, pathological hallmarks observed in PD patient brains. However, it is unknown to what degree midbrain organoids resemble the human midbrain and how a subset of the cells commit to a dopaminergic fate. Here we use longitudinal transcriptional profiling at single cells to understand the identity of all the cells in the organoids and infer how dopaminergic neurons emerge.

BIO

A native of Hang Zhou, China, **Alfred SUN** came to Singapore in 1997 and completed his high school before taking up the A*STAR NSS-PhD scholarship. He obtained B.S. from Duke University and a PhD from Stanford University under the supervision of Dr. Gerald Crabtree. He then returned to A'STAR, Singapore and joined Prof. Bing Lim's and later Prof. Ng Huck-Hui's lab as a postdoc. Prior to starting his own lab in Duke-NUS, Alfred was a junior PI in the National Neuroscience Institute. Alfred is currently leading an independent team using human neural cells to study neurodegeneration, particularly on Parkinson's.



- NOTES -

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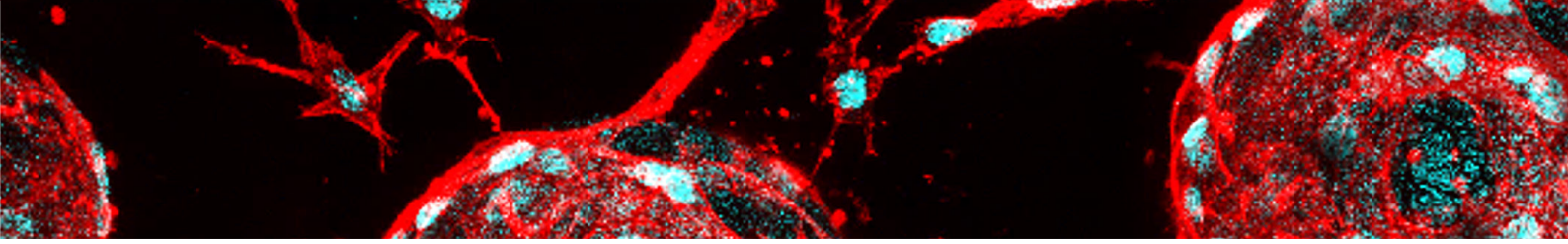
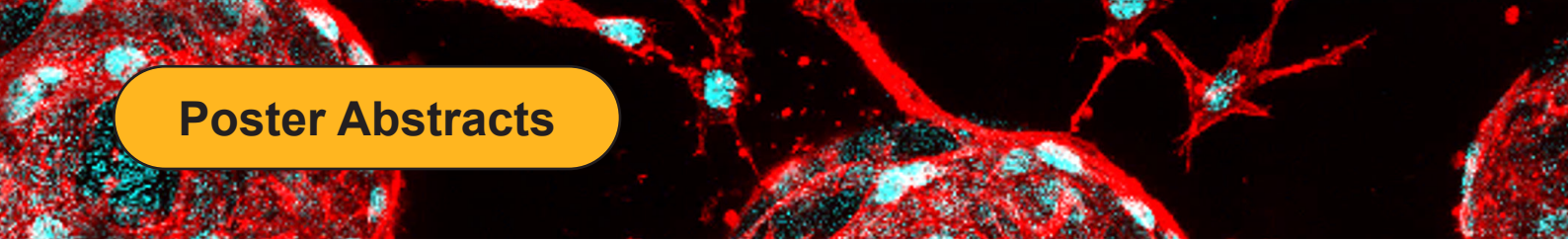


POSTER

ABSTRACTS



Poster Abstracts

P1

Study the functional role of Synaptojanin1 and Auxilin in Parkinson's disease pathogenesis using human stem cell derived neuronal models

Mingkang Liu[1], Hilary S.Y. Toh[1], Alfred Xuyang Sun[1,2], Mian Cao[1,3]

[1]Neuroscience & Behavioural Disorders Programme, Duke-NUS Medical School, Singapore; [2]National Neuroscience Institute, Singapore; [3]Department of Physiology, National University of Singapore, Singapore

Parkinson's disease (PD) is marked by motor deficit due to selective degeneration of midbrain dopaminergic neurons, but the mechanism underlying their vulnerability remains unclear. Recently reported human PD risk genes are found to be involved in pre-synaptic functions, such as synaptic vesicle endocytosis (SVE), implicating an essential role of synaptic dysfunction in PD pathogenesis. Essentially, two synaptic endocytic membrane trafficking proteins, Synaptojanin1 and Auxilin, encoded by PD risk genes SYNJ1 and DNAJC6, work synergistically in SVE and are also linked to early-onset Parkinsonism, further suggesting potential neurodevelopmental PD contributions. To illustrate how these two proteins function in PD pathogenesis, we use human stem cell derived dopaminergic neurons and midbrain-like organoids and CRISPR-Cas9 genome editing to model PD by monitoring pathological changes of SYNJ1KO and DNAJC6KO dopaminergic neurons during development within a humanized cytoarchitecture and extracellular environment. We hypothesize that defective SVE caused by SYNJ1 and DNAJC6 mutations would impair package/sequestration of cytosolic dopamine into the lumen of synaptic vesicles, consequently leading to altered dopamine metabolism and toxicity conferring selective degeneration of dopaminergic neuron in PD. Hopefully, a better understanding of the dysregulated molecular pathway of PD pathology could bring insights into developing future therapeutic interventions for this devastating disorder.

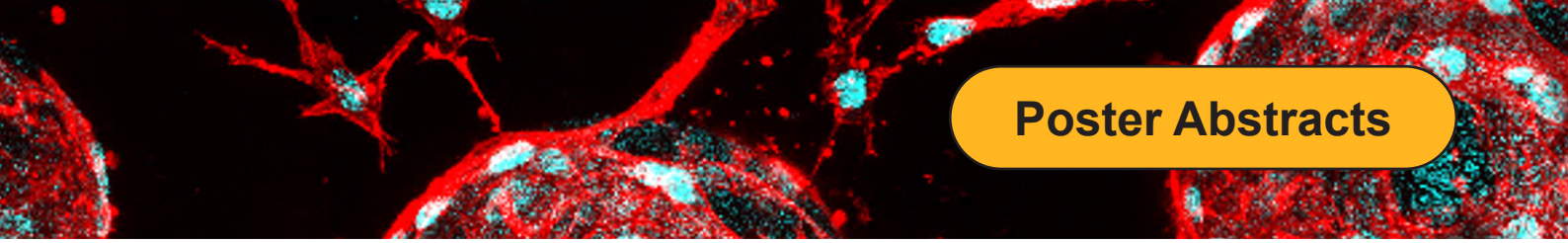
P2

Nuclear Receptor Factor modulates intrinsic development program conserved across blastoids and blastocysts

Ka Wai Wong[1,10], Yingying Zeng[1,2,10], Edison Tay[1], Jia Hao Jackie Teo[1], Yao Yi[1], Haijun Liu[3], Tushar Warrier[1], Qi-Jing Li[4,5], Hu Li[6], Yuin-Han Loh[1,7,8,9,*]

[1]Cell Fate Engineering and Therapeutics Lab, Cell Biology and Therapies Division, Institute of Molecular and Cell Biology (IMCB, Singapore; [2]School of Biological Sciences, Nanyang Technological University, Singapore; [3]Endangered Species Conservation via Assisted Reproduction (ESCAR) Lab, Institute of Molecular and Cell Biology (IMCB), Singapore 138673, Singapore; [4]Institute of Molecular and Cell Biology (IMCB), Singapore; [5]Singapore Immunology Network (SIgN), Singapore; [6]Center for Individualized Medicine, Department of Molecular Pharmacology & Experimental Therapeutics, Mayo Clinic, USA; [7]Department of Biological Sciences, National University of Singapore; [8]NUS Graduate School's Integrative Sciences and Engineering Programme, National University of Singapore; [9]Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [10]These authors contributed equally

Embryonic stem cells possess the remarkable ability to self-organize into blastocyst-like structures upon induction. These synthetic embryo models serve as invaluable platforms for studying embryogenesis and therapeutic developments. Nevertheless, the specific intrinsic regulators that govern this potential for blastoid formation remain unknown. Here we demonstrate a novel intrinsic program that plays a crucial role in both blastoids and blastocysts across multiple species. We first establish metrics for grading the resemblance of blastoids to mouse blastocysts, and identified the differential activation of gene regulons involved in lineage specification among various blastoid grades. Notably, abrogation of nuclear receptor factor (Nrf) drastically reduces blastoid formation. Nrf activation alone is sufficient to rewire conventional ESC into a distinct pluripotency state, enabling them to form blastoids with enhanced implantation capacity in the uterus and contribute to both embryonic and extraembryonic lineages in vivo. Through integrative multi-omics analyses, we uncover the broad regulatory role of Nrf in the transcriptome, chromatin accessibility and epigenome, targeting genes associated with embryonic lineage and the transposable element SINE B1. The Nrf-centred intrinsic program governs and drives the development of both blastoids and early embryos.



Poster Abstracts

P3

Identification of macrophage subtypes within the foetal thymus

Pongsatorn Horcharoensuk[1, 2], Chen Ying[2], Samiyah Shafiq[2], Uma Sangumathi Kamaraj[2], Pradeep Gautam[3], Yuin-Han Loh[1, 2]

*[1]Department of Physiology, National University of Singapore, Singapore; [2]Cell Fate Engineering and Therapeutic Laboratory, Division of Cell Biology and Therapies, A*STAR Institute of Molecular and Cell Biology, Singapore [3]Department of Molecular, Cell, and Cancer Biology, University of Massachusetts Chan Medical School, United States*

Positive and negative selection are two essential decision-making processes necessary for the production of mature T lymphocytes. Subsequently, during the selection processes of T cell development, there is an abundance of cell death in the thymus, which is effectively cleared by thymic macrophages. However, the subtype of macrophage within the human thymus is rarely determined. In this study, we used spatiotemporal enhanced resolution omics-sequencing (Stereo-seq) and single-cell RNA sequencing (scRNA-seq) to identify subtypes of macrophages within foetal thymus. The spatial organization has been separated into three main structures, including the cortex, medulla, and septa, using Pearson correlation. The scRNA-seq data revealed a diversity of cell types within the foetal thymus, including T cells in different stages and other immune cell types such as B cells, mast cells, and macrophages. Subsequently, the macrophages are sub-clustered using UMAP, slingshot pseudotime, and dot plots of marker genes. As a result, there were three subclusters within the macrophage population that can be divided based on the maturation and function of the macrophages, which correlate with spatial transcriptomic location. In conclusion, these findings suggested that stereo-seq and scRNA-seq are effective methods for sub-clustering the cell and pinpointing its location within the thymus.

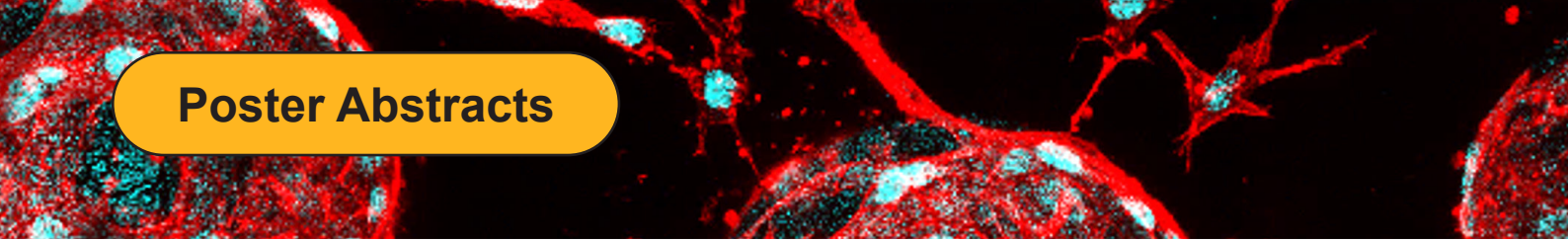
P4

Investigating the function of novel PKD genes in kidney organoids and assessing gender-related effects on cyst formation using patient iPSC-derived organoids

Tian Zhang[1], Chao Zhang[1], Meng Liu[1], Yun Xia[1]

1Lee Kong Chian School of Medicine, Nanyang Technological University Singapore, 11 Mandalay Road, Singapore 308232, Singapore

Polycystic kidney disease (PKD) encompasses two major forms, autosomal dominant (ADPKD) and autosomal recessive (ARPKD), characterized by renal cyst development that can lead to end-stage renal disease. In the first part of this study, we employed CRISPR-Cas9 gene editing to investigate the functional roles of novel PKD-associated genes (GANAB, DNAJB11, DZIP1L) within human kidney organoids. These gene-edited organoids provided critical insights into the mechanisms of cystogenesis. In the second part, we assessed the influence of gender on cyst formation by utilizing patient-specific induced pluripotent stem cell (iPSC)-derived kidney organoids, which were implanted under the renal capsules of both male and female mice. This comprehensive approach not only furthers our understanding of PKD pathogenesis but also sheds light on potential gender-specific variations in cyst development, paving the way for more tailored therapeutic strategies.



Poster Abstracts

P5

Spatiotemporal transcriptomes revealed functional maturation and midkine-mediated vascularization of human grafts in myocardial infarcted pig hearts

Swarnaseetha Adusumalli[1], Samantha Lim[1], Vincent Ren[1], Li Yen Chong[1], Roy Than[1], Ye Lei [2], Yibin Wang[1,3,4], Enrico Petretto[1], Karl Tryggvason[1,4], Lynn Yap[3,5]

[1]Program in Cardiovascular and Metabolic Disorders, Duke-*NUS Medical School, Singapore; [2]University of Alabama, USA; [3]National Heart Center Singapore; 4Duke University, USA; [5]Lee Kong Chian School of Medicine, Singapore*

Ischemic heart disease is the leading cause of death worldwide. During myocardial infarction (MI), there are substantial loss of cardiomyocytes, and heart muscle is replaced with scar tissue leading to heart failure. Regenerative cardiology using stem cell-based therapy holds great potential to replace damaged tissue and improve cardiac function. We used cardiovascular progenitors (CVPs) derived from human pluripotent stem cells differentiated on a laminin 521+221 matrix and transplanted them into acute and chronic MI pig hearts (AMI and CMI). We utilized the 10x Visium platform to define the spatial transcriptomic profile of the transplanted CVPs at AMI 1- and 2- and at CMI 1-, 4- and 12 weeks post-transplantation. In silico analysis showed high transcriptional reproducibility in the biological replicates. Furthermore, the human cells engrafted, matured, and expressed metabolic, ribosomal, T-tubule, and channel-related genes over time. Cell-cell communication analysis revealed Midkine (MDK) signaling as a key pathway that may lead to increased angiogenesis of collaterals in the human graft.

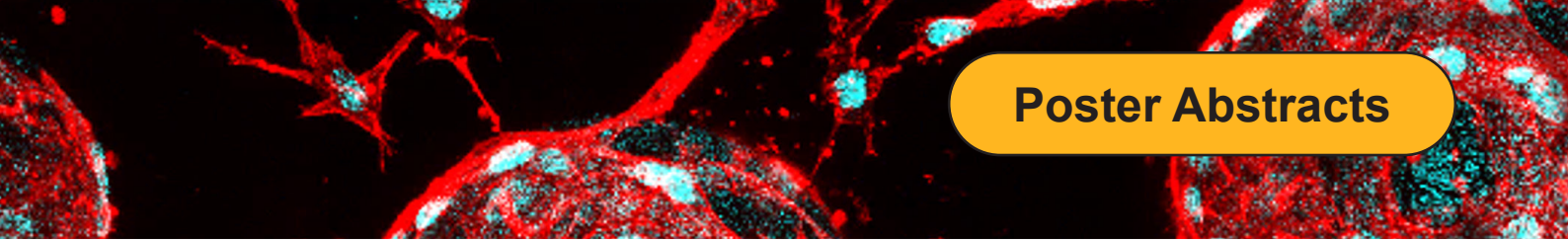
P6

Identification of Long Non-coding RNA regulators of cardiac specification using CRISPRi screening

Ryan Fan[1], Mick Lee[2], Roger Foo[3]

*[1]Cardiovascular Research Institute (CVRI), NUS, Singapore; [2]Genome Institute of Singapore (GIS), A*STAR Biomedical Research Council (BMRC), Singapore*

Long non-coding RNAs (lncRNAs) are a relatively new class of regulatory elements, loosely defined as untranslated transcripts of >200nt. Deep sequencing in the early 2000s has revealed many lncRNAs that have functional roles in human development and disease, and recent collaborative efforts studying the human transcriptome, such as the ENCODE consortium, have annotated thousands of lncRNAs, many of which are currently uncharacterised. One area where lncRNAs are of particular interest is cardiac development. The complex regulatory networks of cardiac specification are yet to be fully elucidated, but lncRNA regulators such as Braveheart, Fendrr, and Meteor have been previously identified in Mus musculus. Therefore, greater investigation of lncRNAs in a human context is paramount, as greater mechanistic understanding of heart development may yield future therapeutic targets. In this project, we opted to focus on cardiomyocyte specification, as dysregulation of the myocardium contributes to many cardiovascular diseases. Here, we report the identification of several putative regulators of cardiomyocyte lineage commitment from a pooled CRISPRi screen, using a library of 322 lncRNAs and an in-vitro hESC-derived cardiomyocyte model. Independent candidate validation was then conducted using individual knockdown lines with the top two sgRNAs by fold change.



Poster Abstracts

P7

Elucidating the mechanism of biologically relevant cardiac laminin-221 in stem cell differentiation

Swarnaseetha Adusumalli[1], Karl Tryggvason[1], Lynn Yap[2]

[1]Cardiovascular and Metabolic Disorders, Duke-NUS Medical School, National University of Singapore; [2]Lee Kong Chian School of Medicine, Nanyang Technological University

Laminins (LN) are heterotrimeric extracellular matrix (ECM) proteins with a high molecular mass and contain three different (□, □ and □) disulfide-linked chains. It is known that LN has tissue-specific expression and modulates cellular phenotypes and stem cell differentiation. RNA transcriptomics analysis of human heart tissue revealed the highly expressed laminin isoform as LN221. In this study, we recapitulated the heart development by coating cell culture plates with LN221 and differentiating pluripotent stem cells towards cardiovascular progenitors (CVPs). A transcriptomic comparison was done with the matrigel-protocol (GiWi) and LN-protocol. The study showed that the LN protocol is more efficient in generating mesodermal precursors and CVPs by comparing expression levels of stage-specific genes. In addition, we also compared the LN protocol with the 3D embryoid body-based differentiation and showed a high correlation (R2>0.8) between both methods. Importantly, our LN221-based differentiation is highly reproducible as evidenced by the single-cell RNA sequencing comparisons between multiple pluripotent stem cell lines. GO terms over-represented in differentially expressed genes from LN221 showed upregulation of the Wnt signaling, organ development, and regulation of heart rate. Suggesting that LN221 is a biologically relevant ECM protein involved in “priming” pluripotent stem cells towards cardiac lineage through activating the Wnt signaling.

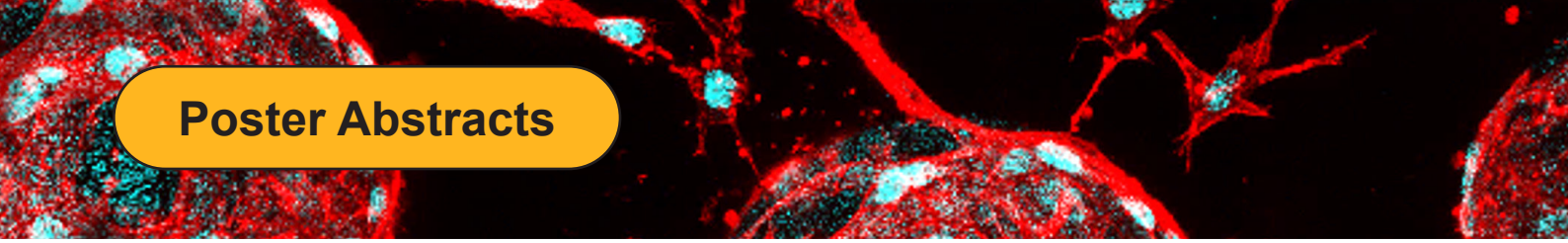
P8

Organoid model of polycystic kidney disease recapitulates clinically relevant symptoms and identifies candidate drugs

Meng Liu[1], Chao Zhang[1], Ximing Gong[1], Tian Zhang[1], Yun Xia[1]

[1]Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore

Polycystic kidney disease (PKD) is one of the most common genetic kidney diseases. Currently, there are limited treatment approaches for PKD. Advances in generating kidney organoids from human pluripotent stem cells (hPSCs) have helped overcome many of the drawbacks of the traditional mouse and 2D cell culture models. Herein, we have generated a collection of kidney organoids from both ARPKD and ADPKD patient-derived iPSCs, as well as genetically engineered hPSCs. Subsequently, we employed stress paradigms to modulate intracellular levels of cAMP or Ca2+ for inducing cystogenesis. We further characterized the structural and functional abnormalities in PKD kidney organoids using a multitude of analyses, including cell biology, molecular biology, biochemistry, and single nuclei RNA-sequencing. Finally, we performed a small-scale drug screening to identify candidate drugs. PKD kidney organoids developed tubular cysts in response to upregulation of intracellular cAMP or downregulation of Ca2+ homeostasis. Multiple structural and functional abnormalities were observed in PKD organoids, including hyper-proliferation of cystic epithelial cells, increased fluid secretion, tubular injury and dedifferentiation, as well as aberrant renin release which are commonly observed in PKD patients. Employing cystic index as the readout, we identified two candidate drugs that can effectively attenuate cyst formation in PKD organoids.



Poster Abstracts

P9

Coordinated Wnt regulation specifies midbrain dopamine neuronal subtypes

Phil Jun Kang[1], Su-Chun Zhang[1]

[1]Program in Neuroscience and Behavioral Disorders, Duke-NUS Medical School, Singapore

During embryonic development, body axis formation is the basis of organogenesis. Antagonistic regulation of signaling pathways precisely controls the body axis formation and generates appropriate cell types for specific organs. Wnt signal is the major player of axial specification, which regulates diverse organogenesis. In this study, we have developed a novel approach to generate pure populations of midbrain floor plate (mFP) progenitors and midbrain dopamine neurons (mDANs) from human pluripotent stem cells in vitro. We applied antagonistic regulation of Wnt signaling pathway to restrict the mFP progenitors to the midbrain identity which co-expressed FOXA2, OTX2 and LMX1A, master regulators of mDAN development. This step eliminates the contamination of forebrain and hindbrain progenitors that plagues previous approaches. Second, we trigger the neurogenesis and prevent the generation of non-neural cells by elevating the concentration of CHIR. As a result, most mDANs derived from stem cells expressed TH which is the major enzymes that synthesize dopamine. The ability to generate pure populations of authentic human mDANs allows modeling disease processes that affect mDANs, testing drugs for these diseases, and developing cell therapy for the diseases through cell transplantation.

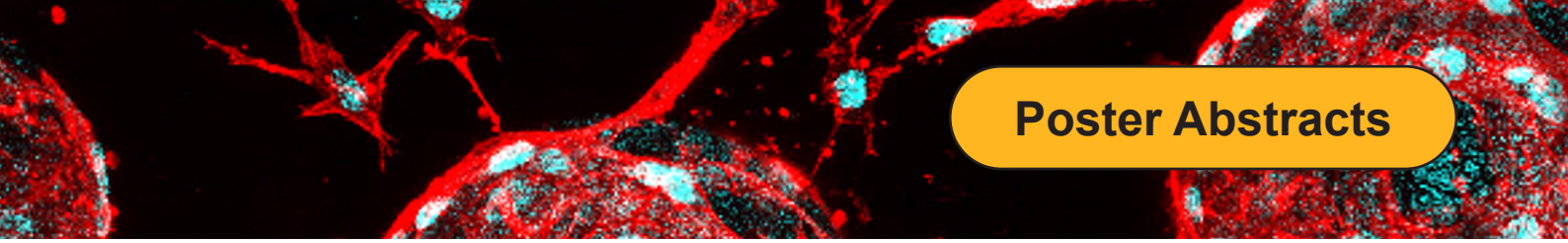
P10

Familial Alzheimer's Disease patient iPSC-derived endothelial cells reveal involvement of dysregulated endothelial cell function and property in Alzheimer's Disease pathogenesis

Yu-Hsin Yen[1], Gabriel Chew[2], Hannah Wee[3], Su-Chun Zhang[1]

[1]Program in Neuroscience & Behavioral Disorders, Duke-NUS Medical School, Singapore; [2]Programme in Cardiovascular and Metabolic Disorders, Duke-NUS Medical School, Singapore; [3]Lee Kong Chian School of Medicine, Nanyang Technology University, Singapore; [4]Department of Neuroscience, Department of Neurology, Waisman Center, University of Wisconsin-Madison, USA

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by an insidious onset of neurocognitive decline, extracellular beta-amyloid (Aβ) plaques, and intracellular tau tangles. Recent studies have highlighted microvascular alterations and transcriptomic changes in endothelial cells (ECs) in post-mortem AD patient brains and AD transgenic mouse models. Microvascular alterations have been observed as early as postnatal day seven in the AD mouse model, suggesting early vascular involvement in AD. However, the specific contribution of ECs to AD in humans and whether these changes are a consequence or a factor in AD development remain unknown. In this study, we hypothesize that dysregulated EC function leads to microvasculature deficits in the brain, contributing to the early stage of AD pathogenesis. Using induced pluripotent stem cells (iPSCs) derived from familial Alzheimer's Disease (FAD) patients with PS1 mutations, we demonstrate innate alterations in ECs, including increased proliferation, migration, and tube formation capabilities, as well as reduced barrier integrity in early passages. In addition, PS1-mutant iPSC-ECs exhibit signs of accelerated cellular senescence and markedly reduced angiogenic properties in later passages. Bulk RNA sequencing analysis of the ECs points to potential alterations in the VEGFA-VEGFR2 pathway. Furthermore, deconvolution of our bulk RNA-seq data with existing single-nucleus RNA sequencing data from sporadic AD patient vasculature shows that PS1 FAD-iPSC ECs are transcriptionally more similar to brain endothelial cells of AD patients than age-matched healthy controls. These findings suggest that the vascular changes observed in our PS1-mutant iPSC-EC model may be a shared phenomenon in AD. The discoveries from this study will enhance our understanding of AD pathogenesis and contribute to the development of novel therapeutics.



Poster Abstracts

P11

Proteome profiling of a transposable element family in mouse embryonic stem cells

Nadia Omega Cipta[1,2], Yingying Zeng[1], Yuin-Han Loh[1,2]

[1]Laboratory for Epigenetics, Stem Cells & Cell Therapy, Programme in Stem Cell, Regenerative Medicine and Aging, ASTAR Institute of Molecular and Cell Biology, Singapore; [2]Department of Biological Sciences, National University of Singapore, Singapore

Transposable elements (TEs) have been increasingly recognized as regulators of genes. In mouse embryonic stem cells (mESCs), the chromatin environment is relatively permissive for TEs to exert their regulatory function. In this study, we attempted to profile the proteins binding on SINE-MIR family in mESCs. Our results revealed enrichment of chromatin organization, pluripotency-associated and epigenetic regulator proteins on this family of TEs. We propose that the cooperative binding of these proteins endows SINE-MIR with regulatory capability.

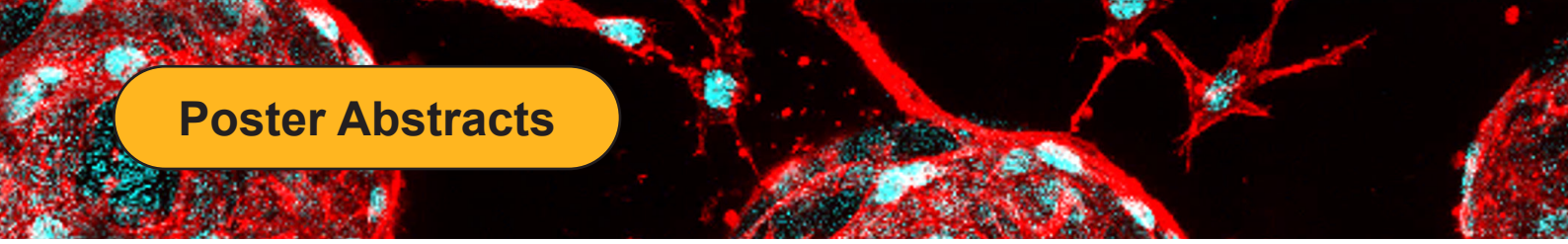
P12

Understanding midbrain dopaminergic cell fate acquisition for Parkinson's Disease therapy

Hilary Toh[1], Audrey Khoo[1,2], Adeline Henry Basil[1,2], John F. Ouyang[1], Alfred Sun[1,2]

[1]Duke-NUS Medical School, Singapore; [2]National Neuroscience Institute, Singapore

Midbrain dopaminergic neurons are preferentially degenerated in Parkinson's disease. Deriving these neurons from human pluripotent stem cells has strong potential for modeling and treating Parkinson's disease. Using single cell transcriptomics, we define major cell types within the midbrain and delineate its developmental trajectories using human midbrain-like organoids (MLOs). These developmental programs are compared to the fetal midbrain. We show that MLOs are able to recapitulate the cell type diversity of the midbrain seen in vivo, and are transcriptionally similar to age-matched counterparts in the fetal brain. We also note the appearance of the A9-like dopaminergic neuronal population, which becomes functionally mature within 6 months in culture. Through our analysis, we identify transcriptional programs that are upregulated in dopaminergic precursors. These results can be used to guide stem-cell therapies of PD to produce better quality grafts of dopaminergic progenitors. Furthermore, we transplanted dopaminergic neurons derived from MLOs into substantia nigra-lesioned mice brains, showing that MLOs have therapeutic potential to correct motor deficits in a mouse model of PD.



Poster Abstracts

P13

Single cell transcriptomic study of tricuspid atresia patient-specific hiPSCs at multiple stages of in vitro cardiac differentiation

Yin-Yu Lam[1], Chun-Ho Chan[1], Lin Geng[1], Wendy Keung[2], Yiu-Fai Cheung[1,2]

[1]Department of Paediatrics and Adolescent Medicine, School of Clinical Medicine, The University of Hong Kong, Hong Kong, China; [2]Dr. Li Dak-Sum Research Centre, The University of Hong Kong - Karolinska Institutet Collaboration in Regenerative Medicine, The University of Hong Kong, Hong Kong, China

Tricuspid atresia (TA), characterized by absence of right atrioventricular connection and hypoplasia of the right ventricle, is a rare cyanotic congenital heart disease. The cause and genetic basis of this malformation in humans remains poorly understood. Mutations in genes underpinning TA knockout animal models have not been found in human TA patients. Identifying the underlying transcriptomic defects might shed light on its pathogenesis. In this study, we established human induced pluripotent stem cells (hiPSCs) from 3 TA patients and 3 healthy controls. At multiple differentiation time points corresponding to different cardiac progenitor and cardiac lineage cell populations, we performed single cell transcriptomics to interrogate transcriptomic abnormalities in TA at different stages. This is the first successful attempt to derive hiPSCs from TA patients. We identified cardiac, endothelial progenitor and cardiac, endothelial lineage descendants at various time points with single cell transcriptomics. TA-hiPSC-cardiac and endothelial lineages were nonetheless comparable to control-derived lineages. The lack of significant transcriptomic differences in cardiac and endothelial lineage suggests that defects in early cardiac differentiation does not play a significant role in the pathogenesis of TA and point towards maldevelopment of neighbouring structures such as endocardial cushions as the initiating event resulting in the cardiac malformation.

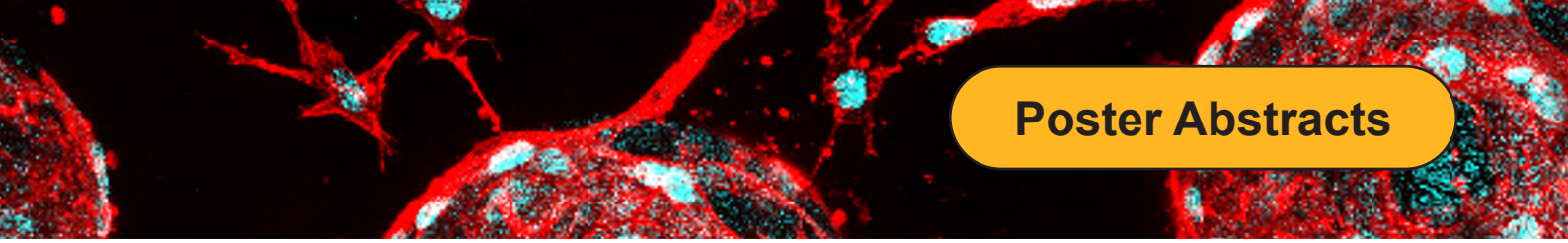
P14

VHL deficient human kidney organoid to model clear cell renal cell carcinoma

Yixuan Wang[1*], Huamin Wang[1*], Ximing Gong[1], Meng Liu[1], Chao Zhang[1], Tian Zhang[1], Angelysia Cardilla[1], Mihir Yogesh Naik[1], Yun Xia[1]

[1]LKCMedicine, Nanyang Technological University, Singapore; [*]These two authors contributed equally to this work

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.



Poster Abstracts

P15

The role of SUMO pathway in neural stem cell reactivation and brain development

Yang Gao[1], Ye Sing Tan[1], Jiaen Lin[1], Liang Yuh Chew[1], Htet Yamin Aung[1], Brinda Palliyana[1], Mahekta Rajeshkumar Gujar[1], Kun-Yang Lin[1], Fengwei Yu[2,3], Shu Kondo[4], and Hongyan Wang* [1,5,6]

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Neural stem cells (NSCs) are crucial for brain development and tissue homeostasis. The delicate balance between quiescence and activation of NSCs is crucial for neurogenesis and NSC maintenance, and dysregulation can cause neurodevelopmental disorders. Posttranslational modification with small ubiquitin-related modifier (SUMO) is a widespread mechanism for rapid and reversible changes in protein function. Although the SUMO pathway is linked to neurodegenerative diseases, its role during NSC reactivation and brain development is not established. Here, we investigated the function of the SUMO pathway in Drosophila and found that its key components are necessary for NSC reactivation and brain development. We further found that the core kinase of the Hippo pathway, Warts, can be SUMOylated, and this modification attenuates its kinase activity and promotes its degradation, leading to inhibition of the Hippo pathway to promote NSC reactivation. Our study uncovers a novel function of the SUMO pathway associated with the Hippo pathway during Drosophila NSC reactivation and brain development.

P16

Coordinated induction of metanephric mesenchyme and ureteric bud to generate human kidney organoids with collecting duct network

Chao Zhang[1], Yun Xia[1]

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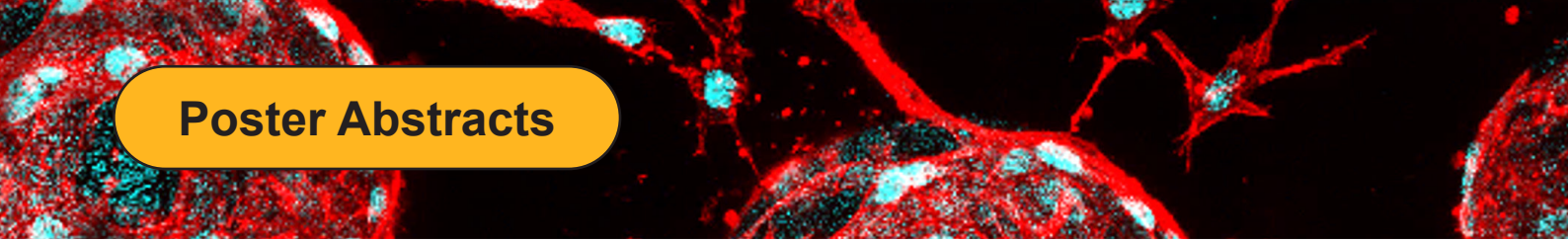
Current kidney organoids can only separately model nephron and collecting duct due to the distinct spatiotemporal origins of these two lineages. Moreover, reliance on uncontrolled, spontaneous self-organization during aggregation results in deficiency in organ-level anatomy, which hamper kidney organoids from recapitulating the organ-level function. In this study, we establish a protocol to coordinately differentiate metanephric mesenchyme (MM) and ureteric bud (UB) from human pluripotent stem cells. Nephrogenic niche with UB tip cells in the center while nephron progenitor cells (NPCs) in the periphery is established. Intrinsic reciprocal signals from UB tip cells and NPCs promote these two lineages to develop into collecting ducts and segmented nephrons respectively, without any need of exogenous growth factors. Importantly, 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 collecting duct network, showing unprecedent structural resemblance to kidney anatomy.



Poster Abstracts

P17

Novel anti-microbial peptides derived from differentiating adipose-derived stem cells

Smarajit Chakraborty[1]; Brain Chia[2], Kamaladasan Kalidasan[3], Prabha Sampath[3], Shigeki Sugii[1]

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Chronic wound is a large and growing problem predominantly due to the emergence of multidrug resistant (MDR) bacterial pathogens with a reported annual spending of US$ 25 billion in United States., We successfully developed a unique co-culture setup between bacteria and ASCs and discovered robust antibacterial properties of conditioned media (CM) derived from intermediate adipogenic differentiating ASCs triggered by bacteria priming, which has never been reported before to our knowledge. Activated ASC-CM exhibited robust bacterial killing, significant antibiofilm effects against Enterococcus faecalis, Pseudomonas aeruginosa and methicillin resistant Staphylococcus aureus (MRSA), accelerated wound-healing and most importantly, prevented antimicrobial resistance (AMR). Through proteomics analysis and confirmatory experiments, we have identified two potent, non-toxic, novel antimicrobial peptides (AMPs). Next, we discovered ultra-short regions (>21-residues) within the AMPs which showed enhanced antimicrobial efficacy, prevented acquisition of AMR, improved migration of human keratinocytes, exhibited reduced cytotoxicity and hemolysis compared to AMP, pexiganan (Phase III clinical trial, NCT01590758) and most antibiotics commonly prescribed for chronic wound management. We aim to develop our lead preclinical peptide into a clinical candidate for the treatment of chronic wound infections.

P18

Small regulatory RNAs in human primordial germ cell development

Navin Brian Ramakrishna[1, 2, 3], Andrea Schorn[4], M. Azim Surani[2], Eric A. Miska[2, 3], et al.

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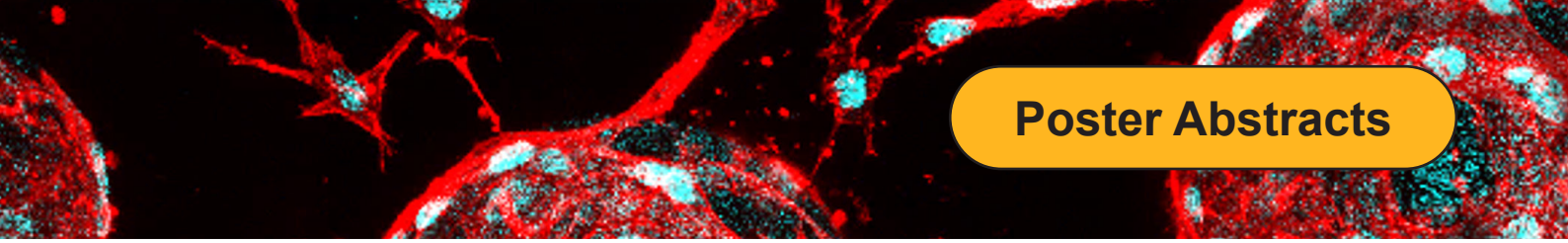
Human Primordial Germ Cells (hPGCs) are the precursors to gametes, emerging at the third week of embryogenesis. Previous studies have detailed their unique transcriptional network, differing from mice, and extensive DNA methylation erasure. Like their mouse counterparts, hPGCs show pluripotency marker retention. While roles for micro-RNAs (miRNAs) have been identified in mouse PGCs, the miRNA-ome and its role in hPGCs remains unknown. Moreover, from Wk10, asynchronous fetal germ cell subgroups (hFGCs) appear alongside hPGCs, with similarly low DNA methylation levels. Yet, transposable elements (TEs) remain repressed, hinting at TE defence mechanisms. Potential defences include post-transcriptional small-RNA silencing. Using small-RNA-seq, I profiled small-RNAs in isolated hPGCs across development, complementing these with in vitro and mouse models. First, I profiled the miRNA landscape of developing hPGCs from Wk6-22 and in vitro hPGCLC germline models, identifying a unique germline miRNA signature with ties to naive pluripotency (in contrast to primed pluripotency). Interestingly, while PIWI-interacting small-RNAs (piRNAs) were absent in hPGC sub-populations, they were readily observed in mature male and female hFGCs as early as Wk12, with divergent characteristics. Meanwhile, more abundant Endogenous Retrovirus (ERV)-mapping tRNA-fragments (tRFs) were observed in hPGCs compared to surrounding somatic cells, in this germ cell subpopulation that lack piRNAs.



Poster Abstracts

P19

Nutritious cultivated fish adipocytes for cellular agriculture

Lamony Chew[1], Cheryl Wong[1], Shigeki Sugii[1,2]

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Cellular agriculture is an emerging field that has attracted more than USD 3 billion of investments. It is described as the production of agricultural products, i.e. meat, using cell culture techniques. Generally, meat contains 60% water, 20% protein, and 20% fat. However, most R&D activities are focused on producing myocytes, and few studies are focused on producing adipocytes. Here, we have successfully established several novel fish-derived adipocytic cell lines, including a Pangasius catfish-derived cell line. This cell line exhibited high growth rate, with doubling time of 13 hours, and stability for more than 200 passages. We have also developed a novel serum-free adipogenic differentiation medium. Using this differentiation medium, we demonstrated that more than 95% of Pangasius catfish-derived cell line differentiated into matured adipocytes, as shown by lipid droplet staining analysis. Whole genome transcriptome analysis of the cultivated adipocytes indicates similarity to original Pangasius fats, without induction of oncogenes. We also established scalable 3D protocols of growing and differentiating cultivated adipocytes. Most importantly, our cultivated adipocytes contain similar fatty acid profiles, including DHA and EPA, as compared to tuna belly sashimi. In summary, we have established scalable fast-growing adipocytic fish cell lines that can enhance the nutrition of cultivated meat.

P20

Investigating the role of microglia in neurodegenerative disease using patient iPSC

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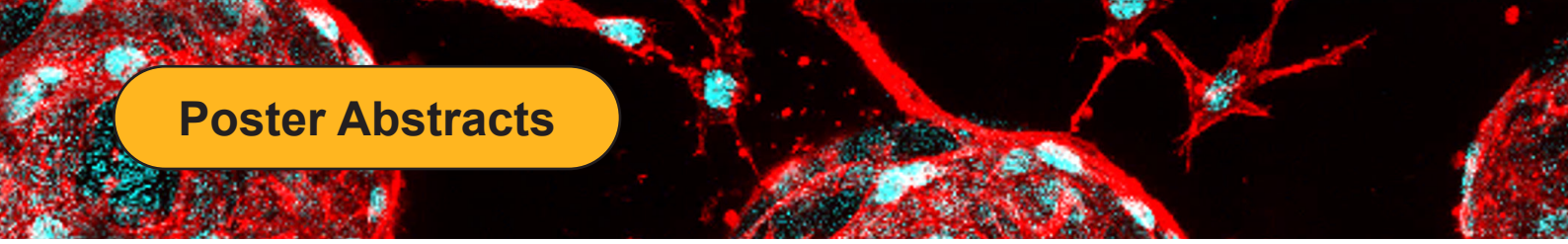
Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) is a rare autosomal dominant neurodegenerative disease characterized by demyelination of white matter, swollen axons, and pigmented glial cells. ALSP is primarily caused by mutations in the CSF1R, which is highly expressed in microglia and is crucial for brain formation and homeostasis. Here, we identified a novel mutation, CSF1R-T567M, in an ALSP patient. To understand the pathogenesis of CSF1R-T567M-associated ALSP, we reprogrammed the peripheral blood mononuclear cells (PBMC) of the ALSP patient into induced pluripotent stem cells (iPSCs) and further differentiated into microglia, neurons, and cerebral organoids. We found that the CSF1R-T567M mutation impaired the migratory ability, phagocytic, and neuroimmune response of iPSC-derived microglia; disrupted the proliferation and differentiation of neural progenitor cells (NPCs) and impaired the neurodevelopment of cerebral organoids at an early stage. In addition, the CSF1R-T567M mutation affected the autophosphorylation of CSF1R at Tyr546 (Y546), which in turn affected downstream signaling pathways that are associated with autophagic flux. All of this closely mimicked the pathological features of CSF1R-T567M-associated ALSP. These results provide insight into the molecular mechanism of the CSF1R-T567M mutation in the pathogenesis of ALSP and pave the way for future precision therapy.



Poster Abstracts

P21

Modeling MT IVNS1ABP-induced cellular senescence by human iPSCs derived NPCs

Fang Yuan[1], Carine Bonnard[2], Bruno Reversade[2], Su-Chun Zhang[1,3]

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Cellular senescence is a cellular state in response to stress. It occurs throughout life, which may contribute to development or aging. In Hutchinson-Gilford Progeria Syndrome (HGPS), mutated lamin A produces a truncated protein progerin, accelerating cellular senescence. Progerin is also detected in normal aging process, highlighting the similarity between premature aging and natural aging. In this study, we have recently encountered a family whose teenagers display progeria symptoms, including dyschromatosis, gray hairs, progressive motor deficits, and intellectual developmental delay. Whole genomic sequencing revealed a homozygous mutation in the IVNS1ABP gene. As an influenza virus nonstructural protein-1 binding protein, IVNS1ABP and its association with aging has never been reported. Here, we acquired dermal fibroblasts from the patients and their family members, generated and created isogenic iPSCs from the fibroblasts, and then differentiated the isogenic iPSCs into neural progenitor cells (NPCs). MT NPCs showed senescent phenotypes and the cytokinesis was disrupted, leading to mitotic failure. Proteomics analyses revealed actin and actin-binding protein alteration. Actin co-sedimentation and bioactivity analysis suggested that IVNS1ABP mutation altered actin dynamics directly, leading to mitotic failure and cellular senescence. Our findings suggest a potential mechanism underlying the aging symptoms of the patients.

P22

Uncovering the role of G6PC2 and the effects of its East Asian-specific S324P variant in glucose metabolism

Lay Shuen TAN[1,2], Gabriel Jing Xiang Ong[1,3], Natasha Hui Jin Ng[1], Craig Nunemaker[4], Xueling Sim[5], Chin Meng Khoo[6], Richard M. O'Brien[7], E Shyong Tai[5, 6, 8, 9, #, *], Adrian Kee Keong Teo[1, 6, 9, 10, #]

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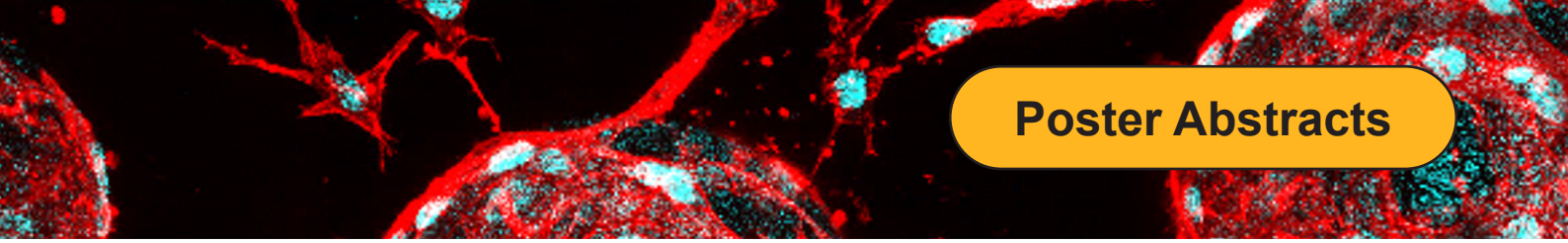
The G6PC2 gene provides instructions for producing the enzyme glucose-6-phosphatase catalytic subunit 2 which plays a role in glucose homeostasis. The mechanism by which G6PC2 influences human glucose metabolism remains obscure, and studies investigating G6PC2 gene regulation and expression are lacking. Recently, the G6PC2 S324P variant has been associated with reduced fasting blood glucose in East Asians, but the mechanism is unclear. Here, we examined key pancreatic transcription factors in the regulation of G6PC2 expression. By conducting luciferase reporter activity assays in vitro, we found that five transcription factors regulating pancreatic development and function, namely NEUROD1, PDX1, HNF1A, MAFA and ZHX3, activated the -376/+4 human G6PC2 promoter in MIN6 cells and human beta cell line EndoC-βH1. Next, we found that overexpressing G6PC2 WT in EndoC-βH1 cells reduced insulin secretion at 2.8 mM, 5.5 mM, 11.1mM and 16.7 mM glucose concentrations, while overexpressing G6PC2 S324P did not lead to the same effects. Further studies would involve the knockout of G6PC2 in human pancreatic beta cells, accompanied by G6PC2 S324P recall-by-genotype studies in humans in vivo, to ascertain how the G6PC2 loss of function can affect insulin secretion and glucose homeostasis.



Poster Abstracts

P23

Primed extracellular vesicles for acute kidney injury

Sungok Hong[1], Hongduk Kim[1], Soo Kim[2], Tae Min Kim[1,3]

[1]Institutes of Green-Bio Science and Technology, Seoul National University, South Korea; [2]Brexogen Research Center, Brexogen Inc., Songpa-gu, Seoul 05855, South Korea; [3]Graduate School of International Agricultural Technology, Seoul National University, South Korea

Acute kidney injury (AKI) is a global health concern. Extracellular vesicles (EVs) produced by induced mesenchymal stem cells (iMSCs) have gained recognition as alternative option for AKI. This study was aimed to examine whether pan-peroxisome proliferator-activated receptor agonist treatment could enhance the therapeutic efficacy of resulting EVs (pan PPAR-iMSC-EV) for AKI. EVs from non-stimulated iMSCs (iMSC-EV) and stimulated iMSCs (pan PPAR-iMSC-EVs) showed typical EV marker expression and morphology. Pan PPAR-iMSC-EVs had enhanced potential for the proliferation of HK-2 cells compared with iMSC-EVs. Using M1-polarized THP-1 cells, we also found that the expression of inflammatory cytokine mRNA was markedly reduced in pan-PPAR-iMSC-EVs those from iMSC-EV-treated group. Compared to iMSC-EVs, pan-PPAR-iMSC-EVs significantly increased renoprotective effects in the mouse model of cisplatin-induced AKI. Pan PPAR-iMSC-EVs specifically decreased tissue inflammatory response, immune cell infiltration, and apoptosis. Finally, renal capillary density was similarly boosted by pan-PPAR-iMSC-EVs. This cell-free approach with cell priming strategy might help create a cutting-edge therapeutic alternative for AKI.

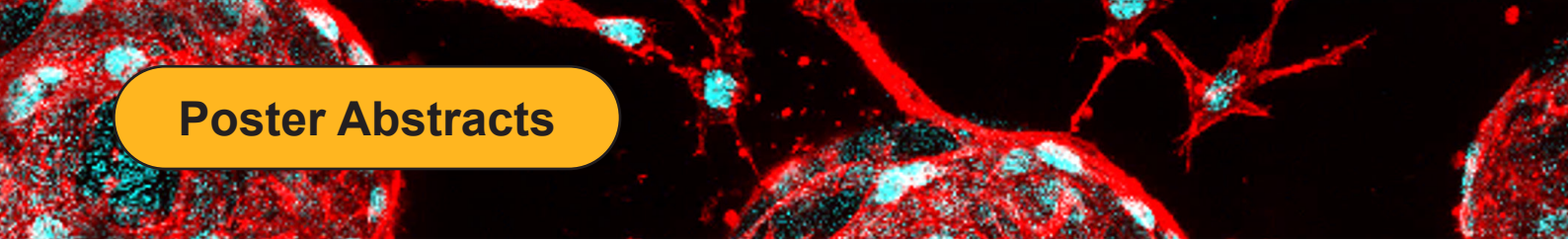
P24

Human stem cell-derived kidney organoid to model diabetic nephropathy (DN)

Huamin Wang[1], Tian Zhang[1], Angelysia Cardilla[1], Jiayi Zhang[2], Meng Liu[1], Yun Xia[1]

[1]Lee Kong Chian School of Medicine, NTU, Singapore; [2]School of Biological Science, NTU, Singapore

Diabetic nephropathy (DN), a devastating complication of diabetes mellitus, is a leading cause of end-stage renal disease (ESRD) worldwide. Despite significant advancements in therapeutic approaches, the underlying mechanisms of DN remain incompletely understood. Kidney organoids are derived in vitro from human pluripotent stem cells (hPSCs) harboring segmentally patterned nephron-like structures and stroma, as well as a vascular network. We investigated the effects of high glucose and free fatty acid on kidney organoid to recapitulate the hyperglycemia and dyslipidemia states observed in diabetes. Histopathological and functional analysis, as well as molecular profiling, are performed to evaluate organoid pathology under these conditions. Diabetic kidney organoids show a range of phenotypes resembling DN at different levels, including deterioration of vascular network, altered stromal cell proportion, acquisition of injury signature, and upregulated inflammatory signaling. Diabetic kidney organoids provide the possibility to specifically study pathogenesis of DN in human patients, which holds great potential for testing new drugs and evaluating novel genetic variants associated with DN.



Poster Abstracts

P25

Human mesenchymal stem cell processing for clinical applications using a closed semi-automated workflow

Alan Lam[1], Premkumar Jayaraman[2], Abigail Becker[2], Ryan Lim[2], Kim Leng Teo[1], Jacqueline Ng[2]

*[1]Bioprocessing Technology Institute (BTI), Agency for Science, Technology and Research (A*STAR); [2]Thermo Fisher Scientific*

Human mesenchymal stem cells (hMSCs) are currently being explored as a promising cell-based therapeutic modality for various diseases, with more market approvals expected for clinical use over the next few years. To facilitate this transition, addressing the bottlenecks of scale, lot-to-lot reproducibility, cost, regulatory compliance, and quality control is critical. These challenges can be addressed by closing the process and adopting automated manufacturing platforms. Here, we demonstrated Wharton's Jelly-derived hMSCs (WJ-1) expansion using regulatory compliant, StemPro® MSC SFM XF medium in closed multi-layered flasks, followed by post-expanded cells harvest directly from the cell factories in a closed, semi-automated manner using the Rotea Counterflow Centrifugation System. WJ1 expanded in the SFM XF medium showed comparable cell proliferation and morphology to that of WJ-1 expanded in serum-containing media. Our closed semi-automated harvesting protocol demonstrated high cell recovery (~98%) and viability (~99%). Cells washed and concentrated using the system maintained hMSC surface marker expression, colony forming units (CFU-F), trilineage differentiation potential, and cytokine secretion profiles. The cell harvesting protocol developed in the study can be easily applied for small- to medium-scale processing of various adherent and suspension cells by directly connecting to different cell expansion platforms to perform volume reduction, wash, and harvest in a low output volume.

P26

Transplantation of cardiovascular progenitors into myocardial infarcted pig heart

Samantha Lim[1,6], Swarnaseetha Adusumalli[1], Min En Cheng[1], Clarissa Tan[1], Li Yen Chong[1], Poh Loong Soong[2], Ye Lei[3], Eric Lim[4], Enrico Petretto[1], Karl Tryggvason[1,5], Lynn Yap[4,7]

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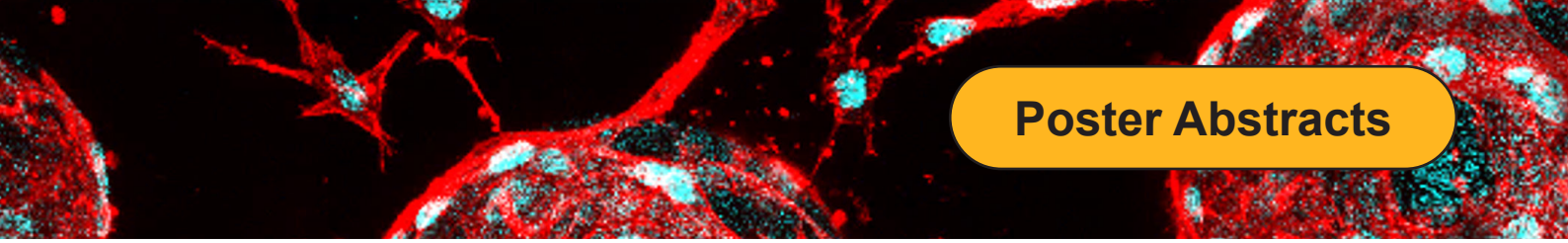
Ischemic heart disease, despite advancements in medicine and technology, remains a global health challenge as it remains the leading cause of illness and death. This study introduces a novel differentiation technique using laminin molecules, specifically LN-221, as a substrate to create Cardiovascular Progenitor Cells (CVPs) from pluripotent stem cells. To mimic human ischemic heart conditions, coronary arteries in immunosuppressed pig models were permanently ligated before CVP transplantation. Promisingly, the transplanted CVPs were successfully integrated into infarcted regions, confirmed via IVIS optical imaging and histological analysis. Magnetic resonance imaging (MRI) demonstrated a significant improvement of 10-15% in left ventricular ejection fraction and reduced infarct size compared to a control group (p-value < 0.05). During a 3 months monitoring period involving ten pigs, four experienced temporary occurrences of ventricular tachycardia (VT), with one pig experiencing persistent VT, while the remaining five maintained a normal sinus rhythm. Importantly, all ten pigs survived the transplantation without VT-related fatalities. A pipeline for 10X spatial transcriptomics analysis was conducted post-transplantation to further investigate the mechanism of regeneration. This study represents a significant step toward addressing ischemic heart disease through CVP transplantation, with potential implications for enhancing patient outcomes.



Poster Abstracts

P27

Altered DNA methylation in HUVECs isolated from pregnancies complicated by gestational diabetes: a contributor to cardiometabolic disease predisposition in the offspring

Dewei Kong[1,2,3], Lillian Yuxian Lim[1], Rajkumar s/o Dorajoo[4], Shirley Suet Lee Ding[1], Patrick Lee[5], Adrian Kee Keong Teo[1,6,7,8*], Shiao-Yng Chan[3,5*]

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Gestational diabetes mellitus (GDM) has long-term effects on the offspring, increasing the cardiometabolic disease risk in the future. However, how it contributes to disease development remains unclear. Using placenta and umbilical cord blood/cells/tissues, studies have found that GDM may change the offspring's methylome. However, cellular heterogeneity in these samples may mask GDM-related marks. Here, we aimed to investigate how GDM could change the offspring's methylome in human umbilical cord vein endothelial cells (HUVECs). Subjects with (N=16) and without GDM (N=16) were recruited and HUVECs were isolated. Illumina EPIC array was performed to determine DNA methylation differences between non-GDM and GDM samples, and with increasing maternal glycaemia. Our preliminary results showed that cell passage number strongly impacted the methylome. Therefore, we stratified our samples into low (P2-4) and high passage (P5-7) groups, and mainly focused on the low passage group. Compared to non-GDM HUVECs, GDM HUVECs showed seven hypomethylated and six hypermethylated CpG sites in Chinese, and one hypomethylated CpG site in Indian samples. We also found that fasting and 2h oral glucose tolerance test (OGTT) levels were associated with different DNA methylation marks. Overall, this project suggested the potential of using HUVECs to understand how GDM affects fetal programming through epigenetic mechanisms and predisposes future cardiometabolic diseases.

P28

Unravelling metabolic defects in macrocephalic ASD knockdown iPSC lines

Zi Jian Khong[1,2], Shi Yan NG[1]

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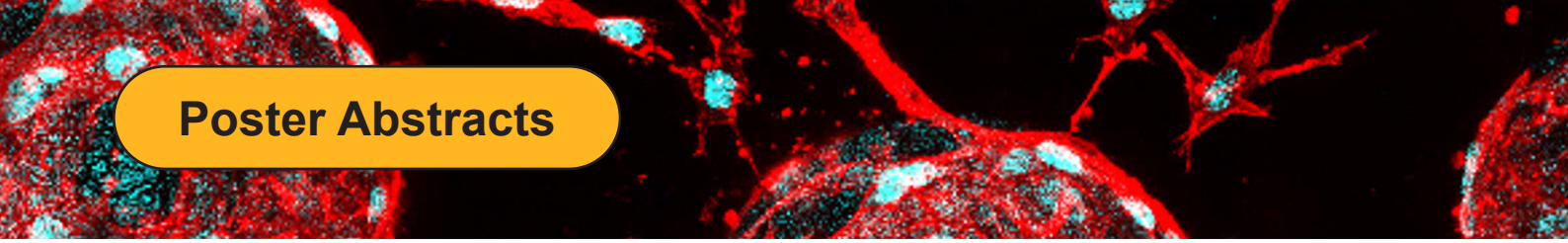
Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that is characterized by a deficit in communication and interaction, with repetitive behaviors. For a subset of ASD patients, they are also diagnosed with macrocephaly, characterized by an abnormally large head size. Both CHD8 and PTEN mutations have been attributed as the cause of macrocephalic ASD. Despite the distinct function of CHD8 and PTEN, disruption in these genes often results in the same set of clinical features. These studies suggest that the downstream effects of these genes might converge on shared pathways that lead to shared behavioral phenotypes observed in ASD. To deconvolute the heterogeneous effects of these genes, we have generated two pairs of inducible shRNA iPSC cell lines, shCHD8 and shPTEN, that produce robust knockdown throughout differentiation. Both shCHD8 and shPTEN early neurons exhibited a significantly higher proliferative rate. Additionally, we have also identified an overactivation of glycolysis that is found exclusively in shCHD8 neurons and an increase in non-glycolytic acidification that is shared between both shCHD8 and shPTEN neurons. These preliminary results suggest that a disruption in glycolysis within macrocephalic ASD cortical neurons could be an underlying cause behind the pathology of macrocephalic ASD.



Poster Abstracts

P29

Elucidating lineage plasticity in pancreatic organogenesis through multiomic profiling of human islets derived from stem cells

Punn Augsornworawat[1], Jeffrey R. Millman[2,3]

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The β-cells in pancreatic islets regulate blood glucose levels by secreting insulin. In type 1 diabetes (T1D), the β-cells in pancreatic islets are destroyed, leading to insufficient insulin production. While islet cell replacement therapy may serve as a long-term cure for T1D, there is limited supply of primary islets from compatible donors. To address this issue, stem-cell derived islets (SC-islets) are a promising alternative as they can be generated in unlimited quantities. However, SC-islets lack proper functional capabilities due to their immature state. We hypothesized that epigenetic and transcriptomic landscape plays a role in determining the maturity state of β-cells. By using multiomic (mRNA and ATAC) single-cell sequencing, we have identified differences in the transcriptome and epigenetic profiles of immature and mature β- cells from in vitro SC-islets, transplanted matured SC-islets, in vitro matured SC-islets, and human primary islets. We have identified crucial factors that define the fate and maturation of β- cells in SC-islets. Through genetic engineering experiments targeting these genes, we have improved SC-islet differentiations. Overall, our study provides valuable insights that can be used to enhance the development of SC-islets for treating type 1 diabetes.

P30

Partial restoration of retina function in pre-clinical models using laminin differentiated photoreceptor progenitors

Wei Sheng Tan[1,2], Swarnaseetha Adusumalli[2], Luanne Chiang Xue Yen[1], Edwin Shepherdson[1], Sai Bo Bo Tun[3], Veluchamy Amutha Barathi[3], Gavin Tan Siew Wei[3], Enrico Petretto[2] and Karl Tryggvason[2,4,] and Hwee Goon Tay[1,3*]

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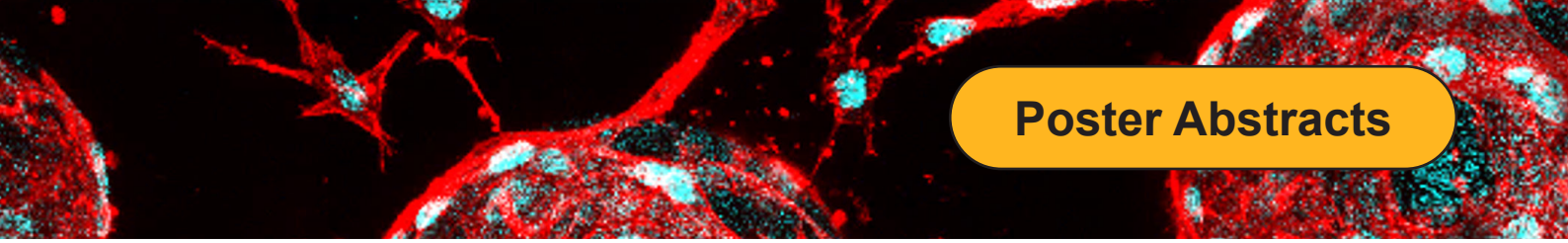
There is rising worldwide prevalence of advanced stages of inherited retinal diseases and age-related macular degeneration. Irreversible photoreceptor loss due to these diseases could lead to blindness. Therefore, stem cell based therapy to replace the loss of photoreceptors is promising to preserve or restore vision in these patients. Our lab has developed a laminin based photoreceptor differentiation method to generate photoreceptor progenitors from human pluripotent stem cells. Comparative single-cell transcriptomic analysis on different laminin isoforms showed that photoreceptor progenitors co-expressing CRX and RCVRN were present as early as Day 32 and significantly higher in the presence of specific laminin isoform 523. We then shortlisted candidate genes we hypothesized to be involved in early retinal cell-fate specification which could also be translated into optimizing our current differentiation protocol. Sub-retinal cell transplantation of Day 32 photoreceptor progenitors in the genetic rd10 mice model showed host-graft integration with extensive synaptic connectivity after 5 months post-transplantation. We also have preliminary result showing subretinal transplanted NHPs exhibited short term retina function improvement which could be a result of functional cell engraftment size. This may constitute an important step towards the future use of human embryonic stem cell derived photoreceptor progenitors to treat vision loss.



Poster Abstracts

P31

Computationally defined genomic safe harbour loci enable stable, as well as inducible, transgene expression in human pluripotent stem cells

Matias I. Autio[1,2]

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Genomic safe harbour (GSH) loci have been proposed as safe sites in the human genome for directed transgene integration. We identified 25 unique putative GSH loci that reside in active chromosomal compartments. We validated stable transgene expression and minimal disruption of the native transcriptome in three GSH sites in vitro using H1 and H9 human embryonic stem cells (hESCs). By engineering landing pad integration cassettes into the GSH sites we have generated pluripotent stem cell lines with easy integration of transgenes or of complex expression cassettes. Using the landing pad GSH cells we have generated hES lines with targeted inducible expression of genes of interest. These cells have also allowed us to establishe a genome integrated platform for dissecting promoter and enhancer activity in pluripotent stem cells as well as their differentiated progeny.

P32

Identification of NEUROD1 targets in differentiating human pancreatic islet cells

Andreas Alvin Purnomo Soetedjo[1,2], Elhadi Iich[1,3], Choon Tat Ng[1], Walter Hunziker[2,4], and Adrian Kee Keong Teo[1,3,5,6]

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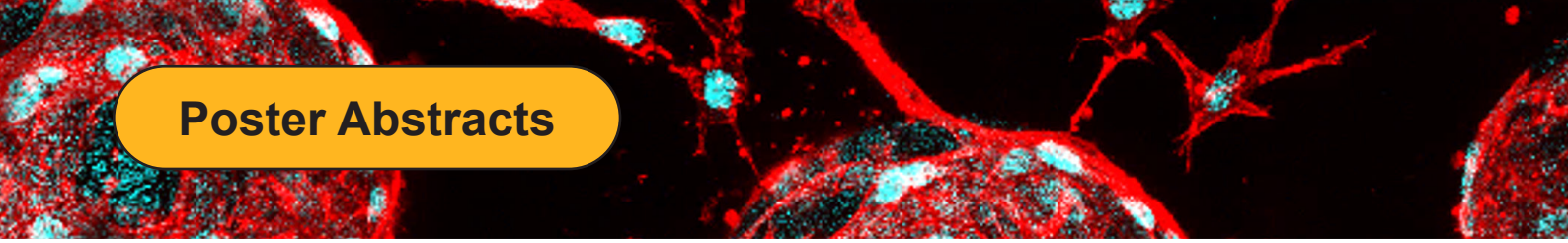
NEUROD1, a basic helix-loop-helix transcription factor, plays a pivotal role in the proper development of the pancreas. Mutations in NEUROD1 gene have been associated with monogenic diabetes subtype 6 (MODY6) in humans, characterized by insulin deficiency and neurological abnormalities, though the underlying mechanisms remain elusive. While the loss of NeuroD1 in mouse model impairs pancreatic beta cell development and function, it is less understood in humans. In our study, we sought to emulate the loss of NEUROD1 in vitro by generating NEUROD1 knockout allelic series in human embryonic stem cells (hESCs) and differentiating them into pancreatic islet-like cells. Additionally, we also knocked out NEUROD1 in human beta cell line EndoC-βH1 to identify targets and pathways regulated by NEUROD1. We performed RNA-Sequencing (RNA-Seq) and chromatin immunoprecipitation-Sequencing (ChIP-Seq) on NEUROD1 in differentiating human pancreatic islet-like cells and identified a set of putative NEUROD1-targets that could be involved in pancreatic beta cell function. Further follow-up studies seek to determine novel targets that may directly contribute to defective human beta cell biology in diabetes patients, such as those with MODY6. Overall, this study contributes valuable insights into the molecular mechanisms underlying NEUROD1-associated diabetes and its manifestations, potentially paving the way for novel therapeutic approaches.



Poster Abstracts

P33

Fetal liver CD34+ contain endothelial cells that induce a distinct liver pathology upon transplantation in NOD/Shi-scid Il2rgnull mice

Teja Celhar[1], Li Xinyi[1], Tay Hui Chien[1], Zhao Yunqian[1], Andrea Lee[1,2], Liew Hui Hua[3], Ravisankar Rajarethinam[4], Jerry Kok Yen Chan[3], Amit Singhal[1,2] and Takeshi Takahashi[5]

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Immunodeficient NOD/Shi-scid Il2rgnull (NOG) mice are excellent recipients for human transplants, including CD34+ hematopoietic stem and progenitor cells (HSPC). Here we have compared the human immune cell reconstitution in NOG mice upon transplantation with CD34+ isolated from cord blood (CB-CD34+) or fetal liver (FL-CD34+). We show that CB-CD34+ are a relatively uniform population of lineage negative HSPC, while FL-CD34+ consist of at least three different populations, including CD14+ endothelial cells. Our data suggests that these CD14+CD34+ cells are capable of reconstituting Factor VIII-producing liver sinusoidal endothelial cells (LSEC). Additionally, CD14+CD34+ enhance the reconstitution of the human immune system in NOG mice and contribute to hepatic immune cell infiltration. FL-CD34+-transplanted mice (FL-CD34+-NOG) with elevated immune infiltration in liver, spleen and lungs have a concomitant increase of serum inflammatory mediators and develop distinct liver lesions at 20 weeks post-transplantation. Visual features of this pathology are disseminated dark spots that are histologically associated with sinusoidal dilatation (SD), hemorrhages and necrosis, resembling sinusoidal obstruction syndrome (SOS). FL-CD34+-NOG could be a useful new model to study hepatic SD and involvement of LSEC in liver pathologies such as SOS

P34

High-density microbioreactor process designed for automated point-of-care manufacturing of CAR T cells

Wei-Xiang Sin[1], Narendra Suhas Jagannathan[2], Denise Bei Lin Teo[1], Faris Kairi[1], Dedy Sandikin[1], Ka-Wai Cheung[1], Yie Hou Lee[1], Rajeev J. Ram[1,3], Lisa Tucker-Kellogg[1,2], Michael E. Birnbaum[1,4]

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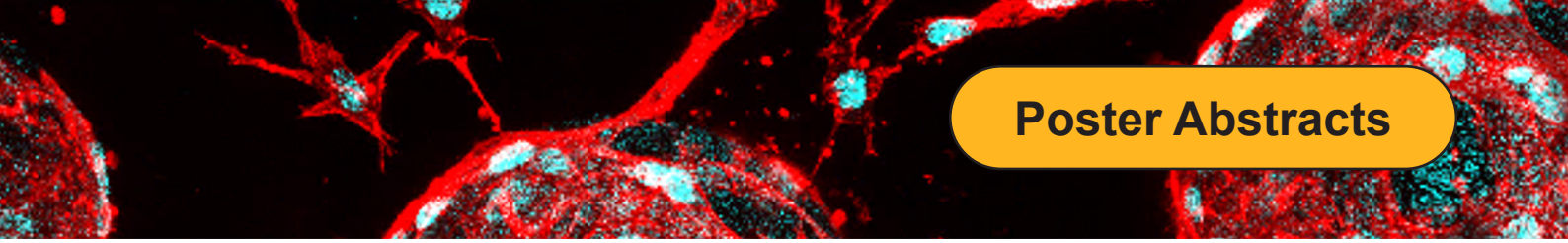
Current autologous CAR-T manufacturing faces challenges in scaling to meet patient demands. Most automated closed-system bioreactors currently used for CAR-T production suffer from large footprints and working volumes, thus hindering process development and scaling-out. Here, we present a means of conducting CAR-T culture-on-a-chip. We show that T cells can be activated, transduced, and expanded to densities exceeding 150 million cells/mL in a 2-mL perfusion-capable microfluidic bioreactor, thus enabling CAR-T production at clinical dose levels in a small footprint. Key phenotypical and functional attributes were comparable to T cells generated in a gas-permeable well. The process intensification and miniaturization offered by the microbioreactor could facilitate high-throughput process optimization studies and enable the efficient scale-out of cell therapy manufacturing.



Poster Abstracts

P35

Confined migration drives stem cell epigenetics and differentiation

Xu Gao[1], Yixuan Li[2], Nicole Jia Wen Lee[2], Andrew W. Holle[1,2]

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Human mesenchymal stem cells (hMSCs) hold promise for stem cell therapies due to their multilineage differentiation capacity and immunomodulatory properties. In either endogenous or exogenous cells, homing to damaged sites requires hMSCs to navigate complex extracellular matrix (ECM) environments that impose confinement on migrating cells. However, the impact of this ubiquitous confined migration on hMSCs remains poorly understood. To address these questions, we designed a physiologically relevant polydimethylsiloxane (PDMS)-based microchannel system, enabling us to quantitatively investigate the consequences of confined migration in stem cells. We found that stem cells migrated faster under narrow (3 μm) confinement and exhibited significant nuclear deformation. Additionally, altered cell and nuclear morphology were observed in cells exiting the narrow 3 μm microchannels. Nuclear deformation was also found to correlate with major changes in genome regulation in stem cells that traverse narrow confinements; H3K9 acetylation was significantly upregulated post-confinement. More importantly, stem cells subjected to confined migration showed higher expression and nuclear translocation of RUNX2, a transcriptional factor involved in early osteogenesis. Critically, this upregulation was found to be dependent on both the length and width of the confinement. Overall, these results provide new insights into our understanding of adult stem cell responses to confinement.

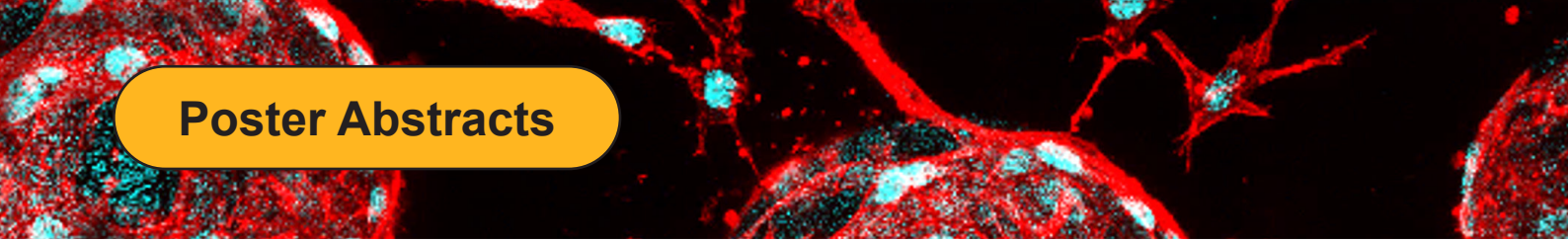
P36

In vitro intra-hepatic bile duct formation via spatiotemporally specified signalling cues

Harini Rajendiran[1,2], Geetika Sahni[1], Aditya Arora[1], Matt Hepburn[3], Brendan Kennedy[3], Flora, Anais monnet, Pakorn Tony Kanchanawong[2], Virgile Viasnoff[1]

[1]Mechanobiology Institute, National University of Singapore, Singapore; [2]NUS Biomedical Engineering, College of Design and Engineering National University of Singapore; Singapore; [3]Harry Perkins Institute (north), QEII Medical Centre, Australia

Mesenchymal stem cells (MSCs) may contribute to cartilage healing by directly differentiating into chondrogenic cells or by secreting a variety of trophic substances that have a paracrine effect on the cells in the vicinity. MSCs respond "environmentally" to biophysical changes and local microenvironmental signals, such as exposure to pulse electromagnetic fields (PEMF). In this study, we demonstrated enhancement of MSC chondrogenesis and paracrine function through PEMF exposure using a custom designed PEMF delivery device that generates a precise magnetic field of low intensity and frequency. The MSC culture platform (scaffold-free pellet culture, hydrogel or fibrous scaffold culture) as well as the pulse intensity, duration, and dosage have a highly dependent relationship with the PEMF inductive effect. Additionally, chondrocyte and MSC migration was improved by PEMF-induced MSC secretome, which could also reduce cellular inflammatory response and death. Our *in vivo* investigation using a rabbit osteochondral injury model demonstrate that brief exposure to low-amplitude PEMF enhances MSC-based cartilage healing capacity. Overall, our findings suggest that PEMF stimulation may have significant clinical and practical implications for enhancing and restoring cartilage regeneration.



Poster Abstracts

P37

Rapid alternatives to compendial sterility and mycoplasma test methods – Case studies on implementation, validation and adoption

Yong Jian Lee

Charles River Laboratories, Singapore

In pharmaceutical manufacturing, the harmonized compendial sterility test (USP <71; EP 2.6.1) and mycoplasma test (USP <63>) are among the longest microbiological Quality Control test which takes 14-21 days for test completion. These tests require an extended incubation duration to provide sufficient time for slow-growers and/or stressed microorganisms to be recovered – this is to provide safety assurance that the finished drug products administered to patients are sterile and free from contaminating objectionable microorganisms. Novel treatments in Advanced Therapeutic Medicinal Products (ATMPs) such as Cell and Gene Therapy products often have very short shelf-lives and limited sample quantities, and there is increasing regulatory awareness that compendial tests may not be suitable for such products. Rapid microbial methods, or RMMs, is the solution by providing an alternative to the compendial test methods, thereby reducing the turnaround time and expediting drug delivery to the patients in need. In this presentation, we review the available alternatives to the compendial test methods and examine several case studies of successful implementations of RMM technologies in the pharmaceutical scene. Shifts in regulatory acceptance towards rapid methods will also be discussed. The switch to robust and validated RMMs is the key in expediting treatments to patients.

P38

Anomaly detection for microbial contamination in mesenchymal stromal cell culture

Shruthi Pandi Chelvam[1], Alice Ng Jie Ying[1], Huang Jiayi[1], Elizabeth Lee[1], Derrick Yong[1,2,3], Rajeev Ram[1,4], Stacy Springs[1,5], Rohan B.H. Williams[1,6,7]

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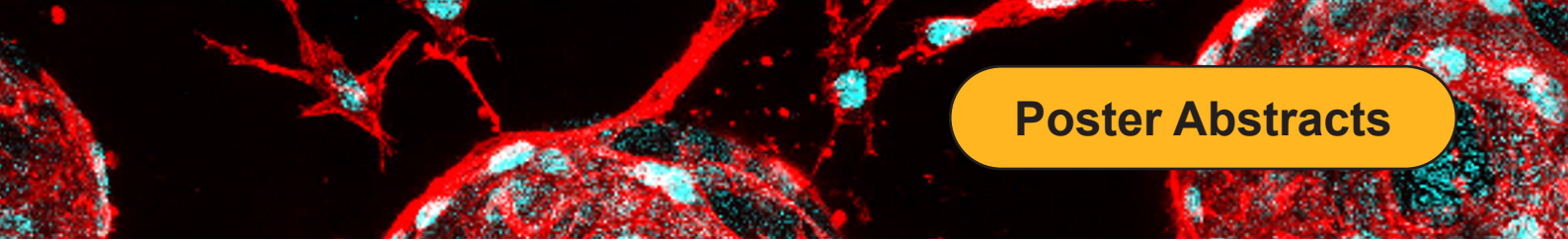
Mesenchymal stromal cells (MSCs) are promising for cell therapies due to their immune regulatory response. Monitoring product quality and safety during manufacturing is crucial and ideally performed with at-line technologies for continuous feedback. Presence of microbial contamination renders products unsafe and timely corrective actions can reduce costs. However, compendial sterility tests and alternate methods have long incubation periods, rendering them incompatible to be integrated with at-line technologies to evaluate short shelf-life products. We propose an anomaly detection aided label-free UV spectroscopy method to detect contamination in MSC cultures within 20 minutes post-incubation. MSCs were cultured in Dulbecco's Modified Eagle Medium without antibiotics and spent medium was collected on intermediate days and split into pairs of clean and bacteria-inoculated samples. A commercial UV-Vis spectrometer was used to measure the absorbance spectra of samples. The spectra of clean spent medium samples were used to train a one-class support vector machine, and bacteria-inoculated samples were used to validate model performance. 90% average prediction accuracy was achieved in detecting 7 USP <71> organisms (n=361), with a limit-of-detection of 10 CFU/mL. Blinded studies with 38 contaminated and 50 sterile samples show that the model has a 100% true positive and 88% true negative prediction accuracy.



Poster Abstracts

P39

Novel roles of two peptides in Drosophila neural stem cell reactivation

Jiaen Lin[1], Justin A Bosch[2], Norbert Perrimon[2,3], Hongyan Wang[1,4,5*]

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The ability of neural stem cells (NSCs) to switch between quiescent and proliferative states is crucial for brain development and regeneration. Dysregulated NSC reactivation might be associated with neurogenesis defects and neurodevelopmental disorders. As current knowledge about the mechanism of NSC reactivation is limited, studies on identifying its novel regulators are necessary. Short open reading frame (sORF)-encoded polypeptides (SEPs) have recently emerged as important regulators of many cellular processes. However, little is known about the roles of SEPs in NSC reactivation. Here, we showed that two previously uncharacterized peptides, Simba1 and Simba2, encoded by conserved sORFs, are novel regulators in Drosophila neural stem cell (NSC) reactivation. Despite their high amino acid identity (97.1%), Simba1 and Simba2 exhibit differential expression in NSCs and glial cells; Simba1 is detected in the nucleus of both NSCs and glial cells, while Simba2 is localized to the nucleus of surface glial cells, but not NSCs. Knocking down simba1 in NSCs causes a delay in NSC reactivation, while depletion of simba2 in surface glial cells results in reactivation defects. Therefore, Simba1 is primarily required in NSCs, while Simba2 acts predominantly in blood-brain-barrier glial cells to promote NSC reactivation. Our findings will shed new light on the novel roles of SEPs in NSC reactivation and contribute to our understanding of brain development.

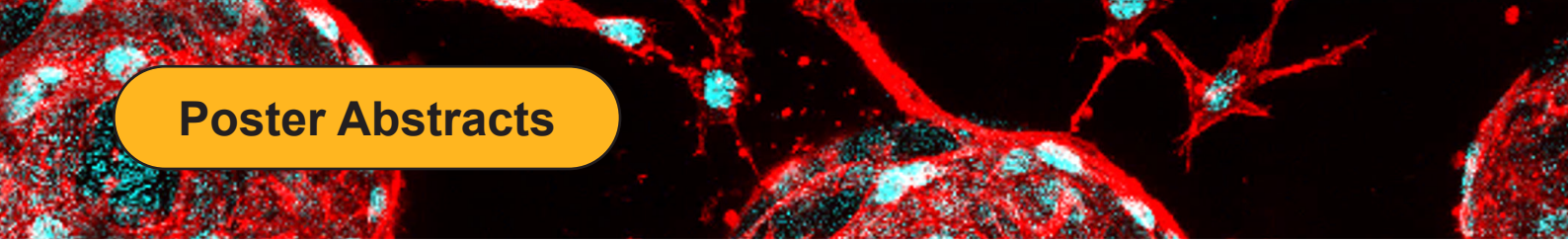
P40

HNF1A binds and regulates the expression of SLC51B to facilitate the uptake of estrone sulfate in human renal proximal tubule epithelial cells

Claire Wen Ying Neo[1,2,#], Jun Wei Chan[1,#], Soumita Ghosh[2], Hyungwon Choi[2], Su Chi Lim[3,4,5], E Shyong Tai[2,4,6], and Adrian Kee Keong Teo[1,2,6,7]

*[1]Stem Cells and Diabetes Laboratory, Institute of Molecular and Cell Biology (IMCB), Agency for Science, Technology and Research (A*STAR), Singapore; [2]Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [3]Khoo Teck Puat Hospital, Singapore; [4]Saw Swee Hock School of Public Health, National University of Singapore, Singapore; [5]Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore; [6]Precision Medicine Translational Research Programme, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [7]Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore; #Co-*authors

Renal defects in maturity onset diabetes of the young 3 (MODY3) patients and Hnf1α -/- mice suggest an involvement of HNF1A in kidney development and/or function. Although Hnf1α/- mice had been used to infer some transcriptional targets and function of HNF1A in mouse kidneys, species-specific differences obviate a straightforward extrapolation of findings to humans. Here, we leveraged on human in vitro kidney cell models to characterize the expression profile of HNF1A and found HNF1A to be expressed in the proximal tubule cells. HNF1A ChIP-seq followed by a qPCR screen were performed to identify putative targets of HNF1A, one of which is SLC51B, a solute transporter responsible for estrone sulfate (E1S) uptake in proximal tubule cells. We confirmed reduced SLC51B expression in HNF1A-depleted human renal proximal tubule epithelial cells (RPTECs) and in MODY3 human induced pluripotent stem cell (hiPSC)-derived kidney organoids. Importantly, we demonstrated that SLC51B-mediated E1S uptake was abrogated in these HNF1A-deficient cells and showed that MODY3 patients exhibited significantly higher expression of urinary E1S. As E1S serves as the main storage form of nephroprotective estradiol in humans, lowered E1S uptake may reduce the availability of nephroprotective estradiol in the kidneys, contributing to renal disease in MODY3 patients.



Poster Abstracts

P41

Astrocytes control quiescent NSC reactivation via GPCR signaling-mediated F-actin remodeling

Kun-Yang Lin[1], Mahekta R. Gujar[1], Jiaen Lin[1], Wei Yung Ding[1], Jiawen Huang[1], Yang Gao[1], Ye Sing Tan[1], Xiang Teng[2], Yusuke Toyama[2, 3], Hongyan Wang[1, 4, 5]

[1]Neuroscience & Behavioral Disorders Programme, Duke-NUS Medical School, Singapore; [2]Mechanobiology Institute, Singapore; [3]Department of Biological Sciences, National University of Singapore, Singapore; [4]Dept. of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [5]Integrative Sciences and Engineering Programme, National University of Singapore, Singapore

Cell therapy biomanufacturing is a growing industry. Cell therapy products have the potential to provide life changing treatment for patients. Yet challenges remain, these are derived from the short product shelf-life, small lot size and subsequent strict sterility requirements during release testing. Our aim is to meet and solve the challenges of rapid, sensitive and unbiased detection of adventitious agents by combining third generation nanopore amplicon sequencing alongside machine learning. We generated samples incorporating T-cells spiked with bacterial and fungal species. Following sequencing, the sequenced reads are processed, host reads are removed and potential contaminant species identified in an untargeted metagenomics approach. The analysed sequencing data are aggregated to build machine learning models that seek to classify samples and predicted contaminants in order to answer the two following questions (1) is my sample contaminated? (2) is my contaminant genuine? Taken together we are able to prepare a 1 mL spiked sample and detect contaminants at 10 CFU within 24 hours.

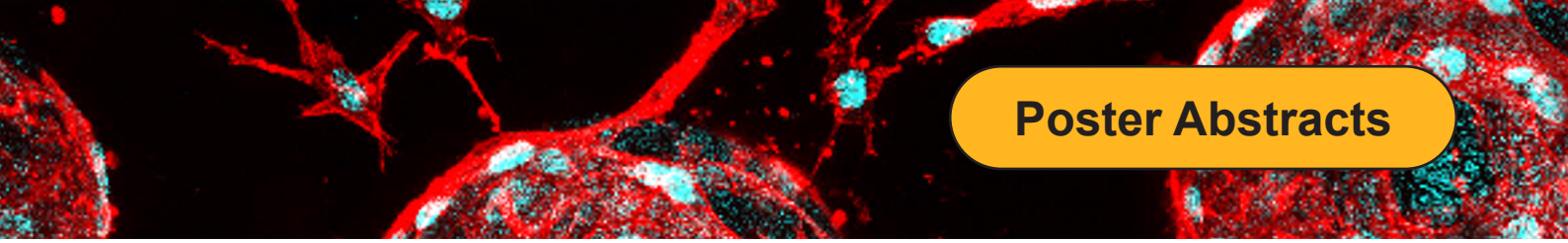
P42

Arl2 regulates neurogenesis via microtubule organization during the mouse cortical development

Dong-Liang Ma[1]*, Kun-Yang Lin[1], Suresh Divya[1], Htet Yamin Aung[1], Ye Sing Tan[1], Jiaen Lin[1], Hongyan Wang[1,2,3,*]

*[1]Program in Neuroscience and Behavioural Disorders, Duke-NUS Medical School, Singapore; [2]Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [3]Integrative Sciences and Engineering Programme, National University of Singapore; *Correspondence: hongyan.wang@duke-nus.edu.sg, dongliang.ma@duke-nus.edu.sg*

ADP ribosylation factor-like GTPase 2 (Arl2) is crucial for controlling mitochondrial dynamics, tubulin polymerization, and the formation of new microtubules and maintaining centrosome integrity. Our previous research demonstrated that Arl2 is vital for regulating microtubule growth, which is a novel mechanism that influences the asymmetric division of neural stem cells in Drosophila. To understand the potential role of Arl2 in mammalian brain development, particularly in relation to mitochondria and/or microtubules, we conducted experiments by using short hairpin RNA (shRNA) to knock down Arl2 in both mouse neural progenitor cells (mNPCs) in vitro and the mouse brain in vivo. Following Arl2 KD, we observed reduced mNPCs proliferation and defective asymmetric division of NPCs, which subsequently affected NPC differentiation in the mouse brain. Arl2 knockdown in mNPCs significantly decreased centrosomal microtubule regrowth and led to a decrease in centrosomal proteins including γ-tubulin and Cep215 in mNPCs. Interestingly, we found that Arl2 physically associates with Cep215 and that overexpression of Cep215 could rescue the neurogenesis defects caused by Arl2 knockdown. These data indicate that mouse Arl2 is required for neurogenesis by regulating microtubule growth in mNPCs. On the contrary, the role of mouse Arl2 is independent of its function in mitochondria fusion, as Arl2(K71R) mutant that caused mitochondrial fragmentation phenotype had no effect on microtubule growth or neurogenesis. Taken together, Arl2 plays an important role in proliferation and neurogenesis during mouse brain development through microtubule growth with the centrosomal protein Cep215 rather than mitochondrial dynamics.



Poster Abstracts

P43

Delayed inflammation and immune rejection of xeno-transplanted human iPSC-RPE monolayers in non-human primates

Zengping Liu[1,2]; Daniel Soo Lin Wong[1], Veluchamy Amutha Barathi[1,3] Gopal, Lingam[1,4]; Kah Leong Lim[5], Xinyi Su[1,2]

[1]Department of Ophthalmology, National University of Singapore, Singapore; [2]Institute of Molecular and Cell Biology (IMCB), Agency for Science, Technology and Research, Singapore; [3]Singapore Eye Research Institute, Singapore; [4]Department of Ophthalmology, National University Hospital, Singapore; [5]Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore

Cell therapy biomanufacturing is a growing industry. Cell therapy products have the potential to provide life changing treatment for patients. Yet challenges remain, these are derived from the short product shelf-life, small lot size and subsequent strict sterility requirements during release testing. Our aim is to meet and solve the challenges of rapid, sensitive and unbiased detection of adventitious agents by combining third generation nanopore amplicon sequencing alongside machine learning. We generated samples incorporating T-cells spiked with bacterial and fungal species. Following sequencing, the sequenced reads are processed, host reads are removed and potential contaminant species identified in an untargeted metagenomics approach. The analysed sequencing data are aggregated to build machine learning models that seek to classify samples and predicted contaminants in order to answer the two following questions (1) is my sample contaminated? (2) is my contaminant genuine? Taken together we are able to prepare a 1 mL spiked sample and detect contaminants at 10 CFU within 24 hours.

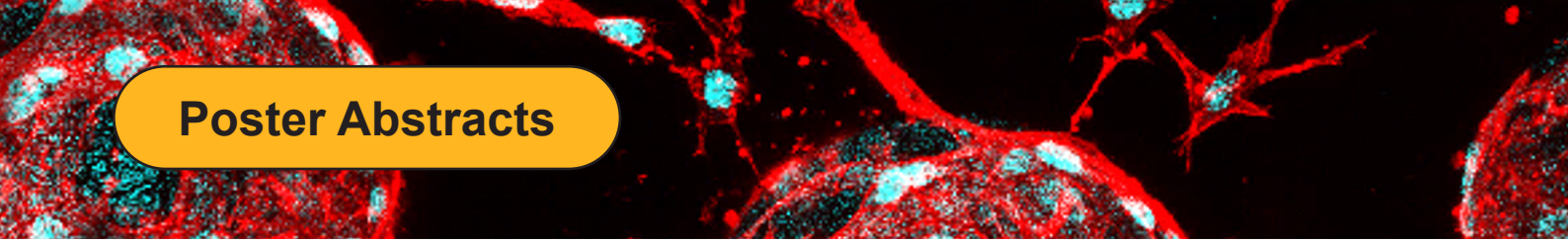
P44

Exploring the significance of single-cell mRNA dynamics in advancing stem cell research using DynaSCOPE

Rachael Lee[1], Sujatha Narayanankutty[1], Jonathan Scolnick[1]

[1]Singleron Biotechnologies Pte Ltd, Singapore

Understanding the intricate molecular mechanisms that govern stem cell fate decision is crucial for harnessing their therapeutic potential. Single-cell RNA-sequencing (scRNA-seq) has emerged as a powerful tool for dissecting cellular heterogeneity in stem cell populations. For the investigation of cellular trajectories from single-cell data, several bioinformatical methods have been developed, but experimental data is lacking. This is overcome with the DynaSCOPE® kit from Singleron. By chemical labelling of nascent mRNA, newly transcribed and pre-existing RNAs are discriminated in single cells, adding a time resolution to single-cell RNAseq experiments. Stem cell fate decisions are highly dynamic processes. Monitoring mRNA dynamics over time enables the tracking of gene expression changes during differentiation, reprogramming, and response to environmental cues. By analyzing the single-cell mRNA data, the accurate measurement of freshly synthesized and long-lasting mRNA will provide insights into regulatory networks and signaling pathways that govern stem cell behavior. These discoveries provide targets for modulating cell fate and enhancing the efficiency of stem cell-based therapies.



Poster Abstracts

P45

Robust lentivirus and AAV manufacturing platforms for cell and gene therapies

Steve KW Oh, Sukh Chopra, Paula Lam, Shekar Ganesa, Eric Soon

CellVec Pte Ltd., Singapore

Chimeric antigen T cell receptors (CAR-T) are receptor proteins that have been engineered to give T cells the new ability to target a specific antigen on cancer cells. To enable success of such therapies in the clinic, viral vectors such as lentivirus and AAV have to be designed, and manufactured according to GMP standards. Cellvec a leading CDMO in Singapore has developed a duet of robust lentivirus and AAV viral vector manufacturing platforms for clients to meet their viral vector requirements suitable for all phases of clinical development (pre-clinical to GMP manufacturing). For the lentiviral vector platform, we have developed patented helper plasmids for the production of CD19, BCMA and CD20 CAR transgenes, produced in a fully characterised bacterial cell bank free from animal components that meet FDA and EMEA regulatory standards. Designing of the vector construct to GMP manufacturing in a closed system, downstream purification and analysis of quality attributes can be accomplished between 6-9 months. Functional (FACS) and genomic (qPCR) titers of the purified product are typically 10E+11 TU/ml in 15L compared to competitive technologies that only achieve 1.5E+11 TU/ml in 50L suspension cultures. The purified lentiviral vectors are highly potent, and is able to transduce 30% of CD3+ T cells at a low multiplicity of infection equivalent to 1, with a low vector integrant copy number. This manufacturing platform can be readily scaled currently to 40L with a potential to scale to 100L in the future. For suspension based viral vector technologies, Cellvec has partnered with SGVector. Using rAAV2-RPE65 as a gene of interest, SGVector has developed an efficient 6 day total HEK293 suspension bioreactor process (3 days of cell expansion + 3 days for viral transfection). This upstream platform suspension process at the 2L scale has consistently demonstrated harvest viral titers (qPCR) yields of 2E+12 vg/ml, 20-fold higher compared to a leading CDMO's published AAV2 process yield of 1E+11 vg/ml. The robust AAV downstream process demonstrates effective clearance of impurities that meets FDA and EMEA regulatory expectations for safety, identity, strength, purity, and quality attributes (SISPQ).

P46

A GMP-Compliant CRISPR-based gene-editing process for the manufacturing of universal CAR-T (OMNICAR) cells

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We have developed a GMP-compliant process for non-viral based gene-editing of potent T cells for a universal immune receptor system, OmniCAR. Here, we demonstrated the use of Neon™NxT electroporation system to deliver the CRISPR-Cas9 protein and sgRNA to knock-out the TRAC locus region, and knock-in the ssDNA encoded with the CAR-Spycatcher, where the expression of SpyCatcher was driven by the endogenous TRAC promoter. CD3+ T cells were directly isolated from a frozen leukopak using CTS™ CD3/CD28 Dynabeads™ on the CTS™ DynaCellect™ magnetic cell seperation system. Further, the isolated and activated CD3+ T cells were expanded in CTS™ OpTmizer™Pro SFM media and de-beaded on day 2 for electroporation buffer exchange using closed CTS™ Rotea™ counterflow centrifugation and followed by gene editing. Engineered OmniCAR T cells were further expanded for up to 12 days, harvested using CTS™ Rotea™ and analyzed for gene editing efficiencies, phenotype and cytotoxicity. OmniCAR T cells showed >98% TRAC knock-out efficiency and OmniCAR knock-in efficiency of ~40% over the time of culture. When engineered OmniCAR-T were armed with a SpyTagged Her2+ binder, ~50% killing efficiency of Her2+ breast cancer cell lines (MDA-231) was observed.

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