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GUANGZHOU REGENERATIVE MEDICINE AND HEALTH GUANGDONG LABORATORY

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Stem Cells and Regenerative Medicine

12-14 January 2022

ISSCR

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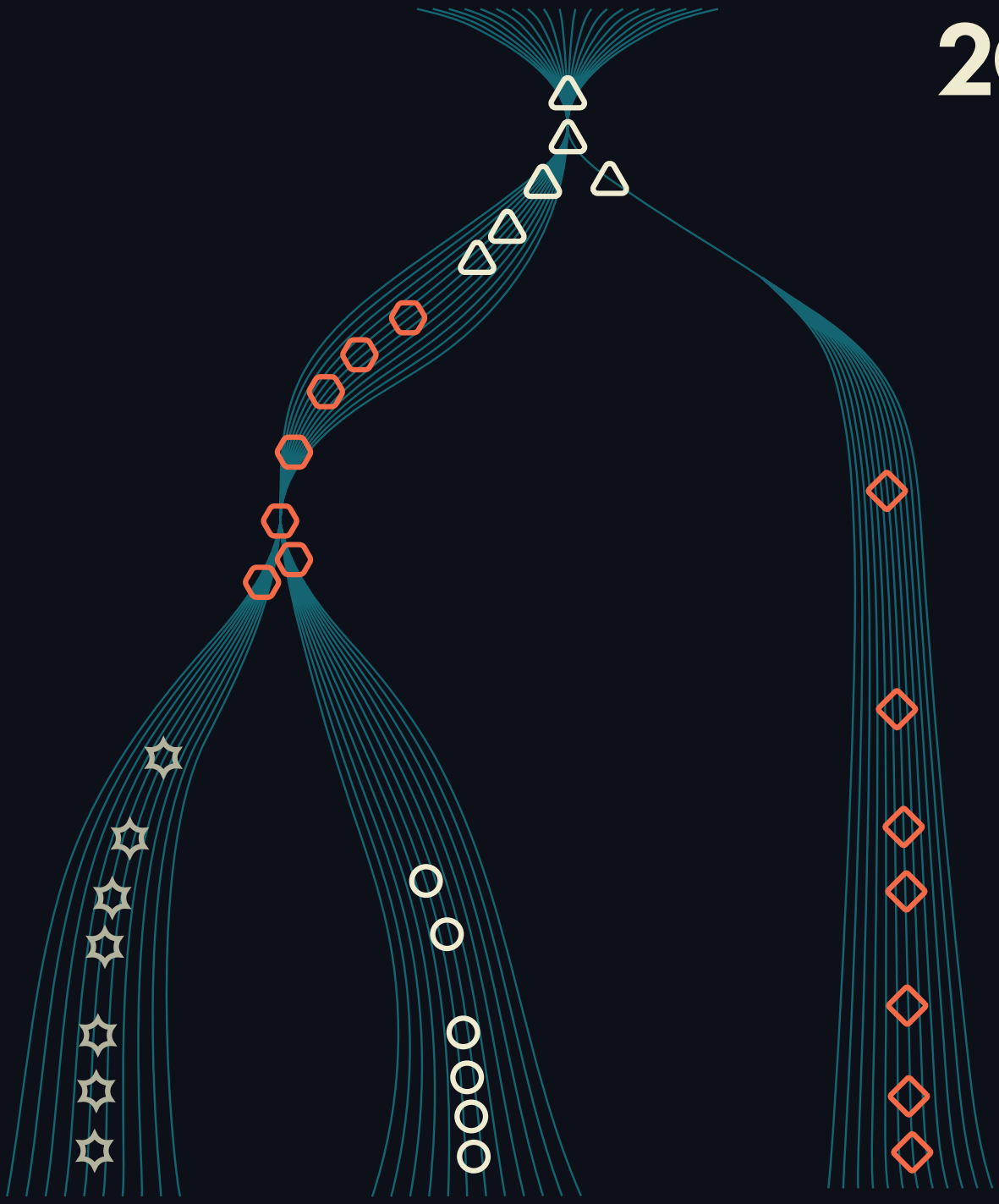

#ISSCR2022

ISSCR ANNUAL MEETING 2022

SAN FRANCISCO

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Welcome

Dear Colleagues,

On behalf of the International Society for Stem Cell Research (ISSCR), ShanghaiTech University, and Guangzhou Regenerative Medicine and Health we warmly welcome you to the International Symposium, “Stem Cells and Regenerative Medicine.” The health care industry is growing rapidly in Shanghai, and large investments in research and innovation are leading to flourishing academic institutions and biotechnology companies. This major hub of education and technology is the perfect backdrop for this important virtual meeting.

Rapid advances in stem cell science show great potential to transform human health. Our diverse and engaging program explores emerging science that is driving the field. This scientific program was created to illustrate how stem cells are increasing our understanding of fundamental biology and regenerative medicine. Over the next few days, leading researchers will discuss their innovative work and how it pushes the boundaries of our field.

Leading edge scientists will highlight the latest advances in pluripotency and reprogramming, insights into stem cell-niche interactions, and different ways to target and enhance regeneration. Speakers will also delve into cutting-edge research using stem cells to model disease and identify new drugs, develop novel technologies, and bring cell therapies to the clinic. Scientific discoveries continue over poster presentations, which foster discussions and potential new collaborations. In addition, innovative companies will display the latest tools and technologies in the Virtual Exhibit Hall, a vibrant hub of meeting activities.

There are abundant opportunities to learn about new areas of research, delve into exciting approaches to stem cell science, expand your network of colleagues and friends, and build new relationships at this International Symposium. Thank you for your important work and for attending what promises to be an inspirational event focused on new advances in the field of regenerative medicine.

Sincerely,

Shanghai International Symposium Organizing Committee

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ABOUT THE ISSCR

The mission of the International Society for Stem Cell Research (ISSCR) is to promote excellence in stem cell science and applications to human health.

The ISSCR is the largest society in the world dedicated to the advancement of responsible stem cell research – a field that strives to advance scientific understanding, treatments, and cures that better human health. We foster junior scientists, give voice and visibility to scientific advancement, and encourage a positive global environment for future discovery and treatment. Our promise is to help the field of stem cell research reach its potential.

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ShanghaiTech is a young resource-rich university with a modern residential campus in the heart of Shanghai Pudong's Zhangjiang Hi-Tech Park. With an academic focus on STEAM research, ShanghaiTech is committed to serving China's national challenges in economic and social development with the focus on science and technology, and nurturing the next generation of innovative scientists, inventors and entrepreneurs.

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ABOUT GUANGZHOU REGENERATIVE MEDICINE AND HEALTH GUANGDONG LABORATORY

Guangzhou Regenerative Medicine and Health Guangdong Laboratory (Bioland Laboratory) was founded on 22nd December 2017, it is operated as a public research institution as one of the first provincial laboratories to develop as the reserves of national laboratories in Guangdong Province.

Bioland Laboratory is supported by the Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences (GIBH, CAS), joining hands with superior scientific research units in Guangdong, Hong Kong and Macao and top international scientific research resources. It aims at addressing major strategic needs of Guangdong Province and the whole country through 5 research areas. Bioisland Laboratory invited world-renowned scientists and their team to join in, including EMBO members and CAS members. Meanwhile, with our tremendous efforts, Bioisland Laboratory has attracted numerous young talents to work in Regenerative Medicine, with a high standard and organized team composition.

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Program Schedule

Wednesday, 12 January

08:00 AM – 08:50 AM OPENING KEYNOTE SESSION

Welcoming Remarks
Haifan Lin, *Yale University, USA*

Introduction to the International Symposium
Keith Alm, *CEO, ISSCR, USA*

Keynote Address
Christine Mummery, *Leiden University Medical Center, Netherlands*
CARDIOVASCULAR DISEASE MODELS BASED ON HUMAN PLURIPOTENT STEM CELLS

08:50 AM – 10:05 AM PLURIPOTENCY

Chair: **In-Hyun Park**, *Yale University, USA*

08:50 AM – 09:20 AM
Robert Blelloch, *University of California, San Francisco, USA*
TRANSCRIPTIONAL REPRESSOR FOXD3 SAFEGUARDS GENOME INTEGRITY IN EMBRYONIC STEM CELLS

09:20 AM – 9:50 AM
Ge Guo, *University of Exeter, UK*
MODELLING EARLY HUMAN EMBRYO DEVELOPMENT USING NAÏVE PLURIPOTENT STEM CELLS

09:50 AM – 10:05 AM
Xiaohua Shen, *Tsinghua University, China*
A GLIMPSE OF THE NONCODING GENOMES IN CHROMATIN AND TRANSCRIPTION REGULATION

10:05 AM – 10:15 AM BREAK

**All times are listed in China Standard Time*

Wednesday, 12 January

10:15 AM – 12:30 PM CELL FATE AND POTENCY

Chair: **Hongkui Deng**, Peking University, China

10:15 AM – 10:45 AM
Danwei Huangfu, *Memorial Sloan Kettering Cancer Center, USA*
A STEM CELL APPROACH TO HUMAN DEVELOPMENT, DISCOVERY OF GENETIC AND EPIGENETIC REGULATORS OF DEVELOPMENT THROUGH GENOME-SCALE CRISPR SCREENS IN HUMAN PLURIPOTENT STEM CELLS

10:45 AM – 11:15 AM
Kathrin Plath, *University of California, Los Angeles School of Medicine, USA*
REGULATION OF X CHROMOSOME DOSAGE COMPENSATION IN PLURIPOTENT CELLS

11:15 AM – 11:30 AM
Jinsong Li, *Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, China*
SPERM LIKE STEM CELL-MEDIATED GENOME EDITING

11:30 AM – 12:00 PM
Ying Jin, *Shanghai Jiao Tong University School of Medicine, China*
THE ROLE OF ALTERNATIVE SPLICING FOR MAINTAINING SURVIVAL AND GENOMIC STABILITY OF HUMAN EMBRYONIC STEM CELLS

12:00 PM – 12:30 PM
Duanqing Pei, *Westlake University, China*
INDUCTION OF PLURIPOTENCY BY JGES

12:30 PM – 12:45 PM BREAK

12:45 PM – 02:00 PM ABSTRACT SELECTED SPEAKER SESSION I

Chair: **Yi Arial Zeng**, *Shanghai Institute of Biochemistry & Cell Biology, Chinese Academy of Sciences, China*

12:45 PM – 01:00 PM
Tongguang Wang, *National Institute of Neurological Disorders and Stroke, USA*
DERIVATION OF BRAIN ORGANOIDS WITH MICROGLIAL INCORPORATION FROM IPSCS

01:00 PM – 01:15 PM
Bing Zhang, *Westlake University, China*
PSYCHOLOGICAL STRESS DRIVES MELANOCYTE STEM CELL EXHAUSTION THROUGH ACTIVATION OF THE SYMPATHETIC NERVOUS SYSTEM

01:15 PM – 01:30 PM
Yu Qing, *Chinese Academy of Sciences, China*
PROCR+ ENDOTHELIAL PROGENITORS IN VASCULAR DEVELOPMENT AND QUIESCENCE ACQUISITION

01:30 PM – 01:45 PM
Kathy Lui, *The Chinese University of Hong Kong, Hong Kong*
REGULATORY T CELLS MEDIATE CARDIOVASCULAR REPAIR: MECHANISM AND THERAPEUTIC IMPLICATION

01:45 PM – 02:00 PM
Yan Huang, *Sun Yat-sen University, China*
TIN2 INSUFFICIENCY LEADS TO ALT-ASSOCIATED PHENOTYPES AND DIFFERENTIATION DEFECTS IN EMBRYONIC STEM CELLS

Program Schedule

Thursday, 13 January

08:00 AM – 10:00 AM TISSUE STEM CELLS IN THE NICHE AND CANCER

Chair: **Robert Blelloch**, *University of California, San Francisco, USA*

08:00 AM – 08:30 AM
Valentina Greco, *Yale Stem Cell, Yale Medical School, USA*
PRINCIPLES OF REGENERATION CAPTURED BY IMAGING THE SKIN OF LIVE MICE

08:30 AM – 09:00 AM
Lijian Hui, *Center for Excellence in Molecular Cell Science, CAS, China*
CELL IDENTITY CONVERSION AND LIVER REGENERATION

09:00 AM – 09:30 AM
Linheng Li, *Stowers Institute for Medical Research, USA*
DEFINING HEMATOPOIETIC STEM CELL-NICHE INTERACTIONS IN FETAL LIVER AT CELLULAR RESOLUTION USING SPATIAL TRANSCRIPTOMICS

09:30 AM – 10:00 AM
Yi Arial Zeng, *Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, China*
GENERATION OF MOUSE PANCREATIC ISLET ORGANOIDS USING RESIDENT PROCR PROGENITORS

10:00 AM – 10:10 AM BREAK

10:10 AM – 12:10 PM REPROGRAMMING, REGENERATION, AND REPAIR

Chair: **Kathrin Plath**, *University of California, Los Angeles, USA*

10:10 AM – 10:40 AM
Helen Blau, Stanford University, USA
REVERSING AGING: REGENERATING AND REJUVENATING AGED MUSCLES

10:40 AM – 10:55 AM
Christopher Antos, *School of Life Science and Technology, ShanghaiTech University, China*
HOW AN ELECTROPHYSIOLOGICAL SIGNAL IS INTEGRATED INTO SCALING A VERTEBRATE DEVELOPMENTAL FIN/LIMB PROGRAM

10:55 AM – 11:25 AM
Anthony Oro, *Stanford University, USA*
USING CHROMATIN DYNAMICS FOR TISSUE REGENERATION APPLICATIONS

11:25 AM – 11:40 AM
Pengyu Huang, *Institute of Biomedical Engineering, Chinese Academy of Medical Sciences, China*
3D-BIOPRINTING LIVER FOR IN VIVO STUDIES

11:40 AM – 12:10 AM
Hongkui Deng, *Peking University, China*
ESTABLISHING A NOVEL INTESTINAL ORGANOID CULTURE SYSTEM MODELING INJURY-ASSOCIATED EPITHELIAL REGENERATION

Thursday, 13 January

12:10 PM – 12:30 PM **BREAK**

12:30 PM – 01:30 PM **POSTER SESSION**

01:30 PM – 01:45 PM **BREAK**

01:45 PM – 03:00 PM **ABSTRACT SELECTED SPEAKER SESSION II**

Chair: **Christopher Antos**, *School of Life Science Technology, ShanghaiTech University, China*

01:45 PM – 02:00 PM
Xi He, *Stowers Institute, USA*
TUMOR INITIATING STEM CELL SHAPES ITS MICROENVIRONMENT INTO AN IMMUNOSUPPRESSIVE BARRIER AND PRO-TUMORIGENIC NICHE

02:00 PM – 02:15 PM
Yangfei Xiang, *ShanghaiTech University, China*
INVESTIGATING RETT SYNDROME USING TWO-DIMENSIONAL AND THREE-DIMENSIONAL HUMAN BRAIN MODELS

02:15 PM – 02:30 PM
Jie Na, *Tsinghua University, China*
CONSTRUCTION OF GASTRULA EMBRYOID FROM REPROGRAMMED CELLS

02:30 PM – 02:45 PM
Jun-An Chen, *Academia Sinica, Taiwan*
PROBING AND TARGETING MOTOR NEURON SUBTYPE DIFFERENTIAL VULNERABILITY IN ALS VIA MICRORNA

02:45 PM – 03:00 PM
Linzhao Cheng, *University of Science and Technology of China, China*
HUMAN IPSC-DERIVED EXTRACELLULAR VESICLES: PROPERTIES AND APPLICATIONS

Program Schedule

Friday, 14 January

08:00 AM – 10:15 AM ADVANCES IN TECHNOLOGY, DISEASE MODELING, AND DRUG DISCOVERY

Chair: **Helen Blau**, *Stanford University, USA*

08:00 AM – 08:30 AM
Nissim Benvenisty, *Hebrew University, Israel*
THE ESSENTIALOME OF HUMAN PLURIPOTENCY

08:30 AM – 08:45 AM
In-Hyun Park, *Yale University, USA*
DEVELOPMENT OF THE INTEGRATED HUMAN BRAIN ORGANOIDS TO STUDY BRAIN DEVELOPMENT AND DISEASES

08:45 AM – 09:15 AM
Samantha Morris, *Washington University School of Medicine, USA*
NEW GENOMIC TECHNOLOGIES TO DISSECT DEVELOPMENT AND REPROGRAMMING

09:15 AM – 09:45 AM
Tannishtha Reya, *University of California, San Diego, School of Medicine, USA*
STEM CELL SIGNALS IN CANCER HETEROGENEITY AND THERAPY RESISTANCE

09:45 AM – 10:15 AM
Yi Sun, *University of California, Los Angeles, USA*
MODELING RETT SYNDROME USING GENE-EDITED MECP2 MUTANT CYNOMOLGUS MONKEYS

10:15 AM – 10:30 AM BREAK

10:30 AM – 11:30 AM STEM CELL-BASED THERAPIES

Chair: **Duanqing Pei**, *Westlake University, China*

10:30 AM – 11:00 AM
Tobias Deuse, *University of California, USA*
ALLOGENEIC HYPOIMMUNE CELL THERAPEUTICS AS SCALABLE LIVING MEDICINES

11:00 AM – 11:30 AM
Malin Parmar, *Lund University, Sweden*
DEVELOPING A STEM CELL-BASED THERAPY FOR PARKINSON'S DISEASE

11:30 AM – 11:40 AM BREAK

11:40 AM – 12:20 PM CLOSING KEYNOTE SESSION

Keynote Address
Michele de Luca, *University of Modena and Reggio Emilia, Italy*
COMBINED CELL AND GENE THERAPY FOR EPIDERMOLYSIS BULLOSA

Closing Remarks
Duanqing Pei, *Westlake University, China*

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30-31 March 2022

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21-23 September 2022

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Sponsored Content: Mini-Symposia

SPONSORED INNOVATION SHOWCASE

Characterizing the Activity of Human iPSC-Derived Neurons Using High-Content Electrophysiology

Organized by Maxwell Biosystems

Presenters: Marie Obien, PhD, MaxWell Biosystems, Silvia Ronchi, PhD, MaxWell Biosystems, and Naihe Jing, PhD, Shanghai Institute of Biochemistry and Cell Biology

Human induced pluripotent stems cell (hiPSC)-derived neuronal networks cultured on-a-dish are emerging as promising tools for investigating brain development, disease progression, and for pharmacology/toxicology studies in-vitro. In order to adopt hiPSC-derived neuronal networks for rapid and cost-effective studies and drug screenings, it is necessary to assess their cell type composition, gene expression patterns, and physiological function. In this innovation showcase, the speakers will highlight how the neuronal activity in different hiPSC lines can be easily captured, labelfree, at single-cell resolution by using MaxWell Biosystems' high-density microelectrode array (HD-MEA) platforms, MaxOne and MaxTwo. With these high-content electrophysiology platforms, one can extract the functional metrics of the neuronal network across multiple scales: full neuronal activity map, population activity, and the propagation of action potentials along axons of individual cells cultured on-a-dish.

Overall, the presentations will provide an overview on how HD-MEA technology enables detailed phenotypical characterization of hiPSC-derived brain models. The combination of both hiPSC and HD-MEA technologies can potentially be utilized as a screening platform in the early phases of drug discovery for neurodegenerative diseases.

Versatile In Vitro Culture Solutions for Your Respiratory Research

Organized by STEMCELL Technologies Inc

Presenters: Juan Hou, MD, PhD, STEMCELL Technologies Inc

In vitro models for culturing human pulmonary epithelial cells have emerged as powerful tools for studying lung cell biology. This session will provide an overview of how different regions of the airway can be modeled using STEMCELL's current and upcoming products. Our kits can be used for both expanding and differentiating primary tissue-derived bronchial epithelial and alveolar cells. In addition, we will highlight new tools for the directed differentiation of human pluripotent stem cells (hPSCs) into a variety of lung epithelial cells. Examples will be given on how these models can be used to study cystic fibrosis and viral infections. Our standardized culture media and protocols will enable researchers to focus efforts on addressing their scientific questions regarding diseases such as COVID-19 and chronic obstructive pulmonary disease rather than optimizing and troubleshooting reagents to ensure consistency and quality.

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EPISODE 7
THE INTERSECTION OF STEM CELLS AND ENGINEERING

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PETER LOSKILL, PHD
EBERHARD KARLS UNIVERSITY TÜBINGEN, NATURAL AND MEDICAL SCIENCES INSTITUTE, GERMANY

EPISODE 8
MENDING A BROKEN HEART

CHARLES MURRY, MD, PHD
UNIVERSITY OF WASHINGTON AND SANA BIOTECHNOLOGY, USA

KENTA NAKAMURA, MD
UNIVERSITY OF WASHINGTON AND VA PUGET SOUND HEALTHCARE SYSTEM, USA

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Speaker Abstracts

WEDNESDAY, 12 JANUARY

Opening Keynote Session

CARDIOVASCULAR DISEASE MODELS BASED ON HUMAN PLURIPOTENT STEM CELLS

Mummery, Christine L.

Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands

Our lab creates models for cardiovascular disease based on pluripotent stem cells (hPSCs). We use these for understanding disease mechanisms and cardiotoxic effects of drugs. Simple monolayer cultures of hPSC-cardiomyocytes or vascular cells are usually adequate for this purpose even though the cells are immature and fetal like. For example, by measuring contraction, action potential and calcium transients simultaneously in hPSC-cardiomyocytes, we demonstrated that we could predict the toxic effects of test drugs with almost 80% accuracy, compared with less than 70% in primary rabbit cardiomyocytes. For some purposes though, immature cells in 2D culture are insufficient to capture disease phenotypes. Examples that show the benefits of 3D culture of multicell type structures given: one model combines cardiomyocytes, cardiac fibroblasts and cardiac endothelial cells in “microtissues” consisting of just 5000 cells. In these microtissues, cardiomycoytes show maturation including the formation of (post-natal) T-tubules. By replacing each of the cell types in the microtissues with a diseased variant, it was shown that cardiac fibroblasts carrying a PKP2 desmosomal gene mutation actually induce arrhythmia in microtissues in which the cardiomyocytes are normal and healthy. This indicates that fibroblasts in the heart can contribute to the phenotype in patients with arrhythmogenic cardiomyopathy. Another example is of a vascular disease in which the vascular cells behaved identically as the healthy isogenic controls in 2D vascular networks but the lumenized vessels were distinctly abnormal in 3D microfluidic “Organ-on-Chip” models. These more complex cell systems based on hPSCs are paving the way forward for a new generation of disease models for understanding disease mechanisms and drug discovery.

Funding Source

The Netherlands Organ-on-Chip Initiative which is an NWO Gravitation project (024.003.001) funded by the Ministry of Education, Culture and Science of the government of the Netherlands.

Keywords: Cardiovascular disease, hiPSC, cardiac microtissues, cardiotoxicity

Pluripotency

TRANSCRIPTIONAL REPRESSOR FOXD3 SAFEGUARDS GENOME INTEGRITY IN EMBRYONIC STEM CELLS

Blelloch, Robert

Urology, University of California, San Francisco, CA, USA

Embryonic stem cells (ESCs) divide rapidly and are transcriptionally hyperactive resulting in high levels of replication stress. Yet, being the source of all adult cells, they must maintain their genomic integrity. How ESCs achieve this feat remains unknown. We have uncovered an essential role for the developmentally regulated transcription factor FOXD3. Loss of FOXD3 results in the accumulation of cells in G2/M cell cycle phase, increased replication stress markers (gamma-H2AX, 53BP1, RAD51, ssDNA) and compromised genome integrity (micronuclei, chromatin bridges) leading to P53 dependent apoptosis. FOXD3 bound sites are highly enriched for OCT4-SOX2-NANOG binding and are near many of the most highly expressed genes in ESCs. Loss of FOXD3 results in a further increase in transcription, H3K27ac and chromatin accessibility in late-S/early-G2 cell cycle phase. These sites also gain the DNA damage marker H2BK120ac. Transient chemical inhibition of POL2 transcriptional initiation rescues progression through late S/G2 phases and diminishes markers of replication stress in FOXD3-depleted cells. These results reveal a hitherto unknown role for a developmentally regulated transcriptional repressor in safeguarding the genome integrity of rapidly dividing pluripotent stem cells, doing so by temporarily inhibiting transcription enabling faithful DNA replication.

Funding Source

National Institute of General Medical Sciences of the National Institutes of Health grant R01GM125089.

Keywords: Embryonic Stem Cells, Genome Fidelity, Replication Stress

Speaker Abstracts

MODELLING EARLY HUMAN EMBRYO DEVELOPMENT USING NAÏVE PLURIPOTENT STEM CELLS

Guo, Ge

Living System Institute, University of Exeter, UK

The blastocyst is the hallmark structure formed in the early development of mammals. It consists of three tissues; trophectoderm, hypoblast and epiblast. Trophectoderm forms first. Morphological segregation of trophectoderm and inner cell mass (ICM) marks the initiation of blastocyst development. Within the ICM the hypoblast and naïve epiblast are then specified. Naïve pluripotent stem cells in vitro resemble the naïve epiblast in the fully expanded blastocyst. We recently discovered that human naïve epiblast cells can regenerate authentic trophectoderm. This plasticity is maintained in human naïve pluripotent stem cells, which are also able to produce hypoblast. We further established a 3D human blastocyst model, blastoid, by harnessing the tri-lineage differentiation potential. This versatile and scalable system provides a robust experimental model for human embryo research. I will discuss the human blastoid model and present new findings from molecular genetic dissection of a key signalling pathway controlling early human embryo morphogenesis.

Keywords: human naive pluripotent stem cells, 3D blastocyst model

A GLIMPSE OF THE NONCODING GENOMES IN CHROMATIN AND TRANSCRIPTION REGULATION

Shen, Xiaohua

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Much of the developmental complexity and biodiversity of higher eukaryotes is thought to arise from gene regulation. RNA represents a hidden layer of regulatory information in complex organisms. I will discuss our recent progress in exploring fundamental functions of genomic repeats, noncoding RNA, and RNA-binding protein in the regulation of transcription and genome organization.

Keywords: RNA homeostasis, transcription, genome organization, stem cell pluripotency

A STEM CELL APPROACH TO HUMAN DEVELOPMENT, DISCOVERY OF GENETIC AND EPIGENETIC REGULATORS OF DEVELOPMENT THROUGH GENOME-SCALE CRISPR SCREENS IN HUMAN PLURIPOTENT STEM CELLS

Huangfu, Danwei

Developmental Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA

My lab interrogates human development and disease mechanisms through combining genetic tools with stem cell biology. We have used efficient and precise genome editing techniques in human pluripotent stem cells (hPSCs) to investigate mechanisms of human pancreatic development and diabetes including monogenic forms of type 2 diabetes. In parallel to our interest in genetic regulators of development, we are interested in the roles of epigenetic regulation in development with a particular focus on the roles of DNA methylation. I will talk about the discovery of genetic and epigenetic regulators of development through genome-scale CRISPR screens in hPSCs, including a newly discovered regulator of DNA methylation.

Funding Source
NIH, DoD, JDRF, ADA.
Keywords: hPSCs, hESCs, CRISPR screens, DNA methylation, pancreatic development, diabetes

Cell Fate and Potency

REGULATION OF X CHROMOSOME DOSAGE COMPENSATION IN PLURIPOTENT CELLS

Plath, Kathrin

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X chromosome dosage compensation represents an epigenetic phenomenon where coordinated regulation of a whole chromosome is required to compensate the imbalance of X-linked gene dosage between the sexes. We study this process in placental mammals, where X chromosome dosage compensation occurs through X chromosome inactivation (XCI), which results in the formation and maintenance of the silent nuclear compartment of the inactive X-chromosome (Xi). XCI is an essential developmental process in which roughly a thousand genes are silenced by the non-coding RNA Xist and therefore offers the unique opportunity to understand mechanistically how RNA molecules can establish a distinct nuclear compartment in pluripotent cells. Our recent advances in studying the complex interplay among Xist RNA, interacting proteins, chromatin and transcription will be presented.

SPERM LIKE STEM CELL-MEDIATED GENOME EDITING

Li, Jinsong

State Key Laboratory of Cell Biology, Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China

From androgenetic haploid blastocysts derived by injection of sperm into enucleated oocytes, we generated mouse androgenetic haploid embryonic stem cells (AG-haESCs) that can support full-term embryonic development upon injection into oocytes, leading to the production of semi-cloned (SC) mice (semi-cloned technology). However, one major drawback of this technology is the very low birth rate of healthy SC mice (2% of total SC embryos transferred). Recently, we established AG-haESCs carrying H19-DMR and IG-DMR deletions (DKO-AG-haESCs) that can efficiently support the generation of SC pups at a rate of 20% ("sperm-like stem cell"). Sperm-like stem cell-mediated SC technology, combined with CRISPR-Cas9 technologies, enables one-step generation of mouse models that mimic multiple gene dosage reduction in human Myotonic Dystrophy type 1 (DM1); identification of novel mutations involved in human neural tube defects; medium-scale targeted screening of critical factors involved in bone development; base mutagenesis of a specific protein-coding gene to identify critical amino acids for protein function in vivo; and efficient generation of mice carrying tagged proteins at genome-scale (Genome Tagging Project, GTP). Moreover, we established haploid ESCs from monkey and human parthenogenetic embryos and most recently human artificial spermatids from androgenetic embryos. In summary, haESCs provide powerful tools for genetic analyses in mammals at both cellular and organismal levels.
Keywords: Sperm-like stem cells, haploid embryonic stem cells, CRISPR-Cas9, genome editing

THE ROLE OF ALTERNATIVE SPLICING FOR MAINTAINING SURVIVAL AND GENOMIC STABILITY OF HUMAN EMBRYONIC STEM CELLS

Jin, Ying

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Human embryonic stem cells (hESCs) can serve as an excellent model for investigating early human embryonic development and provide a renewable source to obtain specialized cells for disease modelling, drug screening and regenerative medicine. However, the genomic instability observed in the prolonged culture hampers their full application. Understanding molecular mechanisms controlling hESC genomic stability and survival is critical for the generation of highly qualified hESCs. During the past decades, my group has focused on the study of molecular regulation of hESC self-renewal at levels of gene transcription and signaling pathways. Here, we report the role of alternative splicing for the control of hESC survival and genomic stability at a posttranscriptional level. We find that pre-mRNA processing factors, PRPF6 and PRPF8, are both required for the maintenance of hESC survival. However, they act through distinct mechanisms. PRPF6 knockdown (KD) in hESCs triggers apoptosis and DNA damage. There are changes in the alternative splicing (AS) of genes- related to genome stability in PRPF6 KD hESCs. Unexpectedly, we find the interaction between PRPF6 and components of the m6A RNA methyltransferase complex. The results of our further studies indicate that PRPF6 can modulate both m6A modification and AS of certain genes- related to genomic stability to safeguard hESC genome stability and self-renewal. Interestingly, PRPF8 KD in hESCs also induces apoptosis, however, it does not trigger DNA damage. Significant up-regulation of genes associated with cell death and p53 apoptotic signaling pathway is found upon PRPF8 KD. Moreover, PRPF8 KD results in a drastic increase in the protein level of total p53 due to enhanced p53 protein stability. In line with this, concomitant p53 KD blocks the cell death caused by PRPF8 KD. The results of our further studies indicate that PRPF8 regulates AS of p53 E3 ubiquitination ligase Pirh2 and in turn the p53 protein level to inhibit cell apoptosis and maintain hESC self-renewal. Taken together, our findings provide novel insights into how hESC genome stability and survival are maintained through post-transcriptional mechanisms and link pre-mRNA processing to hESC fate determination.
Keywords: Human Embryonic Stem Cells, PRPF6/8, Genome Stability, Alternative Splicing, m6A RNA Modification, p53 protein stability, ubiquitin E3 ligase

INDUCTION OF PLURIPOTENCY BY JGES

Pei, Duanqing

School of Life Sciences, Westlake University, China

The conversion of differentiated somatic cells to pluripotency represents a model system to understand cell fate control. Here we describe a 4 factor system, Jdp2, Glis1, Esrrb and Sall4 or JGES, that can efficiently convert E13.5 MEFs to naive pluripotent. Compared to the classic Oct4, Sox2, Klf4 and Myc or OSKM, JGES appears to drive MEFs to pluripotency differently based on RNAseq, ATACseq and sensitive to growth factors even though the resulting iPSCs are indistinguishable from those from OSKM nor mESCs. We further generated an inducible secondary system in which mice bearing JGES were obtained and MEFs can be readily isolated for induction of pluripotency. Technical details will be presented to show that JGES offers a unique pathway for reprogramming.
Keywords: reprogramming, Jdp2, Glis1, Esrrb, Sall4, pluripotency

Speaker Abstracts

Abstract Selected Speaker Session I

DERIVATION OF BRAIN ORGANOIDS WITH MICROGLIAL INCORPORATION FROM IPSCS

Wang, Tongguang, Gastfriend, Benjamin, McDonald, Valerie, Nath, Avindra

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3D brain organoids are important alternative tools for the research of human brain development and pathogenesis. However, lacking the incorporation of certain cell types, especially microglial cells, in the iPSC (induced pluripotent stem cells)-derived human brain organoids make them difficult to be used to mimic neuroinflammation. Current protocols to addressing this issue either incorporated fully differentiated microglia into the brain organoid, missing the stage when microglial interacts with the adjacent neural environment during differentiation; or induced the microglial differentiation together with the neural induction from the early stage of 3D organoid formation, technically difficult to produce consistent organoids in terms of the quantity and quality of microglia. To model brain organoids with microglia to study the early interactions between microglial and neuronal development, we tested different approaches and successfully derived high purity of CD34+ cells first from human iPSCs, then incorporated the CD34 cells into iPSC-derived embryoid bodies to make brain organoids. Using immunostaining and flow cytometry analysis, we confirmed that CD34 cells incorporated into the 3D organoids which eventually developed into brain organoids with both Iba1+ microglia and beta-III tubulin+ neurons. Compared to brain organoids without CD34 incorporation, this approach produces significantly more microglial incorporation in the brain organoids. This novel 3D organoid model consisting of both microglial and neural development properties can be used to study the early interactions between the innate immune and nervous system development and potentially as a model for neuroinflammation.

Funding Source

This work is supported by NIH/NINDS intramural research fund.

Keywords: 3D brain organoid, Microglia, CD34 cells

PSYCHOLOGICAL STRESS DRIVES MELANOCYTE STEM CELL EXHAUSTION THROUGH ACTIVATION OF THE SYMPATHETIC NERVOUS SYSTEM

Zhang, Bing[1], Ma, Sai[2], Rachmin, Inbal[3], He, Megan[2], Baral, Pankaj[4], Choi, Sekyu[2], Gonçalves, William A.[5], Shwartz, Yulia[2], Fast, Eva M.[2], Su, Yiqun[3], Zon, Leonard I.[2], Regev, Aviv[6], Buenrostro, Jason D.[2], Cunha, Thiago M.[7], Chiu, Isaac M.[4], Fisher, David E.[3], Hsu, Ya-Chieh[2]

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Psychological stress negatively affects tissue homeostasis and regeneration, but whether and how stress perception leads to profound changes in tissue biology remains poorly understood. Here, we investigate this question in the skin. Psychological stress has been anecdotally associated with hair graying, but a scientific evidence linking the two is lacking. By adapting approaches to induce stress in mice, including physical pain and restraining, we showed that psychological stress leads to gray hair formation through rapid depletion of melanocyte stem cells (MeSCs). Combining denervation, endocrine surgeries, cell ablation, and cell-type specific gene deletions, we showed that stress-induced hair graying is independent of stress hormones or the immune system, but relies on the activation of the sympathetic nervous system. Sympathetic nerve terminals innervate the MeSC niche. Under stress, sympathetic nerve activation leads to burst release of neurotransmitter norepinephrine, which targets MeSCs directly. Norepinephrine drives MeSCs proliferation, leading to their rapid exhaustion. Inhibition of MeSC proliferation or MeSC-specific deletion of norepinephrine receptors rescue stress-induced hair graying. Our study shows that psychological stress-induced neural activity can alter somatic stem cells directly, and identifies strategies that might be exploited for therapeutic purposes in the future.

Keywords: Melanocyte stem cell, niche, hair follicle

PROCR+ ENDOTHELIAL PROGENITORS IN VASCULAR DEVELOPMENT AND QUIESCENCE ACQUISITION

Yu, Qing Cissy

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Vascular growth and remodeling are continuous processes that persist throughout adulthood, coordinating with organ growth and repair. We identified Protein C receptor-expressing (Procr+) ECs as endothelial progenitors that actively contribute to angiogenesis, ensuring organ development and homeostasis maintenance. Procr+ VESCs exhibit robust clonogenicity in culture, high vessel reconstitution efficiency in transplantation, long-term clonal expansion in lineage tracing, active participation in injury repair. Moreover, Procr+ VESCs are bipotent, giving rise to de novo formation of ECs and pericytes. Recently, we reveal Procr as a molecular marker for endothelial progenitors during vessel initiation in the early developing embryos. More so, we identified an intrinsic molecular regulator which drives the quiescence acquisition of endothelial progenitors, ensuring the function and homeostasis of formed vasculature.

Keywords: Endothelial Progenitor, Vessel formation, quiescence entry

REGULATORY T CELLS MEDIATE CARDIOVASCULAR REPAIR: MECHANISM AND THERAPEUTIC IMPLICATION

Lui, Kathy

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Accumulating evidence has demonstrated that immune cells such as macrophages play an important role in the regulation of cardiovascular repair. After injury, danger signals released by the damaged tissues trigger the initial pro-inflammatory phase essential for removing cellular debris that is later replaced by the anti-inflammatory phase responsible for tissue healing. Impaired immune regulation can lead to excessive scarring and fibrosis that are detrimental for the restoration of heart function. Our earlier work has shown that regulatory T-cells (Treg) respond to heart injury that are indispensable for heart repair particularly during neonatal regeneration. In this talk, we will summarize their regenerative role in directing macrophages after injury, and their direct function in enhancing replication of cardiovascular cells during repair and regeneration. We will also demonstrate the possible molecular mechanisms by which Treg mediate cardiovascular repair through regulating the transcriptomic and epitranscriptomic events in cardiovascular cells. Altogether, our findings may suggest some clinically relevant insights into the development of Treg therapy targeting cardiovascular repair and regeneration in the future.

Funding Source
National Natural Science Foundation of China (81922077, 82070494); Research Grants Council of Hong Kong (14100021, 14108420, C4026-17WF, M-402-20)
Keywords: Cardiovascular Repair, Regulatory T cells, Transcriptomic and Epitranscriptomic regulation

TIN2 INSUFFICIENCY LEADS TO ALT-ASSOCIATED PHENOTYPES AND DIFFERENTIATION DEFECTS IN EMBRYONIC STEM CELLS

Yan, Huang, Yin, Shanshan, Huang, Yan, Songyang, Zhou

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Telomere integrity is critical for embryonic development, and core telomere-binding proteins such as TIN2 are key to maintaining telomere stability. Here we report that a premature termination mutation of Tin2 in mice (homozygous Tin2 S341X) resulted in embryonic lethality at E3.5-E7.5 and reduced expression of TIN2 in the derived mouse embryonic stem cells (mESCs). Homozygous mutant mESCs were able to self-renew and remain undifferentiated but displayed many of the phenotypes associated with alternative lengthening of telomeres (ALT), including excessively long and heterogenous telomeres, increased ALT-associated PML bodies (APBs), and unstable chromosomal ends. These cells also showed upregulation of Zscan4 expression and elevated targeting of DAXX/ATRX and H3K9me3 marks on telomeres. Furthermore, the mutant mESCs were impeded in their differentiation capacity. Upon differentiation, DAXX/ATRX and PML disassociated from telomeres in these cells, where elevated DNA damage was also apparent. Our results combined reveal differential responses to telomere dysfunction in mESCs versus differentiated cells and highlight the critical role of TIN2 in telomere integrity during embryonic development.

Keywords: telomere, mouse embryonic stem cell, alternative lengthening of telomeres

Speaker Abstracts

THURSDAY, 13 JANUARY

Tissue Stem Cells in Niche and Cancer

PRINCIPLES OF REGENERATION CAPTURED BY IMAGING THE SKIN OF LIVE MICE

Greco, Valentina

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Our regenerative organs, like skin, intestine and blood, undergo continuous cellular turnover: fueled by stem cell self-renewal and differentiation. However, due to technical limitations, this highly dynamic process was initially examined by static approaches. These approaches cannot track the same cells over time, and thus could not identify the cells and behaviors that sustain an organ over time. Yet, this knowledge is critical to decipher the mechanisms of homeostasis. To fill this gap in knowledge, my lab along with collaborators developed novel methods to visualize and manipulate stem cells and their niche in the skin epithelium of an intact, uninjured mouse. These approaches allow to address the following fundamental questions in organ biology: First, how are tissue dynamics regulated at the single stem cell level? Stem cells must choose between two fundamentally different behaviors: self-renewal or differentiation. We aim to determine how the genomic and metabolic activities of stem cells relate to their choice to self-renew versus differentiate. Second, how do other constituent cells influence stem cells and homeostasis? Apart from epithelial stem cells, our skin comprises diverse cell types (e.g. immune cells, fibroblasts, endothelial cells) and structures (e.g. extracellular matrix). Our novel approaches allow us to dissect the contributions of these individual components to stem cell regenerative potential and organ homeostasis. Third, how do cumulative mutations affect homeostasis? During normal aging, epithelial stem cells acquire mutations that are often oncogenic and yet tolerated within apparently phenotypically normal tissue. How tissues function despite diverse mutant populations is unclear. With the ability to create a mosaic of mutant clones and track individual stem cell and niche cells in vivo, along with their metabolic and transcriptional changes, we will determine how mutant clones are maintained and function within the skin. The data presented at this conference will discuss our current understanding of how distinct neighboring niche cells influence the coexistence of mutant subpopulations within the epithelium as well as support this tissue ability to maintain a homeostatic steady state and function throughout a lifetime.

Keywords: stem cell, live imaging, skin, epithelial cells, mutation, tolerance

CELL IDENTITY CONVERSION AND LIVER REGENERATION

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To understand the mechanistic regulation underlying tumorigenesis of normal cells is a long-term interest of our lab. Recently, taken hepatocytes as the experimental system, our lab has initiated studies on cell lineage conversion for regeneration, namely transdifferentiation and dedifferentiation in vitro and in vivo. Striving to understand these two seemingly different phenomena, we find ourselves in querying the essential scientific question: How is cell identity maintained through preventing the conversion of terminally differentiated cells to other cell types, including cell lineage conversion and transformation to tumor cells; or in a reversed term, how is the cell plasticity regulated? In this talk, I will present our latest findings to demonstrate a role of hepatocyte reprogramming in liver regeneration and tumorigenesis.

Keywords: cell lineage conversion; hepatocyte reprogramming; liver regeneration and tumorigenesis

DEFINING HEMATOPOIETIC STEM CELL-NICHE INTERACTIONS IN FETAL LIVER AT CELLULAR RESOLUTION USING SPATIAL TRANSCRIPTOMICS

Li, Linheng

Stowers Institute for Medical Research, Kansas City, MO, USA

To understand the spatial distribution and interactions between hematopoietic stem cells (HSCs) and the microenvironment or niche cells in fetal liver, we introduced two spatial transcriptomic methods, slide-seq and 10x Visium, in our study on E14.5 mouse fetal liver. By integrating with a parallel single cell sequencing analysis, we revealed spatial transcriptomics of HSCs and potential niche cells, including hepatocytes, endothelium cells, macrophages, megakaryocytes, and mesenchymal hepatic stellate cells (MhSCs). Of note, MhSCs were characterized by enriched N-cadherin expression. Both slide-seq and 10x Visium showed that the N-cadherin-expressing MhSCs are enriched in the portal vessel area. Importantly, most fetal liver HSCs are near N-cadherin-expressing MhSCs, indicating a supportive role of N-cadherin-expressing MhSCs in HSC maintenance. Subsequent CellPhoneDB (CPDB) analysis demonstrated that N-cadherin-expressing MhSCs are major niche-signaling senders with an enriched expression of niche factors, such as CXCL12 and KITL, and stemness pathway-related ligands, such as IGF1, IGF2, TGFβ2, TGFβ3, JAG2, and DLK1. This finding was consistent

with our previous finding that N-cadherin-expressing bone and marrow stromal progenitor cells can maintain reserve HSCs in adult bone marrow. Other niche cells, including endothelium cells, macrophages, and megakaryocytes, may support HSCs with different signaling modules. For example, endothelium cells have enriched expression of KITL, IGF2, DLL1, TGFβ1 and TGFβ2; macrophages have enriched expression of KITL, IFNγ, and TGFβ1; megakaryocytes have enriched expression of PF4, JAG2 and TGFβ1. Furthermore, using genetic mouse models N-cadCreER;Cxcl12 and N-cadCreER;Scf, we conditionally knocked out (CKO) CXCL12 or SCF, in N-cadherin-expressing cells. Unexpectedly, we found that CKO of either CXCL12 or SCF resulted in an increase in the number of HSCs. Moreover, conditional knockout of Cxcxl12 in N-cadherin-expressing cells also resulted in a myeloid-biased differentiation. We postulate that knockout of Cxcl12 or Scf in N-cadherin-expressing cells leads to the migration of HSCs towards other potential niche cells, such as macrophages and megakaryocytes, which may induce HSC expansion and biased differentiation.
Keywords: HSC expansion, HSC niche cells, spatial transcriptomics

GENERATION OF MOUSE PANCREATIC ISLET ORGANOIDS USING RESIDENT PROCR PROGENITORS

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It has generally proven challenging to produce functional β cells in vitro. Our recent study uncovers a novel Procr cell population in adult mouse pancreatic islets. The cells do not express differentiation markers and feature epithelial-to-mesenchymal transition (EMT) characteristics. By genetic lineage tracing, Procr islet cells undergo clonal expansion and generate all four endocrine cell types during adult homeostasis. Sorted Procr cells, representing ~1% of islet cells, can robustly form islet-like organoids when cultured at clonal density. Exponential expansion can be maintained over long time periods by serial passaging, while differentiation can be induced at any time point in culture. Beta cells dominate in differentiated islet organoids, while α, δ and PP cells occur at lower frequencies. The organoids are glucose-responsive and insulin-secreting. Upon transplantation in diabetic mice, the organoids reverse disease. These findings demonstrate that the adult pancreatic islet contains a population of Procr progenitors. We will also describe the physiological relevance of Procr progenitors during postnatal islet development and homeostasis.
Keywords: adult stem cells, pancreatic islet, beta cells, organoids

Reprogramming, Regeneration, and Repair

REVERSING AGING: REGENERATING AND REJUVENATING AGED MUSCLES

Blau, Helen

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Regenerative medicine holds great promise for local enhancement of skeletal muscle repair to treat muscular dystrophies and aging-associated muscle wasting. Muscle stem cells (MuSCs) are a potent population that resides within muscle tissues, poised to repair muscle damage throughout life. However, the therapeutic utility of MuSCs is currently limited by their rarity and their inefficient survival, self-renewal, and differentiation after injection into muscle tissue. We have devised bioengineering strategies and discovered novel molecular regulators to surmount these hurdles. By defining the myogenic stem cell progression by single cell mass cytometry (CyTOF), we can target metabolic functions that dictate cell fate transitions. By fabricating biomimetic hydrogels with differing elasticity matching muscle tissue, we can overcome the loss of stem cells on traditional plastic cultureware. Fibrosis, which causes dysfunction and ultimate failure of numerous tissues with aging, is characterized by increased tissue stiffness. We have developed a dynamic hydrogel platform to enable mechanistic studies of cellular dysfunction as fibrosis progresses in real time. Cell autonomous defects in MuSC function accompany aging. By targeting these molecular pathways, we can rejuvenate stem cell function. As an alternative to cell therapy, we are seeking to stimulate the function of endogenous quiescent satellite stem cells within muscle tissues. Through an in silico screen, we identified a potent regulator that robustly augments stem cell function and may serve as a novel therapeutic agent to induce muscle regeneration and counter debilitating muscle wasting in the elderly.
Funding Source
National Institutes of Health, California Institute for Regenerative Medicine, American Heart Association, Li Ka Shing Foundation, Donald E and Delia B Baxter Foundation, SPARK.
Keywords: stem cells, muscle, regenerative

Speaker Abstracts

HOW AN ELECTROPHYSIOLOGICAL SIGNAL IS INTEGRATED INTO SCALING A VERTEBRATE DEVELOPMENTAL FIN/LIMB PROGRAM

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All animals control stem and progenitor cells to generate anatomical structures to specific body dimensions. While mechanisms that scale organs and appendages are known to involve hormones, growth factors and specific signal transduction cascades, it is remains unknown how such factors are coordinatedly controlled to generate complete and exactly scaled anatomical structures. My talk will show how hormone information (retinoic acid) is linked to the control of a phosphatase (calcineurin) to regulate an electrophysiological signal (changes in intracellular K+) that itself regulates the expression of the several morphogens to scale vertebrate fins/limbs in a coordinated manner. My lab is also defining how these intracellular K+ changes regulate these coordinated changes by controlling the transcription of a developmentally important morphogen, the shh ligand. I will also show what these intracellular K+ changes look like in vivo.

Funding Source
ShanghaiTech University, Deutsche Forschungsgemeinschaft.
Keywords: Zebrafish, Electrophysiology, Scaling anatomical structures

USING CHROMATIN DYNAMICS FOR TISSUE REGENERATION APPLICATIONS

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Understanding chromatin dynamics during tissue development unlocks the ability to harness this information for tissue regeneration, insights into the logic of tissue morphogenesis and for elucidating the basis of developmental disorders. Proper ectodermal patterning into skin requires surface ectoderm commitment and proper inductive signals from regional mesoderm. In collaboration with Google, we have created a knowledge graph called Biomedical Data Commons that allows public search of 'omics data. Using an inference network from embryonic stem cells to skin that ranks the coordinate effects of TF-accessible regulatory element-target gene expression triplets, we have identified two critical transition periods, surface ectoderm initiation and keratinocyte maturation, and identified TFAP2C and p63 as the ectoderm lineage initiation and maturation factors, respectively. Further exploration of the mechanism by which ectoderm and mesoderm factors stably pattern lineage commitment led to identification of several craniofacial disease-associated chromatin regulators required for proper dermal maturation epithelial-mesenchymal communication, resulting in defective keratinocyte stratification. Finally, we have used chromatin dynamic information to develop Dystrophic Epidermolysis Bullosa Cell Therapy (DEBCT), the first GMP-compatible, scalable viral and integration-free platform to produce CRISPR-corrected, autologous clinical grade iPSC-derived tissue stem cells to treat the incurable wounds of DEB patients.

Funding Source
National Institutes of Health, California Institute for Regenerative Medicine, EB Research Partnership.
Keywords: Induced pluripotent cells, skin, craniofacial diseases

3D-BIOPRINTING LIVER FOR IN VIVO STUDIES

Huang, Pengyu

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The shortage of organ donors, a critical challenge for the treatment of end-stage organ failure, has motivated the development of alternative strategies, such as three-dimensional (3D) bio-printing, to generate organs in vitro. Despite many years of bioprinting development, the generation of functional bioprinted organs for regenerative medicine is still challenging. Here, we describe the hepatorganoids, which is a liver tissue model generated by three-dimensional (3D) bioprinting of HepaRG cells and investigate its liver functions in vitro and in vivo. HepaRG cells in three-dimensional bioprinted hepatorganoids (3DP-HOs) were differentiated into hepatocytes in vitro, and the 3DP-HOs acquired several liver functions such as ALBUMIN secretion, drug metabolism, and glycogen storage after 10 days of differentiation. After transplantation of 3DP-HOs into the abdominal cavity of the Fah-/-Rag2-/- liver injury mouse model, 3DP-HOs further matured with increased synthesis of liver-specific proteins. In particular, 3DP-HOs acquired human-specific drug metabolism in mice. Functional vascular systems were also formed in 3DP-HOs at 14 days after transplantation, further supporting material transport and functions of 3DP-HOs. Most importantly, transplantation of 3DP-HOs significantly improved the survival of the liver injury mouse model. Thus, our results demonstrated the proof-of-principle that organs generated by 3D bioprinting function in vivo and provide a promising new approach for the treatment of liver diseases.

Keywords: 3D printing, bio-artificial liver, liver failure

ESTABLISHING A NOVEL INTESTINAL ORGANOID CULTURE SYSTEM MODELING INJURY-ASSOCIATED EPITHELIAL REGENERATION

Deng, Hongkui

Cell Biology, Peking University, Beijing, China

The development of organoids, which mimic physiological 3D tissue organization and functionality through the self-organization of stem cells, has shown great applicative potential in studying development and diseases. One major limitation of current organoid technology is that it only reflects the self-organization of homeostatic stem cells and their derivatives, but not regenerative tissues upon injury. Importantly, increasing evidence indicates that the properties of stem cells during in vivo regeneration are largely distinct from their characteristics during homeostasis. As a result, the development of an in vitro organoid culturing system that can model the in vivo regeneration process remains challenging. We recently developed a novel intestinal organoid culture system, designated hyperplastic intestinal organoids (Hyper-organoids), which has key features of injury-associated intestinal epithelial regeneration. Single-cell RNA sequencing identified different regenerative stem cell populations in our Hyper-organoids that shared molecular features with in vivo injury-responsive Lgr5+ stem cells or Clu+ revival stem cells. Using this system, we found that two small molecules were critical for epigenome reprogramming and regeneration, which functioned through epigenetically regulating YAP signaling. Our studies demonstrated a new in vitro organoid model to study epithelial regeneration, and highlight the importance of epigenetic reprogramming that pioneers tissue repair.

Keywords: Organoid, injury-associated epithelial regeneration, epigenome reprogramming

Abstract Selected Speaker Session II

TUMOR INITIATING STEM CELL SHAPES ITS MICROENVIRONMENT INTO AN IMMUNOSUPPRESSIVE BARRIER AND PRO-TUMORIGENIC NICHE

He, Xi, Li, Linheng

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Tumor initiating stem cells (TSCs) are critical for drug-resistance and immune escape. However, the mutual regulations between TSC and tumor microenvironment (TME) remain unclear. Using DNA-label retaining, scRNA-sequencing, and other approaches, we investigated intestinal adenoma in response to chemoradiotherapy (CRT), thus identifying therapy-resistant TSCs (TrTSCs). We further analyzed signaling modules and revealed bidirectional crosstalk between TSCs and TME using CellPhoneDB analysis. An intriguing finding is that TSCs shape TME into a landscape that favors TSCs for immunosuppression and propagation. To investigate the mechanism, we used adenoma-organoid co-cultures, niche-cell depletion, and lineaging tracing to characterize a functional role of Cox-2-dependent signaling, predominantly occurring between tumor associated monocyte and macrophage (TAMMs) and TrTSCs. We show that TAMMs promoted TrTSC proliferation through the PGE2-PTGER4(EP4) pathway that enhanced the Beta-Catenin activity via AKT phosphorylation. Thus, our study shows that the bidirectional crosstalk between TrTSC and TME resulted in a pro-tumorigenic and immunosuppressive contexture.

Keywords: therapy-resistant TSCs (TrTSCs), PGE2-PTGER4(EP4) pathway, TAMM

INVESTIGATING RETT SYNDROME USING TWO-DIMENSIONAL AND THREE-DIMENSIONAL HUMAN BRAIN MODELS

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Rett syndrome (RTT) is a severe X-linked neurodevelopmental disorder caused by mutations in the methyl-CpG binding protein 2 (MeCP2). Cellular heterogeneity in the brain confounds the understanding of RTT etiology. To date, how MeCP2 mutation affects defined cell types in the human brain remains unclear, and effective therapeutics for RTT are lacking. In this study, we applied CRISPR/Cas9-based gene editing, two-dimensional and three-dimensional human brain models to investigate the molecular and cellular function of MeCP2. We found that

Speaker Abstracts

MeCP2 mutation caused severe abnormalities in human interneurons (INs). Surprisingly, the treatment with a BET inhibitor, JQ1, rescued the molecular and functional phenotypes of MeCP2 mutant INs. We uncovered that BRD4-mediated epigenetic regulation underlies the abnormal transcription in MeCP2 mutant INs, which were recovered to normal levels by BET inhibition. Further, our single cell-based approach revealed the cell type-specific transcriptome impairment in RTT cells from dorsal and ventral human forebrain organoids, which also can be rescued by BET inhibition. Finally, BET inhibition ameliorates RTT-like phenotypes in mice. Overall, we demonstrate that BRD4 dysregulation is a critical driver for RTT etiology, and suggest that targeting BRD4 could be a potential therapeutic opportunity for RTT. Our study also emphasizes the potential of in vitro human brain models, particularly human brain organoids, in furthering our understanding of human brain function, disease, and therapy.
Keywords: Brain organoids, MeCP2, Rett syndrome

CONSTRUCTION OF GASTRULA EMBRYOID FROM REPROGRAMMED CELLS

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Stem cell-based embryo models by cultured pluripotent and extra-embryonic lineage stem cells are novel platforms to model early post-implantation development. In this study, we showed that induced pluripotent stem cells (iPSCs) could form ITS (iPSCs and trophectoderm stem cells) and ITX (iPSCs, trophectoderm stem cells, and XEN cells) embryos, resembling the early gastrula embryo developed in vivo. To facilitate the efficient and unbiased analysis of the stem cell-based embryo model, we set up a machine learning workflow to extract multidimensional features and perform quantification of ITS embryos using 3D images collected from a high-content screening system. We found that different iPSC lines differ in their ability to form embryo-like structures. Through high-content screening of small molecules and cytokines, we identified that BMP4 best promoted the morphogenesis of the ITS embryo. Our study established an innovative strategy to analyze stem cell-based embryo models and uncovered new roles of BMP4 in stem cell-based embryo models.
Funding Source
This work was supported by the National Key R&D Program of China (grants 2017YFA0102802 and 2019YFA0110001) to JN. NSFC grant (32000610) to JG.
Keywords: stem cell-based embryo model, machine learning, gastrulation

PROBING AND TARGETING MOTOR NEURON SUBTYPE DIFFERENTIAL VULNERABILITY IN ALS VIA MICRORNA

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Selective motor neuron (MN) degeneration is the hallmark of amyotrophic lateral sclerosis (ALS). Among degenerated MNs, lateral motor column motor neurons (LMC-MNs) innervating limbs are one of the most vulnerable types. Previously, we reported that deletion of mir-17~92 in MNs leads to selective apoptosis of LMC-MNs in embryos by promoting PTEN nuclear import. Here, we further revealed that mir-17~92 expression is sustained in the adult MNs, and a reduction in mir-17~92 expression, with concomitant nuclear PTEN accumulation, is manifested in spinal MNs before disease onset in SOD1G93A ALS mice. Using a novel double-transgenic reporter system in embryonic stem cells (ESCs), we uncovered down-regulation of mir-17~92 and increased nuclear PTEN in ALS-linked degenerating LMC-MNs, whereas non-LMC-MN subtypes remained relatively unaffected. This dysregulation axis of mir-17~92/nPTEN hallmark is recapitulated in human ALS SOD1+/L144F iPSC-derived MN system. Finally, we demonstrate that overexpression of mir-17~92 can significantly rescue human SOD1+/L144F iPSC-derived MNs and improve motor deficits as well as survival in the SOD1G93A mouse model. These findings envisage mir-17~92 to be a potential prognosis marker for MN degeneration and a promising candidate for therapeutic target in ALS.
Keywords: microRNA, iPSC, gene therapy

HUMAN IPSC-DERIVED EXTRACELLULAR VESICLES: PROPERTIES AND APPLICATIONS

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Extracellular vesicles (EVs), including exosomes and microvesicles, mediate intercellular communications and exert various biological activities via delivering cargos of functional molecules to recipient cells. These membrane-enclosed but nuclei-free EVs are stable for weeks at 4oC and survive quite well after electroporation, unlike their parental mammalian cells. It is widely considered that EVs produced from various cell types have different cellular contents that include membrane and cytosolic proteins, RNA, mRNA and metabolites. Therefore, it is of great interest to examine in details EVs derived from various types of human stem cells. In this study, we examined properties and functions of EVs from human induced pluripotent stem cells (iPSCs) that can be cultured infinitely under a chemically defined medium, free of any exogenous EVs. We collected and purified EVs secreted by human iPSCs and MSCs. Purified EVs produced by both stem cell types have similar sizes (~150 nm in diameter), but human iPSCs produced EVs 16-fold more efficiently than MSCs. When highly purified iPSC-EVs were applied in culture to senescent MSCs that have elevated reactive oxygen species (ROS), they reduced cellular ROS levels and alleviated aging phenotypes of senescent MSCs in 3 different aging models. Our discovery reveals that EVs from human stem cells can alleviate cellular aging in culture, at least in part by delivering intracellular peroxiredoxin enzymes that reduce intercellular ROS levels in recipient cells. We also tested whether human iPSC-derived EVs exert reparative or regenerative capacity in vivo after tissue injuries. The highly purified EVs were electroporated with SPIO magnetic particles. The SPIO-containing EVs were assessed by MRI in vivo after iv injection in 3 animal models with acute injuries, revealing that iPSC-EVs can selectively home to injury sites and confer substantial improvement in treated mice. Our studies provide evidence that EVs from human iPSCs may represent a novel form of cellular therapy (without the use of intact cells) for treating aging/degenerative diseases and acute tissue injuries. We also describe a novel approach to track EVs by non-invasive MRI to assess in vivo whole-body distribution that helps optimize further development of EV-based cellular therapy.

Keywords: Human iPSCs, Extracellular Vesicles, aging

FRIDAY, 14 JANUARY

Advances in Technology, Disease Modeling, and Drug Discovery

THE ESSENTIALOME OF HUMAN PLURIPOTENCY

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Human pluripotent stem cells (hPSCs) can differentiate into all embryonic lineages while having the capacity for self-renewal. We have recently generated haploid hPSCs carrying only one set of chromosomes. Interestingly, we found that a haploid human genome is compatible not only with the undifferentiated pluripotent state, but also with differentiated somatic fates representing all three embryonic germ layers Furthermore, we demonstrated the superior utility of haploid hPSCs for loss-of-function genetic screening. To define the essentialome of hPSCs we generated a genome-wide loss-of-function library in the haploid cells utilizing CRISPR/Cas9 technology using about 180,000 guide RNAs, targeting virtually all coding genes. This library enabled us to define the genes essential for the normal growth and survival of undifferentiated hPSCs. We could also allude to an intrinsic bias of essentiality across cellular compartments, uncover two opposing roles for tumor suppressor genes and link autosomal-recessive disorders with growth retardation phenotypes to early embryogenesis. More recently, we set out to map the essential genes for the differentiation of hPSCs into the three embryonic germ layers by using our loss-of-function library. Through the analysis of essential genes for the differentiation of hPSCs into ectoderm, mesoderm and endoderm, we defined the essentialome of each germ layer separately and also identified commonly essential genes for the transition from pluripotency stage into differentiated cells. Interestingly, the latter group was enriched by genes localized within the endoplasmic reticulum-Golgi network and regulate membrane and secreted molecules, highlighting the key role of signaling events during these dynamic cell state transitions. Our data enabled analysis of all hereditary neurological disorders, uncovering essentiality of a significant fraction of microcephaly-causing genes during early stages of neuroectoderm development. Overall, our work sheds light on the gene networks regulating pluripotency, early gastrulation, and human embryonic disorders by defining essential drivers of specific embryonic germ layer fates and essential genes for the exit from pluripotency.

Keywords: human pluripotent stem cells; genome-wide screening; genetic disorders

Speaker Abstracts

DEVELOPMENT OF THE INTEGRATED HUMAN BRAIN ORGANOIDS TO STUDY BRAIN DEVELOPMENT AND DISEASES

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Brain organoids represent the 3D tissues that recapitulate the structure and function of the developing human brain. Much efforts have been made to advance the regionalization and to utilize the brain organoids to study human diseases. We developed region-specific cortical organoids and used them to study Rett syndrome. Since neuroectoderm differentiation of the human embryonic stem cells (hESCs) is an essential first step in brain organoid formation, the majority of cells in brain organoids are of the neuroectoderm origin. However, the cells that comprise the blood vessel in brain, and the residential immune cells in brain are from the mesoderm. In order to implement the mesoderm cells in brain organoids, we genetically engineered the hESCs to express the transcription factors that facilitate the formation of vascular-like structure and microglia-like cells. The vascular-like structures highly improved the quality of the brain organoids, dramatically decreasing the cell death, and increasing the neural maturation. The microglia-like cells demonstrated the innate immune function such as phagocytosis. Overall, our engineered brain organoids provide the essential mesoderm-derived cells in neuroectoderm-oriented brain organoids that play critical roles in brain function.
Keywords: Brain Organoids, iPSCs, hESCs, cortical organoids

NEW GENOMIC TECHNOLOGIES TO DISSECT DEVELOPMENT AND REPROGRAMMING

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Cell identity is governed by the complex regulation of gene expression, represented as Gene Regulatory Networks (GRN). While network inference has evolved, GRN analysis is often restricted to local pairwise or modular relationships, limiting the phenotypic insights derived at the level of a cell or organism. To address this, we developed CellOracle, a machine learning-based approach leveraging single-cell GRN inference to perform in silico transcription factor (TF) perturbations and predict subsequent changes in cell identity, without requiring experimental perturbation data. We applied CellOracle to two well-established paradigms: mouse hematopoiesis and zebrafish development, correctly predicting reported phenotypic changes due to TF perturbation. The application of CellOracle to direct lineage reprogramming, in combination with single-cell lineage tracing, reveals GRN reconfiguration during the conversion of cell fate. Further, via network analysis, we identify TFs to enhance reprogramming efficiency and fidelity.
Keywords: Reprogramming, single-cell analysis, gene regulatory networks

STEM CELL SIGNALS IN CANCER HETEROGENEITY AND THERAPY RESISTANCE

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Our research focuses on the signals that control stem cell self-renewal and how these signals are hijacked in cancer. Using a series of genetic models, we have studied how classic developmental signaling pathways such as Wnt, Hedgehog and Notch play key roles in hematopoietic stem cell growth and regeneration and are dysregulated during leukemia development. In addition, using real-time imaging strategies we have found that hematopoietic stem cells have the capacity to undergo both symmetric and asymmetric division, and that shifts in the balance between these modes of division are subverted by oncogenes. Further, regulators of this process, including the cell fate determinant Musashi, are critical players in driving progression of solid and liquid cancers and could serve as targets for diagnostics and therapy. Ongoing work is focused on understanding the mechanisms that drive therapy resistance after drug delivery, as well as developing high resolution in vivo imaging approaches to map normal stem cell behavior and interactions within living animals, and to define how these change during cancer formation.
Keywords: leukemia, stem cell, cancer stem cell

MODELING RETT SYNDROME USING GENE-EDITED *MECP2* MUTANT CYNOMOLGUS MONKEYS

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With superb gene-editing technologies, monkey models for neurological and psychiatric disorders with defined genetic causes have begun to manifest their values. Here we report detailed phenotypes of TALEN-edited MECP2 mutant cynomulgus monkeys. Female mutant monkeys display development-dependent brain structural changes, reduced cerebral blood flow, and abnormal electrocardiograms. They showed stereotypic movements with limbs,

social withdrawal, altered reactivity toward environmental stimuli, fragmented sleep and reduced aggression, all of which resemble Rett syndrome (RTT) clinical output. Primate-unique eye tracking assays demonstrated similarity between RTT patients and MECP2 mutant monkeys, and peripheral blood gene expression profiling revealed putative biomarkers for MECP2 deficiency in both human and monkeys, which may facilitate future drug development. Taken together, the stark similarity in these phenotypes and/or endophenotypes between monkeys and patients demonstrate the value of gene-edited RTT mosaic founder monkeys for disease mechanistic studies as well as development and validation of future therapeutic interventions for RTT. Recently, through base-editing, we created T158M point mutant RTT monkeys, which are both genetically and phenotypically similar to the human disease. Using single cell transcriptomic analyses using wild type and RTT monkeys, we discovered new insight into RTT etiology.

Keywords: gene editing, non human primate disease models

Stem Cell-Based Therapies

ALLOGENEIC HYPOIMMUNE CELL THERAPEUTICS AS SCALABLE LIVING MEDICINES

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Cell therapeutics to replace or regenerate impaired organ functions could become a new strategy for the treatment of major diseases. The success will depend on scalability, off-the-shelf availability, accessibility for large patient populations, and economic affordability. The goal is thus to move away from patient-specific, autologous cell products to universal, allogeneic therapeutics. Therefore, we have developed B2M-/- CIITA-/- CD47 transgenic iPSCs that can evade all innate and adoptive allogeneic immune cell responses. This engineered immune evasiveness is based on MHC class I and II deletion to escape T cell recognition and on activating the CD47-SIRPalpha immune checkpoint in innate myeloid and lymphoid immune cells. Such 'hypoimmune' iPSCs can be differentiated into functionally active, hypoimmune cell types to treat diseases in allogeneic recipients without any immunosuppression. Scaling can be achieved at the iPSC stage and, for some proliferating cell types like endothelial cells, at the final product level. We show engraftment and survival of several hypoimmune cell types in fully MHC-mismatched mice. In an attempt to treat heart failure, a mixture of hypoimmune mouse iPSC-derived endothelial cells (iECs) and cardiomyocytes (iCMs) was injected into infarcted mouse hearts and both cell types orthotopically engrafted in the ischemic areas. Cell therapy led to an improvement of invasive hemodynamic heart failure parameters. To use hypoimmune cells as living factories, iECs were transduced to express a transgene for alpha1-antitrypsin (A1AT) and successfully restored physiologic A1AT serum levels in mice with genetic A1AT deficiency. This cell therapy prevented both structural and functional changes of emphysematous lung disease. Human hypoimmune iCMs have been generated that achieved long-term survival in humanized mice reconstituted with allogeneic hematopoietic stem cells and show physiologic responses to chronotropic stimuli. Our study supports the development of hypoimmune, universal regenerative cell products for cost-effective treatments of major diseases.

Keywords: hypoimmune cells, cell therapy, immune editing

DEVELOPING A STEM CELL-BASED THERAPY FOR PARKINSON'S DISEASE

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Cell based transplantation aimed at the replacement of lost dopamine (DA) neurons holds great potential for the treatment of Parkinson's disease (PD). We have developed robust and efficient differentiation protocols resulting in the formation of authentic and functional DA neurons from human embryonic stem cells (hESCs). Here, I will summarize the translational trajectory, pre-clinical validation and regulatory process embarked on to bring this hESC-derived dopamine cell product to clinical trial for PD.

Funding Source
ERC, EU, NYSCF, Novo Nordisk
Keywords: Human embryonic stem cells, dopamine neurons, Parkinson's disease

Speaker Abstracts

Closing Keynote Session

COMBINED CELL AND GENE THERAPY FOR EPIDERMOLYSIS BULLOSA

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LAMB3-dependent generalized Junctional Epidermolysis Bullosa (JEB) was targeted by transplantation of epidermal cultures originated from transgenic epidermal stem cells. We report life-saving regeneration of the entire epidermis on a seven-year-old JEB child suffering from a devastating form of JEB. The regenerated transgenic epidermis remained stable throughout the entire follow-up period and did not form blisters, even upon shear force. The proviral integration pattern was maintained in vivo and epidermal renewal did not cause any clonal selection. Clonal tracing showed that the human epidermis is sustained by a limited number of long-lived stem cells, detected as holoclones, that can extensively self-renew and produce short-lived progenitors that replenish terminally differentiated keratinocytes. In studying the different behaviour of JEB and COL7A1-dependent generalized Dystrophic EB (RDEB) cultures we discovered a pivotal role of YAP in sustaining human epidermal stem cells, which explains the progressive stem cell loss observed in JEB. Epidermal stem cell depletion of primary JEB keratinocytes is due to perturbation of the YAP/TAZ pathway and consequent alteration of the expression of FOXM1. YAP/TAZ and FOXM1 expression is significantly decreased in JEB keratinocytes, which do not contain nuclear YAP but only phosphorylated, inactive YAP. The JEB phenotype is recapitulaled by Laminin 5 ablation and consequent YAP/TAZ down-regulation in normal cells. Restoration of adhesion properties by Laminin 5-gene therapy rescues normal nuclear levels of YAP/TAZ and clonogenic potential. Both enforced YAP and FOXM1 recapitulate Laminin 5-gene therapy in JEB cells, thus uncoupling adhesion from proliferation in epidermal stem cells. This work has important clinical implication for an efficient ex vivo gene therapy of JEB.

Keywords: stem cell, cell therapy, gene therapy

Poster Abstracts

102

ELEMENTOMIC CHARACTERIZATION ANALYSIS OF NORMAL AND DYSFUNCTIONED HUMAN SPERMATOZOA USING SINGLE-CELL ICP-MS

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Sperm dysfunction is the main cause of male infertility nowadays, but the diagnostic failure rate still accounts for 30% to 70% of clinical cases, most of which are still labelled as idiopathic. This is partly because the traditional assessment methods of sperm function cannot fully meet the clinical needs. In an attempt to determine whether the element bioavailability profile in single human spermatozoa can be an approach to clarify the functional association with male factor infertility, we employed single-cell inductively-coupled plasma mass-spectrometry (sc-ICP-MS). To this end, we characterized the elementomic bioavailability profiles of human normal spermatozoa and dysfunctioned sperm with oligoasthenozoospermia. We found that not only the elementomic profiles, but the unique signatures of specific elements of human spermatozoa were related to the risk of oligoasthenozoospermia. We propose that the single-cell elementomic characterization analysis approach can be used to analyze spermatozoal function and other health conditions related to male fertility.

Funding Source
ShanghaiTech University
Keywords: Human sperm elementomics; single-cell ICP-MS profile; oligoasthenozoospermia risk

103

SARS-COV-2 INFECTION AND REPLICATION IN HUMAN GASTRIC ORGANOIDS

Giobbe, Giovanni G.[1], Bonfante, Francesco[2], Jones, Brendan C.[3], Gagliano, Onelia[4], Luni, Camilla[5], Zambaiti, Elisa[4], Perin, Silvia[3], Laterza, Cecilia[4], Busslinger, Georg[6], Stuart, Hannah[4], Pagliari, Matteo[2], Bortolami, Alessio[2], Mazzetto, Eva[2], Manfredi, Anna[7], Colantuono, Chiara[7], Di Filippo, Lucio[7], Pellegata, Alessandro[3], Panzarin, Valentina[2], Thapar, Nikhil[3], Li, Vivian Sze Wing[8], Eaton, Simon[3], Cacchiarelli, Davide[9], Clevers, Hans[6], Elvassore, Nicola[3], De Coppi, Paolo[3]

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COVID-19 typically manifests as a respiratory illness, but several clinical reports have described gastrointestinal symptoms. This is particularly true in children in whom gastrointestinal symptoms are frequent and viral shedding outlasts viral clearance from the respiratory system. These observations raise the question of whether the virus can replicate within the stomach. In this work we have generated human gastric organoids from fetal (8-21 post-conception week PCW), pediatric, and adult biopsies as in vitro models of SARS-CoV-2 infection. Cells were extensively characterized for expansion rate. Noggin, WNT-3A and R-spondin1 resulted essential for fetal gastric organoid expansion. Each line was analyzed after 10 passages through single nucleotide polymorphism (SNP) array, showing karyotype and genome stability during expansion. To facilitate viral infection, we induced reverse polarity in the gastric organoids. We found that the pediatric and late fetal gastric organoids are susceptible to infection with SARS-CoV-2, while viral replication was significantly lower in undifferentiated organoids of early fetal and adult origin. We then demonstrated that adult gastric organoids are more susceptible to infection following cell differentiation. We

were able to assess that 15.5% of somatostatin-secreting endocrine delta-cells were infected, while only 3.7% of mucin 5AC-secreting mucous cells were infected by the virus. We performed transcriptomic analysis to reveal a moderate innate antiviral response and a lack of differentially expressed genes belonging to the interferon family. We proved that human gastric organoids are a reliable in vitro tool to model SARS-CoV-2 infection. Collectively, we show that the virus can efficiently infect the gastric epithelium, suggesting that the stomach might have an active role in fecal-oral SARS-CoV-2 transmission.

Funding Source
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Keywords: Human gastric organoids; SARS-CoV-2; In vitro disease modeling

104

ANALYSIS OF METAL ION FLUX DURING CAPACITATION OF MOUSE SPERMATOZOA USING SINGLE-CELL ICP-MS

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Currently, the clinical analyses of male infertility mainly rely on semen analysis and sperm parameters. However, the high diagnostic failure rate indicates that the current evaluation methods are still insufficient, and a new approach for evaluating sperm function still needs to be developed. With increasing evidence showing the role of biometals in reproductive biology, the importance of metal ion homeostasis in sperm function and male fertility is emergent. To determine whether we could determine the changes of metals in single sperm cells during fertilization activities, we employed single-cell inductively coupled plasma mass spectrometry (sc-ICP-MS) technology to measure the metal concentrations of capacitating sperm. This study used a male sterile calcium pump PMCA4 knockout mouse model with abnormal calcium regulation during the fertilization events. Consistently, our resulted showed an abnormal dynamic calcium profile in the PMCA4-KO sperm undergoing capacitation. Overall, our study demonstrates that sc-ICP-MS can be applied for sperm functional analysis.

Funding Source
ShanghaiTech University
Keywords: Sperm function evaluation; Single-cell ICP-MS; Single-cell elementomic analysis

105

INHIBITION OF VASCULARIZED CARTILAGE REGENERATION BY LOADED CURCUMIN SCAFFOLD

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Autologous chondrocytes are important seed cells for cartilage regeneration. But chondrocytes had limited sources. Ectopic ossification of cartilage constructed by BMSCs in subcutaneous environment, ectopic ossification is mainly related to vascularization. Vascularization inhibition might be the key to solve this problem. Curcumin has anti-angiogenesis effect. The aims of this study were to investigate the regeneration of chondrocytes in the presence of curcumin in vivo and clarify how to regulate curcumin loading materials to inhibit angiogenesis without affecting the BMSCs proliferation. The curcumin loaded materials were tests for concentrations of curcumin solution, cell scratch test, cell cycle, and apoptosis tests were carried. The outcome cells of each stage (of serum-free medium group) were then implanted subcutaneously into nude mice. After 3, 6, 12 weeks tests HE, S/O staining, CD31immunohistochemical, CD31 immunofluorescence staining were performed. Seed cells from rabbit Auricular cartilage were Cultured 4weeks in vitro and 3,6 weeks in vivo. Constructs were implanted subcutaneously into the nude mice. The curcumin concentration of 30μM can be considered as appropriate since it can inhibit the growth of vascular endothelial cells without affecting the proliferation of BMSC. With the appropriate concentration, curcumin loaded scaffolds can inhibit the vascular endothelial cells without any obvious effects on BMSCs.

Keywords: cartilage regeneration; curcumin; anti-angiogenesis effect

106

ISOLATON, DIFFERENTIATION, MIGRATION AND ANGIOGENIC PROPERTIES OF RAT HEART-DERIVED PERICYTE

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Pericytes, the mural cells of micro-vessels, are the second most abundant mesenchymal precursor cells in primate hearts and are hypothesized to possess the capacity to differentiate into endothelial cells and vascular smooth muscle cells, playing an active role in angiogenesis. The present study

Poster Abstracts

aimed to develop a simpler and more efficient isolation method for primary rat heart pericytes, and to test the primary pericytes' differentiation, migration, and angiogenic properties. The isolated rat heart pericyte expressed classic pericyte markers, including CD146, NG2, PDGFRβ, but not endothelial cell (EC) markers such as CD31. To demonstrate rat cardiac pericyte differentiation into smooth muscle cells, the expression changes of smooth muscle cell markers such as αSMA and SM22α, were assessed. Primary pericyte migration ability was demonstrated using Transwell chambers. The Matrigel tube formation results indicated their angiogenic properties. Pericytes represent a promising therapeutic candidate for stem cell transplantation; however, such studies for cardiac pericytes are limited. Our data provide an economic, simple, and effective cardiac pericyte isolation method, and revealed their angiogenic capability, thus providing an efficient candidate for application in in vitro and in vivo research.

Keywords: rat heart pericyte; differentiation; smooth muscle cells

107

DIRECT ADMINISTRATION OF MESENCHYMAL STEM CELL-DERIVED MITOCHONDRIA IMPROVES CARDIAC FUNCTION AFTER INFARCTION BY PROMOTING ANGIOGENESIS

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Mitochondrial dysfunction is considered to be a key contributor to the development of heart failure. Replacing injured mitochondria with healthy mitochondria to restore mitochondrial bioenergy in myocardium holds great promise for cardioprotection after infarction. This study aimed to investigate whether direct transplantation of exogenous mitochondria derived from mesenchymal stem cells (MSC-mt) is beneficial and superior in protecting cardiac function in a mouse model of myocardial infarction (MI) compared to mitochondria derived from skin fibroblast (FB-mt), and to explore the underlying mechanisms from their effects on the endothelial cells. The isolated MSC-mt presented intact mitochondrial morphology and activity, as determined by electron microscopy and JC-1 mitochondrial membrane potential assays. Direct injection of MSC-mt into the peri-infarct region in a mouse MI model enhanced blood vessel density, inhibited cardiac remodeling and apoptosis, thus improving heart function compared with FB-mt group. The injected MSC-mt can be tracked in the endothelial cells. In vitro, the fluorescence signal of MSC-mt can be detected in human umbilical vein endothelial cells (Huvecs) by confocal microscopy after coculture. Compare to FB-mt, MSC-mt more effectively protected the Huvecs from oxidative stress-induced apoptosis and reduced mitochondrial production of reactive oxygen species. MSC-mt induced tube formation, enhanced the ATP content and cell proliferation in Huvecs. MSC-mt treatment increased angiogenic ANG-2, bFGF, HGF, and VEGF protein expression by activating the ERK pathway. MSC-mt administration alleviated oxidative stress-induced endothelial senescence by via ERK pathway. These findings suggest that using MSCs as sources of mitochondria is feasible and that proangiogenesis could be the mechanism by which MSC-mt transplantation attenuates MI. MSC-mt transplantation might serve as a new therapeutic strategy for treating MI.

Funding Source

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Keywords: mesenchymal stem cells; mitochondria transplantation; myocardial infarction

108

GENERATION OF FUNCTIONAL SALIVARY GLAND ORGANOIDS FROM ADULT HUMAN MINOR SALIVARY GLAND STEM CELLS: FINDING A WAY TO TREAT XEROSTOMIA

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Xerostomia can be caused by radiotherapy for head and neck cancers as well as Sjögren's syndrome and medications. Current treatments are limited to symptom improvements or substitute medications without real relief. Stem cell therapy brings about an attractive strategy for permanent treatment. Here, we are able to generate functional salivary gland organoids by adult stem cells isolated from human minor salivary glands. With tissue explant culture method we previously reported, we were able to isolate and maintain two distinct populations of stem cells from human minor salivary glands, human minor salivary gland stem cells (hMSGSCs) and human minor salivary gland mesenchymal stem cells (hMSGMSCs). hMSGSCs showed typical epithelial lineage characterizations with positive expression of CD49f and cell keratin proteins. hMSGSCs also served as adult stem cells in salivary glands and showed capacity differentiating into both acinar cells and duct cells. hMSGMSCs shared common mesechymal stem cell markers such as

CD29, CD73, CD90, CD105 and CD166. They were able to differentiate into mesoderm lineage cells and trans-differentiate into neural cells and hepatocytes when treated in inductive system. We labeled two type of stem cells with GFP and RFP respectively and seeded them into Matrigel for 3D culture in vitro. We observed organoids with duct-branching morphology formed in 7 days and hMSGSCs labeled with GFP generated majority part of the organoid including both duct and acinar-like parts. Interestingly, such structure did not form if only hMSGSCs were cultured in Matrigel. Moreover, organoids generated by two cell types showed expression of MUC5B and AMY1B, indicating mature salivary gland function. We then transplanted organoids generated in vitro into mice animal models with submandibular glands surgically removed. We were able to detect expression of KRT19, AQP5 and KRT14 after 2 weeks in vivo. These data suggest that together with tissue-specific mesenchymal stem cells, salivary gland stem cells would generate functional organoids in 3D culture system. Here, we introduce a new method for salivary gland organoid generation with mature structure and function from adult stem cells, which brings about great potential in cell therapy and tissue repair/regeneration.

Keywords: salivary gland organoids; xerostomia; adult human minor salivary gland stem cells

109

JUNCTIONAL PARAPHAGY SENSES LUMINAL LIPOPHILIC SIGNAL AND PROMOTES TRANSEPITHELIAL CHOLESTEROL ELIMINATION THROUGH FCGR2B AND OCCLUDIN

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Epithelial endocytosis is essential for tissue homeostasis. The current dogma is that all endocytotic mechanisms involve only single-membrane vesicles at the plasma membrane. From occludin-null mice, we uncovered an undescribed LC3-associated endocytosis mechanism that forms double-membrane phagosomes from the epithelial tight-junction paracellular membranes, which we term “paraphagy”. We observed that paraphagy is more abundant in the male reproductive system than other mucosal systems. Paraphagy endocytoses ApoJ-chaperoned lipophilic cargoes via binding to the surface low-affinity IgG-receptor Fcgr2b and involves occludin-bound intracellular HDL-receptor Atp5b in the epididymis, the organ for sperm maturation. In this way, extracellular lipophilic signals are sensed and intracellular phagolysosomes are maintained. In this regard, occludin-null mice showed arrested paraphagy and impaired phagolysosome in proximal epididymis, accompanying cholesterol accumulation, ApoJ-deposition and dysregulated physiological microenvironment throughout epithelial cavity. We propose that paraphagy senses and distinguishes the luminal lipophilic signals in epithelia and modulates their function via crosstalk across physiological compartments.

Funding Source
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Keywords: Membrane trafficking and Endocytosis; Lipoprotein sensing and transport; cholesterol transepithelial elimination

110

A MECHANISM IN WHICH POSITION INFORMATION SCALES FIN DURING REGENERATION VIA ELECTROPHYSIOLOGY

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Proportional growth control of stem and progenitor cells is a fundamental phenomenon that not only affects the development and regeneration of anatomical structures but also affects the affects the formation of cancer. We previously showed that the proportional growth of the entire anatomical structure of the zebrafish appendage is regulated by a calcineurin-K+ channel (kcnk5b) mechanism. However, it remains unclear what the signals are from the body that regulate this mechanism to scale the appendages to the appropriate size to the body. We found that a particular hormone is involved in regulating calcineurin, and that this hormone also can regulate the proportional growth of the fish fin appendage. We will show how this hormone is able to regulate calcineurin and thereby control proportional growth.

Funding Source
ShanghaiTech University

Keywords: Proportional growth; calcineurin; zebrafish appendages

Poster Abstracts

111

A CALCINEURIN-MEDIATED SCALING MECHANISM THAT CONTROLS A POTASSIUM LEAK CHANNEL TO REGULATE MORPHOGEN TRANSCRIPTION

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All animals grow organs and appendages to the exact same dimensions. The control of proportional growth is a fundamental yet poorly understood phenomenon that involves the coordinated scaling of all the stem and progenitor cells of each tissue within the organ. Previous findings show that the continued activity of the two-pore potassium-leak channel Kcnk5b maintains allometric juvenile growth of adult zebrafish appendages. However, it remains unknown how this channel maintains allometric growth and how its bioelectric activity is regulated to scale these anatomical structures. We show the activation of Kcnk5b is sufficient to activate several development programs in adult, larva and embryonic structures. The most responsive of which are Shh and Lef1. We provide in vivo transplantation evidence that the activation of these developmental programs is cell autonomous. We also show that this bioelectric signal can induce the expression of different subsets of developmental genes using different cultured mammalian cell lines, indicating that the electrophysiological changes induced by Kcnk5b are not restricted to specific developmental cascades. We also provide evidence that the post-translational modification of serine 345 in Kcnk5b by calcineurin regulates channel activity and controls the fin developmental programs to scale the entire fin anatomical structure. Thus, we show how an endogenous bioelectric program can be regulated to coordinated different developmental signals to generate and scale a vertebrate appendage.

Funding Source
Deutscheforschungsgemeinschaft (DFG) ShanghaiTech University
Keywords: Electrophysiology; Appendage regeneration; Proportional Growth

112

NOTCH SIGNALING PATHWAY IN TRIPLE NEGATIVE BREAST CANCER WITH HIGH AND LOW CONTENT OF CANCER STEM CELLS

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Triple negative breast cancer is at the greatest interest for the study. Cells of this type of breast cancer do not have receptors for steroid hormones or tyrosine kinases. For successful treatment of triple negative cases additional points of application are needed. Such targets could be cancer stem cells. For these types of cells, as well as for other stem cells, there are three main cascades are typical – NOTCH. However, the conducted studies indicate the existence of other signaling mechanisms of regulation. In our work, we investigated the expression of the NF-kB, PI3K signaling pathways, as well as NOTCH in cells of triple negative breast cancer with high and low content of cancer stem cells (ALDH1A1 level). The material of 54 cases of infiltrative breast cancer was used. To determine the presence of stem cells in tumor population, the presence of ALDH1A1 protein in cancer cells was investigated. Expression of steroid hormone receptors, HER-2 and Ki-67 was studied by immunohistochemical method to identify cases of triple negative breast cancer. The expression of signaling molecules PI3K, NF-kB, Notch was also explored by immunohistochemical method. All cases were investigated for ALDH1A1 expression and were divided into two groups – with low (expression of ALDH1A1 was estimated as 0 and 1+) and high (expression of ALDH1A1 was estimated as 2+ and 3+) content of cancer stem cells. It was found that 20% of cases of triple negative breast cancer are positive for ALDH1A1. Expression of explored signaling molecules in cases with high and low content of cancer stem cells is shown in the table. It was found that cases with high content of cancer stem cells are less regulated by signaling pathways NOTCH. Expression of NF-Kb and PI3K signaling molecules (which are responsible for activation of eponymous signaling pathways) appeared in all studied cases, while expression of PTEN phosphatase, which inhibits PI3K signaling pathway, was more common for cases with high content of cancer stem cells.

Keywords: Triple negative breast cancer; NOTCH; NF-kB

113

RETINOIC ACID PARTICIPATES IN CALCINEURIN MEDIATED SCALING MECHANISM

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Vertebrate organs and appendages grow to specific proportions with the body. However, it remains unknown how signals from the body (systemic) and local signals are integrated to scale entire organs and appendages. A previous discovery shows that the increase in activity of the potassium channel kcnk5b leads to zebrafish fins disproportional growth, the fins grow at higher rate (allometrically) than the body. The Antos lab found that inhibition of calcineurin can activate kcnk5b and promote allometric growth of the fins. Because calcineurin is a phosphatase that post-translationally regulates the kcnk5b I want to know what regulates calcineurin to regulate proportional growth. I found that morphogen retinoic acid induces disproportional (allometric) growth of the fins. I also observed that retinoic acid treatment of primordial zebrafish fins can activate potassium channel. Calcineurin phosphatase activity is inhibited by an endogenous small protein regulatory of calcineurin2 (RCAN2). I also found that rcan2 mRNA is up-regulated during the early stages of allometric growth of the regenerating fin when calcineurin activity is low. This result indicates that a transcription mechanism is an important regulator of calcineurin-mediated tissue scaling. I also found that treating fish with retinoic acid promotes the transcription of rcan2, which occurs during the allometric growth phase of fin regeneration, which suggests retinoic acid activates Kcnk5b to scale zebrafish appendages.

Funding Source
ShanghaiTech University
Keywords: Proportional growth; retinoic acid; zebrafish appendage

114

HUMAN IPSCS-DERIVED LIVER BUDS AS A NOVEL PLATFORM FOR ASSESSMENT OF ANTIVIRAL ACTIVITY OF PHARMACEUTICALS AGAINST HCV INFECTION

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Molecular virology and anti-viral treatment for HCV infection is impeded by the lack of efficient and reliable in vitro and in vivo infection models. In order to establish a novel model for HCV infection, the present study aims to generate human induced pluripotent stem cells (iPSC)-derived liver buds or organoids as a platform to assess antiviral activity of anti-HCV pharmaceutical candidates. Liver buds were generated from human iPSC-derived hepatic progenitor cells with HUVEC and mesenchymal stem cells (MSC) following a standard protocol. Expression of cellular receptors in liver buds essential for HCV infection such as occludin was verified by quantitative RT-PCR. Liver buds were infected with HCV vectors (HCVser derived from isolates of HCV-positive patients (genotype 2). Luciferase reporter in the HCV vector was used as an indication of viral infectivity. HCV infectivity in the liver buds was similar to control Huh-7.5 cells, but was sustained at 1.7-fold higher in liver buds than Huh-7.5 cells 7 days after the infection. After treatment with an anti-HCV agent, 2'-C-methyl-adenosine (2CMA) at 0.25 μM for 7 days, HCV copies were decreased significantly in both liver buds and Huh-7.5 cells in a similar trend compared to controls treated with solvent DMSO ($p<0.05$). Liver buds could be generated from human iPSC-derived hepatic progenitor cells with HUVEC and MSC cells, have a sustained HCV infectivity, and are a valid platform for in vitro assessment of anti-HCV efficacy for pharmaceutical candidates. The present study represents a preliminary application of regenerative medicine in the improvement of in vitro HCV infectivity and sustainability for anti-viral assessment.

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Keywords: Human iPSC; Liver bud; HCV infection

Poster Abstracts

115

GENERATION OF NON-IMMUNOGENIC HUMAN EMBRYONIC STEM CELLS (NI-HESCS) FOR XENOGENIC TRANSPLANTATION STUDIES

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Establishment of a universal non-immunogenic human embryonic stem cell (ni-hESC) line holds immense promise for both cell and tissue transplantation-based treatments, and would not be restrictive to individual patients. This can only be achieved if such a ni-hESC line is able to circumvent the host immune response to avoid rejection. This study aims to generate a ni-hESC line that can surpass xenogenic graft rejection and hereby be superior for transplantation purposes for in vivo studies in mice and pig experimental models. To achieve this, human embryonic stem cells (hESC) were CRISPR-Cas9 engineered to obtain cells with knockouts (KO) of human leukocyte antigens (HLA) combined with insertion of the gene encoding murine or porcine CD47, as these species will be used for later in vivo testing. To trace engrafted cells in vivo, a human secreted embryonic alkaline phosphatase gene (hSEAP) was inserted in a safe locus together with the herpes simplex virus thymidine kinase (HSV-TK) suicide gene, to enable elimination of implanted cells. CRISPR-Cas9 engineered cells showed high cutting efficiency for all tested loci (>40%), and PCR confirmed correct insertion of CD47, hSEAP and HSV-TK. A high and stable hSEAP expression and efficient cell elimination by Gangciclovir treatment, was confirmed in vitro. Thus, the CRISPR modifications in hESC lines were successful, establishing knock-out of HLA's and enabling tracing and annihilation of cells in vitro. Next steps include confirmation of a stable expression of CD47 upon hESC differentiation, followed by assessment of the immunogenic response in vivo in mice and pigs. Should implantation prove successful, this will help pave the road towards clinical testing.

Funding Source
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Keywords: Non-immunogenic; Human embryonic stem cells; Gene editing

116

NOVEL SUSPENSION CULTURE MATERIAL CELLHESION® FOR HUMAN MESENCHYMAL STEM CELLS

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Cell therapy using human mesenchymal stem cells (hMSCs) is a breakthrough strategy for the treatment of intractable diseases. Large-scale production of hMSCs is required for further clinical applications. The conventional methods using adherent 2D culture have some limitations, such as restricted scalability, complicated processes for cell seeding and detachment, and difficult for automation. In addition, hMSCs have problems such as loss of proliferative and medicinal potential caused from passaging. 3D suspension culture is attracting considerable attention in order to overcome the problems faced in conventional 2D cell culture. We screened scaffold from natural polysaccharide fibers and acquired Cellhesion®. Cellhesion® can be applied in 3D suspension culture of hMSCs in static condition. Interestingly, cells cultured with Cellhesion® upregulated the expression levels of stemness and migration related genes compared with 2D cells. Also, these cells secreted paracrine factors related to anti-inflammation and angiogenesis more than 2D cells. In addition, these cells secreted much more extracellular vesicles than 2D cells and spheroid cells. In this presentation, we report about 3D cultured MSCs using Cellhesion® focused on cell characteristics.

Keywords: 3D culture; paracrine factor; extracellular vesicles

117

SUSPENSION CULTURE OF ORGANOIDS AND NON-CRYOPRESERVATION OF SPHEROIDS USING FCEM® THREE-DIMENSIONAL CULTURE METHOD

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In recent years, three-dimensional culture are being developed rapidly, especially spheroids and organoids which are expected to be applicable for cell therapy. Mass production and preservation of cell clumps, however, remain problematic. Some unique vessels are useful for culturing large amount of simple spheroids while organoids with complex in vivo-like structures are generally prepared by embedding in Matrigel® which is difficult for large quantity. In addition, the technology for cryopreservation of cell clumps such as spheroids has not yet been established, which limits cell spheroids transplantation to be administrated within the vicinity of CPC facilities. We have developed a polymer FP003 (FCeM® Advance), which enables suspension culture without shaking or agitation after adding to common culture medium. In this study, we investigated organoids culture and non-cryopreservation of spheroids using FP003-containing medium. When crypts derived from mouse small intestine were cultured in FP003-containing medium, organoids were formed in suspension culture without gel embedding, suggesting it is promising for mass production. In addition, spheroids of mesenchymal stem cells and fibroblasts suspended in FP003-containing medium were stored statically in sealed conical tubes under room temperature and maintained high viability even after one week. It indicates the possibility to apply FP003 for non-crypreservation of spheroids for long distance spheroids transportation and therapy. In conclusion, FP003 is expected to be useful material for the development of cell production and preservation technology for the realization of cell therapy using spheroids and organoids.

Keywords: spheroids; suspension culture; non-crypreservation

Author Index