INTERNATIONAL 2017 SYMPOSIA

GUANGZHOU CHINA 2017

10-12 NOVEMBER

STEM CELLS:
THE NEXT GENERATION

PROGRAM GUIDE

ISSCR
INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

GUANGZHOU INSTITUTES OF BIOMEDICINE AND HEALTH CHINESE ACADEMY OF SCIENCES
中国科学院广州生物医药与健康研究院
GIBH

WELCOME

ISSCR INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

Dear Colleagues:

We would like to welcome you on behalf of the International Society for Stem Cell Research (ISSCR) and the Guangzhou Institutes for Biomedicine and Health (GIBH). The ISSCR and GIBH are proud to partner together to bring you Stem Cells: The Next Generation, a joint conference of the ISSCR International Symposia 2017.

Guangzhou, the "South Gate of China," is an international center for science, technology and education. It's a fitting home for GIBH, which was jointly established by Chinese Academy of Sciences, the Guangdong Province People's Government, and the Guangzhou Municipal People's Government, to focus on the study of stem cell and regenerative medicine, chemical biology, infection, and immunity.

For this ISSCR International Symposium, we've created a forward-looking, engaging program that will explore the latest work in the stem cell field, the cutting-edge technology helping to drive research, and thoughtful perspectives from global leaders on where stem cell research is heading.

As always, we thank you for joining us. We hope that you not only gain knowledge and insight from our speakers' presentations, but also make connections with your fellow delegates that lead to future discovery and collaboration. The ISSCR International Symposia are designed to be smaller, intimate settings to facilitate interactions with speakers and attendees, so we encourage you to engage in conversation during breaks, lunches, and poster sessions.

Our sponsors and exhibitors help make this meeting possible. Please thank them for their support by exploring the newest tools and technologies on exhibit during the meeting and recognizing the supporters listed in our acknowledgements.

On behalf of all the organizers and people who made this event possible, we hope you are invigorated by the scientific sessions and the personal connections you cultivate.

Sincerely,

Duanqing Pei
Director, Guangzhou Institutes of Biomedicine and Health, CAS, China

And Members of the Organizing Committee

Hongkui Deng
Peking University, China

Andrew Elefanty
Murdoch Childrens Research Institute, Australia

Gordon Keller
McEwen Centre for Regenerative Medicine
Ontario Cancer Institute, Canada

Duanqing Pei
Guangzhou Institutes of Biomedicine and Health, CAS, China

Kathrin Plath
UCLA Medical School, U.S.

Qi Zhou
Zoology Institute, CAS, China

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ABOUT THE ISSCR

Mission Statement

The International Society for Stem Cell Research (ISSCR) is an independent, nonprofit organization established to promote and foster the exchange and dissemination of information and ideas relating to stem cells, to encourage the general field of research involving stem cells and to promote professional and public education in all areas of stem cell research and application.

History and Philosophy

With more than 4,100 members from over 60 countries, the ISSCR is the preeminent transnational, cross-disciplinary science-based organization dedicated to stem cell research.

Formed in 2002, the Society promotes global collaboration among talented and committed stem cell scientists and physicians, and plays a catalyzing role in the development of effective new medical treatments.

The Society brings together investigators who are engaged in both fundamental and applied research. Their investigations include the use of pluripotent stem cells and stem cells within adult organs and tissues to create applications in specific therapeutic settings.

The ISSCR represents academia and industry on a broad range of issues that affect the well-being of patients and their families, and strives to educate the public and government regulators on the basic principles of stem cell science and the realistic potential for new medical treatments and cures.

The leadership of the ISSCR is acutely aware of the responsibility the Society bears to promote the highest scientific and ethical standards, and is dedicated to integrity in the rigor and quality of the research community's scientific work, the public policy stands it takes on stem cell related issues, and the organization's relations with its key constituents and the public. Only such an abiding commitment to integrity can ensure that as the ISSCR grows, it will continue to serve a fair and trusted advocate by both its internal and external stakeholders.

Contact Us

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www.isscr.org

GENERAL INFORMATION

VENUE

The Stem Cells: The Next Generation International Symposium is located at the DoubleTree Guangzhou—Science City. All program sessions and activities are on floor 1F.

- Program sessions take place in the Grand Ballroom.
- The Exhibit Hall, Registration, and the cloak room are in the ballroom lobby.
- Posters will be in the Hong Kong and Shang Hai Rooms.
- The buffet lunch will be in the Elements Room.

REGISTRATION AND BADGE PICKUP

Attendees and exhibitors must register and receive their name badge at the DoubleTree Guangzhou—Science City ballroom lobby during posted hours at the registration desk.

Name badges are required for admission to all sessions, poster presentations, meals, and the Exhibit Hall.

Since the meeting badge serves as proof of participation, all attendees, speakers and exhibitors are required to wear their badges at all times during the International Symposium. Access to events may be refused if the meeting badge is not displayed.

Registration Desk and Badge Pickup Hours

FRIDAY, 10 NOVEMBER	**10:00 – 18:30**
SATURDAY, 11 NOVEMBER	**8:00 – 18:30**
SUNDAY, 12 NOVEMBER	**8:00 –16:00**

INTERNET ACCESS

Complimentary access to the internet is available within the DoubleTree Guangzhou—Science City. The network name is HHonors and the password is 888888.

As a courtesy to speakers, please be sure to silence any mobile phones and devices and refrain from using the internet during sessions. Please note that the bandwidth of this connection might be limiting.

RECORDING POLICY

Still photography, video and/or audio taping of the sessions, presentations and posters at the International Symposium is strictly prohibited. Intent to communicate or disseminate results or discussion presented at the meeting is prohibited until the start of each individual presentation. Thank you for your cooperation.

EXHIBIT HALL

The Exhibit Hall features leading suppliers and vendors whose support helped help make this International Symposium possible. Please visit their stands in the ballroom lobby.

Exhibit Hall Hours

FRIDAY, 10 NOVEMBER	**12:30 – 17:00**
SATURDAY, 11 NOVEMBER	**8:30 – 17:30**
SUNDAY, 12 NOVEMBER	**8:30 –16:00**

POSTER PRESENTATIONS

Poster presentation will take place in the Hong Kong and Shanghai Rooms on Saturday 11 November and Sunday 12 November from 13:30 – 14:30. Poster presenters will be at their posters during these times to answer your questions about their research. Abstracts can be found beginning on page 19 of this guide.

POSTER SET UP AND TAKE DOWN:

Posters must be set up by 17:00 on Friday, 10 November. Please do not remove your poster prior to Sunday, 12 November at 14:30. If you wish to keep your poster, you must take it down prior to the end of the final session on Sunday, 12 November, at 16:00.

ORGANIZERS & REVIEWERS

ORGANIZING COMMITTEE

Hongkui Deng, PhD
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ABSTRACT REVIEWERS

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RIKEN Center for Developmental Biology, Japan

Wang Tao, PhD
Guangzhou Institutes of Biomedicine and Health, CAS, China

Hui Zheng, PhD
Guangzhou Institutes of Biomedicine and Health, China

Bin Zhou, PhD
Shanghai Institutes for Biological Sciences, CAS, China

PROGRAM SCHEDULE

FRIDAY, 10 NOVEMBER

13:00 - 14:00 WELCOME AND KEYNOTE

13:00 - 13:15 **Welcome**

Duanqing Pei, *Guangzhou Institutes of Biomedicine and Health, CAS, China*

Nancy Witty, *CEO, International Society for Stem Cell Research*

13:15 - 14:00 **Keynote Address:**

Rudolf Jaenisch, *Whitehead Institute for Biomedical Research, U.S.*

EPIGENETICS, STEM CELLS AND DISEASE RESEARCH

14:00 - 15:15 STEM CELL STATE AND CELL DIFFERENTIATION

Chair: **Qi Zhou**, Institute of Zoology, CAS, China

14:00 - 14:30 **Nissim Benvenisty**, *Hebrew University, Israel*

HUMAN HAPLOID PLURIPOTENT STEM CELLS: DERIVATION AND APPLICATION

14:30 - 14:45 **Charles Hernandez**, *Yale University, U.S.*

CHROMATIN-ASSOCIATED FACTORS OF THE DPPA2/4 FAMILY ARE THE KEY REGULATORS OF CELLULAR REPROGRAMMING

14:45 - 15:15 **Jose Polo**, *Monash University, Australia*

TRANSIENT AND PERMANENT RECONFIGURATION OF CHROMATIN AND TRANSCRIPTION FACTOR OCCUPANCY DRIVE REPROGRAMMING

PROGRAM SCHEDULE

15:15 - 15:45 BREAK

15:45 - 17:00 STEM CELL STATE AND CELL DIFFERENTIATION

Chair: **Qi Zhou**, Institute of Zoology, CAS, China

15:45 - 16:15 **Stephanie Protze**, *McEwen Centre for Regenerative Medicine, Canada*
TRANSLATING HUMAN HEART DEVELOPMENT TO NOVEL BIOLOGICAL PACEMAKER THERAPIES

16:15 - 16:30 **Ying Jin**, *Shangai Institutes of Biological Sciences, CAS, China*
SOX21 PLAYS A CRITICAL ROLE FOR TELENCEPHALON SPECIFICATION DURING NEURAL DIFFERENTIATION OF HESCS

16:30 - 17:00 **Xiaoyang Zhao**, *Southern Medical University, China*
MAMMALIAN GERM CELL REGENERATION

PROGRAM SCHEDULE

SATURDAY, 11 NOVEMBER

9:00 - 10:30 ORGANOIDS AND DISEASE MODELING – SESSION I

Chair: **Zheng Qin Yin**, *Third Military Medical University, China*

9:00 - 9:30 **Minoru Takasato,** *RIKEN Center for Developmental Biology, Kobe, Japan*
REGULATING THE PATTERNING OF MESODERM AND GENERATING KIDNEY ORGANOIDS BY THE DIRECTED DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

9:30 - 9:45 **Zi-Bing Jin**, *Wenzhou Medical University, China*
DISEASED PHOTORECEPTORS INTERROGATED IN RETINITIS PIGMENTOSA PATIENT IPSC-DERIVED 3D RETINAE

9:45 - 10:15 **Bennett Novitch**, *University of California, Los Angeles, U.S.*
ORGANOID MODELING OF HUMAN BRAIN DEVELOPMENT & CONGENITAL ZIKA VIRUS SYNDROME

10:15 - 10:45 BREAK

10:45 - 12:00 PANEL DISCUSSION: ETHICS IN GENOME ENGINEERING

Moderator: **Jeremy Sugarman**, *Johns Hopkins University, U.S.*
Panelists: **Nissim Benvenisty**, *Hebrew University, Israel*
Rudolf Jaenisch, *Whitehead Institute for Biomedical Research, U.S.*
Duanqing Pei, *Guangzhou Institute for Biomedicine and Health, China*
Douglas Sipp, *RIKEN, Japan*

10:45 - 11:00 **Panel Introductions**

11:00 - 11:15 **Jeremy Sugarman**, *Johns Hopkins University, U.S.*
ETHICS, POLICIES, AND GENE EDITING

11:15 - 12:00 **Moderated Discussion**

12:00 - 14:30 LUNCH AND POSTER VIEWING

PROGRAM SCHEDULE

13:30 - 14:30 POSTER SESSION (POSTER PRESENTERS AT THEIR POSTERS)

14:30 - 15:45 NEW TOOLS FOR STEM CELL RESEARCH

Chair: **Nissim Benvenisty**, *Hebrew University, Israel*

14:30 - 15:00 **Fuchou Tang**, *Peking University, China*
SINGLE-CELL RNA-SEQ ANALYSIS MAPS DEVELOPMENT OF HUMAN GERMLINE CELLS AND GONADAL NICHE INTERACTIONS

15:00 - 15:15 **Jian Shu**, *Broad Institute of MIT and Harvard, U.S.*
A CONTINUOUS MOLECULAR ATLAS OF REPROGRAMMING TO IPSCS BY HIGH-THROUGHPUT SINGLE CELL RNA-SEQ

15:15 - 15:45 **Pentao Liu**, *Wellcome Trust Sanger Institute, U.K.*
ESTABLISHMENT OF CULTURES OF MAMMALIAN EXPANDED POTENTIAL STEM CELLS

15:45 - 16:15 BREAK

16:15 - 17:00 NEW TOOLS FOR STEM CELL RESEARCH

Chair: **Nissim Benvenisty**, *Hebrew University, Israel*

16:15 - 16:30 **Xiaodong Liu**, *Monash University, Australia*
CHARACTERISATION OF DISTINCT STATES OF HUMAN NAIVE PLURIPOTENCY GENERATED BY REPROGRAMMING IDENTIFIES KLF4 AS A CONDUIT FOR PRIMED TO NAIVE CONVERSION

16:30 - 17:00 **Bin Zhou**, *Institute for Nutritional Sciences, SIBS, CAS, China*
GENERATION OF A DUAL RECOMBINASE SYSTEM FOR TRACING ENDOGENOUS CARDIAC STEM CELLS

PROGRAM SCHEDULE

SUNDAY, 12 NOVEMBER

9:15 - 10:30 ROAD TO THE CLINIC – SESSION I

Chair: **Rudolf Jaenisch**, Whitehead Institute for Biomedical Research, U.S.

9:15 - 9:45 **Michael Laflamme**, *University Health Network, Canada*
HEART REGENERATION WITH HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

9:45 - 10:00 **Jianhong Zhu**, *Fudan University Huashan Hospital, China*
CLINICAL AND PRECLINICAL IMAGING REVEALS THE DYNAMIC BEHAVIOR OF THE EXOGENOUS STEM CELLS TRANSPLANTED AND ENDOGENOUS STEM CELLS IN THE NERVOUS SYSTEM

10:00 - 10:30 **Jinghua Piao**, *Memorial Sloan Kettering Cancer Center, U.S.*
THE SELECTIVE DERIVATION OF SOMATOTROPHS FROM HUMAN EMBRYONIC STEM CELLS

10:30 - 11:00 BREAK

11:00 - 12:00 ORGANOIDS AND DISEASE MODELING – SESSION 2

Chair: **Zheng Qin Yin**, Third Military Medical University, China

11:00 - 11:15 **Carl Ernst**, *McGill University, Canada*
DISRUPTION OF GRIN2B IMPAIRS NMDA-INDUCED CELL DIFFERENTIATION IN HUMAN NEURONS

11:15 - 11:30 **Ling Li**, *CHA University, South Korea*
MODELING OF ALZHEIMER'S DISEASE USING IPSC TECHNOLOGY

11:30 - 12:00 **Wei Li**, *Institute of Zoology, CAS , China*
NOVEL STEM CELL TYPES FOR VERSATILE BIOLOGICAL APPLICATIONS

PROGRAM SCHEDULE

12:00 - 14:30 LUNCH AND POSTER VIEWING

13:30 - 14:30 POSTER SESSION (POSTER PRESENTERS AT THEIR POSTERS)

14:30 - 15:45 ROAD TO THE CLINIC – SESSION II

Chair: **Rudolf Jaenisch**, Whitehead Institute for Biomedical Research, U.S.

14:30 - 15:00 **Baoyang Hu**, *Institute of Zoology, CAS, China*
CLINICAL GRADE hESCS AND DERIVATIVES FOR THERAPY

15:00 - 15:15 **Douglas Sipp**, *RIKEN Center for Developmental Biology, Japan*
THE STEM CELL DARK ECONOMY: IMPLICATIONS FOR THE FIELD

15:15 - 15:45 **Zheng Qin Yin**, *Third Military Medical University, China*
SUBRETINAL TRANSPLANTATION OF HESC-RPE: CLINCAL TRIAL IN THE TREATMENT OF WET AGE-RELATED MACULAR DEGENERATION (WAMD) – ONE YEAR FOLLOW-UP

15:45 - 16:00 CLOSING REMARKS

Duanqing Pei, *Guangzhou Institute for Biomedicine and Health, CAS, China*

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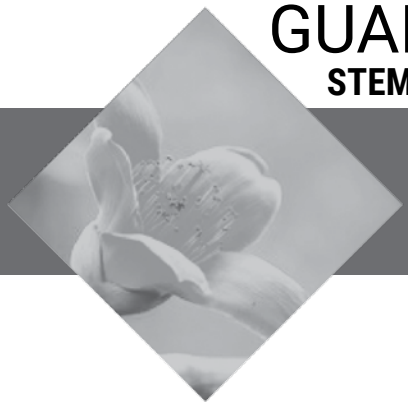

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Contact: Zheng Guipeng
Telephone: 136-000-77740
Website: www.saliai.com

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For more details, please contact: zhangjianquan@cib.cc or service@cib.cc

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SPEAKER ABSTRACTS

FRIDAY, 10 NOVEMBER

KEYNOTE ADDRESS

EPIGENETICS, STEM CELLS AND DISEASE RESEARCH

Rudolf Jaenisch

Whitehead Institute for Biomedical Research, U.S.

The development of the iPS cell technology has revolutionized our ability to study development and diseases in defined in vitro cell culture systems. The talk will focus on the use of gene editing for the study of epigenetic regulation in development and disease.

1. Monitoring the dynamics of DNA methylation at single cell resolution during development and disease: DNA methylation is a broadly studied epigenetic modification that is essential for normal mammalian development. Current methods to quantify methylation provide only a static "snap shot" of DNA methylation, thus precluding the study of real-time methylation dynamics during cell fate changes. We have established a new approach that enables monitoring loci-specific DNA methylation dynamics at single-cell resolution.

2. Editing DNA methylation in the mammalian genome: The functional significance of specific methylation events in development and disease remains elusive due to lack of experimental approaches to edit these events. We developed a DNA methylation editing toolbox that fusion of either the catalytic domain of Tet1 or Dnmt3a protein to a catalytic inactive Cas9 (dCas9) to achieve targeted DNA methylation editing with co-expression of target-specific guide RNAs. (i) We first validated this tool by turning on or off two endogenous methylation reporters. (ii) With application of dCas9-Tet1, we observed that active de-methylation can be induced in BDNF promoter IV to activate its expression in mouse cortical neurons, and showed (iii) that de-methylation of MyoD distal enhancer facilitates reprogramming fibroblasts into myoblasts and subsequent myotube formation. (iv) We show that dCas9-Dnmt3a can open CTCF-mediated chromatin loops by targeted methylation of specified CTCF anchor site thus blocking its binding. Our results established that a modified CRISPR system with dCas9 fused by DNA modification enzymes can be assembled into DNA methylation editing tools to study the functional significance of specific methylation event in the mammalian genome. (v) Finally, we show that these tools can be used to reactivate the FMR1 gene that is silenced by DNA methylation in patients with Fragile X Syndrome.

STEM CELL STATE AND CELL DIFFERENTIATION

HUMAN HAPLOID PLURIPOTENT STEM CELLS: DERIVATION AND APPLICATION

Nissim Benvenisty

The Azrieli Center for Stem Cells and Genetic Research, The Hebrew University, Israel

Diploidy is a fundamental genetic feature in mammals, in which haploid cells normally arise only as post-meiotic germ cells that serve to insure a diploid genome upon fertilization. However, haploid cells provide valuable tools for delineating genome function through loss-of-function genetic screening. We have recently generated haploid human embryonic stem (ES) cells from unfertilized human oocytes. The haploid human ES cells exhibited typical pluripotent stem cell characteristics, such as self-renewal capacity and a pluripotency-specific molecular signature. Although haploid human pluripotent cells resembled their diploid counterparts by several aspects, they also displayed distinct properties including differential regulation of X chromosome inactivation and genes involved in oxidative phosphorylation, alongside reduction in absolute gene expression levels and cell size. 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 both in vitro and in vivo. Furthermore, we demonstrated the utility of haploid human ES cells for loss-of-function genetic screening by analyzing a haploid gene-trap mutant library for genes conferring resistance to the purine analog 6-thioguanine. In order to define the essentialome of human pluripotent stem cells we have generated a genome-wide loss-of-function library in haploid human ES cells utilizing the CRISPR/Cas9 technology using about 180,000 guide RNAs, targeting more than 18,000 coding genes, with 10 guide RNA constructs for each gene. Using this library we have characterized the essential gene in human pluripotent stem cells, showed the relative role of each of the cellular compartments in promoting or restricting cell growth, and categorized human genetic disorders according to their role in early embryogenesis. Thus, haploid human ES cells hold a great potential for biomedically-relevant functional genomics to unravel genotype-phenotype interactions in the context of human development and disease.

SPEAKER ABSTRACTS

CHROMATIN-ASSOCIATED FACTORS OF THE DPPA2/4 FAMILY ARE THE KEY REGULATORS OF CELLULAR REPROGRAMMING

Charles Hernandez, Zheng Wang, Yu-Wei Lee, Cheryl Dambrot, Francesco Strino, Yuval Kluger, and Natalia Ivanova

Yale University, U.S.

Following seminal discovery by Takahashi and Yamanaka, pluripotency can now be induced in somatic cells by expression of OSKM factors. While the technology is being increasingly utilized to produce iPSCs, its mechanistic understanding remains incomplete. In particular, the process of epigenetic remodeling, shown to be a major rate-limiting step of the reprogramming process, remains poorly characterized. Recent work in the field identified a set of predictive markers (Utf1, Esrrb, Lin28 and Dppa2) whose expression labels the rare cells which have a higher probability of becoming iPSCs. We show that Dppa2 and its close homolog Dppa4 are the essential components of the chromatin remodeling network and govern the transition to pluripotency. Dppa2/4 are induced in pre-iPSCs and are required for generation of iPSCs. Ectopic expression of single Dppa2/4 transgene in OSKM-MEFs significantly increases the number of iPSC colonies compared to OSKM alone. Expression of both Dppa2 and Dppa4 (Dppa2/4-OE) results in additional and drastic increase in colony numbers. In a single-cell assay, more that 75% of Dppa2/4-OE MEFs give rise to Nanog+ iPSCs compared to less than 2% in the OSKM cultures. The emergence of reprogramming intermediates is also greatly accelerated, resulting in formation of fully-reprogrammed iPSCs in 2-4 days. Dppa2/4-OE iPSCs exhibit correct pattern of pluripotency marker expression and robustly generate germline-competent chimeric mice by blastocyst injection and tetraploid complementation. Importantly, overexpression of DPPA2/4 drastically improves both the efficiency and the kinetics of human iPSC generation. Mechanistically, Dppa2 and Dppa4 function as a dimer that recognizes a conserved DNA motif at the promoters and intergenic regions located within large H3K9me3 heterochromatin domains. Biochemical and bioinformatics analyses revealed that Dppa2/4 binding leads to the recruitment of several chromatin remodeling complexes, gain of H3K4me3 marks, and erasure of H3K9me3 marks, increase in DNA accessibility and rapid activation of endogenous pluripotency network. Our findings reveal a novel mechanism responsible for generation of accessible chromatin during reprogramming and provides a useful strategy to enhance iPSC generation for clinical and research use.

TRANSIENT AND PERMANENT RECONFIGURATION OF CHROMATIN AND TRANSCRIPTION FACTOR OCCUPANCY DRIVE REPROGRAMMING

Jose Polo

Monash University, Australia

Somatic cell reprogramming into pluripotent stem cells (iPSC) through the forced expression of defined factors induces changes in genome architecture reflective of the embryonic stem cell state. However, only a small minority of cells typically transition to pluripotency, which has limited our understanding of what defines cells that successfully reprogram. Here, we characterize the changes that occur across the DNA regulatory landscape during reprogramming by time-course profiling of isolated sub-populations of reprogramming intermediates poised to become iPSC. Widespread reconfiguration of chromatin states and transcription factor occupancy occurs early during reprogramming, and cells that fail to reprogram partially retain regulatory elements active in their somatic cell state regardless of their transcriptional output being similar to cells that undergo reprogramming. Of note, we uncovered a transient binding of Oct4 and Sox2 with concomitant genome wide chromatin accessibility changes which leads to a progressive increase of chromatin accessibility during reprogramming. A second wave of reconfiguration occurs just prior to cells achieving pluripotency, where many of the changes that define the pluripotent state become established. Our comprehensive characterization of the molecular changes that occur during reprogramming broaden our understanding of the reprogramming process by providing crucial insights into iPSC generation, and shed light on how transcription factors in general access and change the chromatin during cell fate transitions

TRANSLATING HUMAN HEART DEVELOPMENT TO NOVEL BIOLOGICAL PACEMAKER THERAPIES

Stephanie Protze[1], Udi Nussinovitch[2], Jie Liu[3], Lily Ohanna[3], Peter Backx[3], Lior Gepstein[2], and Gordon Keller[1]

[1]McEwen Centre for Regenerative Medicine, Canada, [2]Technion-Israel Institute of Technology, Israel, [3]Department of Biology, York University, Canada

The human heart rate is established by the sinoatrial node (SAN) that functions as a primary pacemaker throughout life. Dysfunction of the SAN results in bradyarrhythmias, and is routinely treated by implantation of an electronic pacemaker that has disadvantages including lack of autonomic

SPEAKER ABSTRACTS

responsiveness and limited adaption to growth in pediatric patients. Given these limitations, biological pacemakers derived from human pluripotent stem cells (hPSCs) represent a promising alternative to the electronic devices. To generate SAN-like pacemaker cells (SANLPCs) from hPSCs we have used a developmentally guided differentiation strategy that involves activation of BMP and RA signaling in combination with inhibition of FGF signaling at the cardiac mesoderm stage. Enriched populations of SANLPCs (>80%), isolated as SIRPA+CD90- cardiomyocytes expressed significant higher levels of SAN markers including TBX3, TBX18, SHOX2, HCN4 and lower levels of the ventricular markers MLC2V and IRX4 compared to hPSCs-derived ventricular cardiomyocytes (VLCMs). Furthermore, SANLPCs displayed typical pacemaker action potentials, ion-currents and responses to autonomic signals. To evaluate the potential of SANLPCs for biological pacemaker applications we tested their ability to pace cardiac-tissue both in-vitro and in-vivo. For the in vitro analyses, we showed that aggregates of SANLPCs can act as pacemaker for monolayers of hPSC-derived VLCMs. Our in vivo studies demonstrated that SANLPCs transplanted into the apex of the rat heart could pace the ventricle following induction of transient atrioventricular block. In contrast, transplanted control VLCMs displayed no pacemaker capacity. We are currently extending these in vivo experiments to a pre-clinical large animal pig model to further evaluate the long-term reliability and safety of a SANLPC biological pacemaker.

SOX21 PLAYS A CRITICAL ROLE FOR TELENCEPHALON SPECIFICATION DURING NEURAL DIFFERENTIATION OF HESCS

Ying Jin and Zhuoqing Fang

Shanghai Institutes of Biological Sciences, CAS, China

During central nervous system development, neural progenitors acquire distinct identities according to their characteristic spatiotemporal order and controlled by the combinatorial actions of signaling gradients. The molecular circuitry underlying these important processes is still poorly understood. Here, through time-series analysis for transcriptomes of early neural differentiation from human embryonic stem cells (hESCs), we report the identification of transcription factor SOX21 as an inhibitor of Wnt signaling to promote the telencephalon specification. Our experimental data indicate that the expression of SOX21 is repressed by OCT4, being silenced in undifferentiated hESCs, but upregulated transiently at the early stag of neural differentiation. Overexpression of SOX21 in hESCs disrupts self-renewal and leads to neural differentiation. Deletion of SOX21 using the CRISPR/Cas9 approach does not affect hESC self-renewal, whereas it activates WNT signaling and represses the expression of forebrain markers (FOXG1, SIX3, WNT8B), leading to the failure of telencephalon specification during hESC neural differentiation. Mechanistically, SOX21 binds to the regulatory region of important Wnt pathway components, such as WNT8B, WLS, to enhance the activity of the canonical Wnt-β-catenin signaling pathway. Collectively, this study reports highly dynamic transcriptomes of hESC neural differentiation and uncovers the early activation and critical roles of transcription factor SOX21 during human neuroectoderm development and for promoting the irreversible fate specification of human pluripotent cells toward the telencephalon fate.

MAMMALIAN GERM CELL REGENERATION

Xiaoyang Zhao

Southern Medical University, China

Around 15% of couples suffer from infertility. Many cases could be treated with assisted reproductive technology, while some cases failed to get babies due to poor quality gametes. Our previous work indicates that infertility which due to genetic mutations can be treated by genomic editing and stem cell technology in mice. In addition, we reported that pluripotent stem cells could differentiate into spermatids like cells via meiosis in vitro. Recently, we established a 3D methylcellulose (MC) induction system which could generate hPGCLCs more efficiently and economically, and more importantly, in large scale. hPGCLCs from MC group retained similar gene expression profiles, germ cell specific markers, epigenetic properties and cellular states with these characteristics of hPGCLCs from U96 group. Furthermore, hPGCLCs also could be generated from iPSCs which induced from testes cells of an azoospermia patient with deletion of AZFc fragments in equivalent efficiency of hESCs. The results suggested the deletion of AZFc fragments, one common problem for azoospermia, have no effect on induction of human primordial germ cell fate. Thus, we provide a modified system for obtaining hPGCLCs from pluripotent stem cells, which will facilitate the study of human germ cell development and stem cells based reproductive medicine.

SPEAKER ABSTRACTS

SATURDAY, 11 NOVEMBER

ORGANOIDS AND DISEASE MODELING – SESSION I

REGULATING THE PATTERNING OF MESODERM AND GENERATING KIDNEY ORGANOIDS BY THE DIRECTED DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

Minoru Takasato

RIKEN Center for Developmental Biology, Kobe, Japan

Our knowledge about human development is much poorer than that of model animals such as mouse and chicken. This is mainly because of the difficulty to utilise human bodies or embryos for experiments to study their development. However, recent progresses of stem cells research, especially about the directed differentiation of human pluripotent stem cells (hPSCs) into various organs/tissues, make us enable to access a new approach for the study of human development. Previously, we succeeded to generate kidney organoids by directing the differentiation of hPSCs. During that differentiation, hPSCs developed into the primitive streak followed by the intermediate mesoderm and kidney progenitors. This process is consistent with findings about kidney development in model animals. Furthermore, by modifying the culture condition of the directed differentiation, we could regulate the patterning of mesoderm developed from hPSCs. In such modifications, we preferentially induced the posterior or anterior primitive streak, and regulated the direction of primitive streak differentiation into mesoderm along anteropsterior and mediolateral axes. When the posterior intermediate mesoderm was induced, it developed to kidney organoids. On the other hand, ureteric epithelia and bipotential gonads-like cells were generated from the anterior intermediate mesoderm as expected. These results indicate that this directed differentiation protocol mimics the real development of human mesoderm, and can be a platform to study about human development in vitro.

DISEASED PHOTORECEPTORS INTERROGATED IN RETINITIS PIGMENTOSA PATIENT IPSC-DERIVED 3D RETINAE

Zi-Bing Jin, Wen-Li Deng, and Mei-Ling Gao

Wenzhou Medical University, China

Retinal pigmentosa (RP) is an irreversible, inherited retinopathy in which nyctalopia is observed at early onset, eventually followed by complete blindness as the disease progresses. Despite the genetic heterogeneity of RP, Retinitis Pigmentosa GTPase Regulator (RPGR) mutations are the most common causes of this disease in patients. Here, we generated iPSCs from an RP patient with a frameshift mutation in the RPGR gene, which were then differentiated into well-structured retinal organoids. We observed significant defects in photoreceptor development in terms of photoreceptor morphology, localization and transcriptional profiling. Furthermore, shorted cilium was found in both the patient iPSCs and 3D retinal organoids. CRISPR-Cas9-mediated correction of the mutation not only rescued photoreceptor development but also reversed the observed ciliopathy. Additionally transcriptome-based analysis revealed that gene expression in the mutation-corrected retinal organoids was in accordance with that in the normal control. This study recapitulated the pathogenesis of RPGR using patient-specific organoids for the first time and achieved targeted gene therapy of RPGR mutations in a dish as proof-of-concept evidence.

ORGANOID MODELING OF HUMAN BRAIN DEVELOPMENT AND CONGENITAL ZIKA VIRUS SYNDROME

Bennett Novitch

University of California, Los Angeles, U.S.

The human cerebral cortex possesses distinct structural and functional features that are not found in the lower species traditionally used to model brain development and disease. Accordingly, considerable attention has been placed on the development of methods to direct pluripotent stem cells to form human brain-like structures termed organoids. However, many organoid differentiation protocols are inefficient and display marked variability in their ability to recapitulate the three-dimensional architecture and course of neurogenesis in the developing human brain. Here, we report optimized organoid culture methods that efficiently and reliably produce cortical and basal ganglia structures similar to those in the human fetal brain in vivo, with functional neurons that exhibit network-like activities. We will also describe factors that can influence success or failure in organoid formation and growth. Lastly, we will present recent work using the cortical organoid system to model the teratogenic effects of Zika virus on the developing brain and identify new candidate receptors for viral entry and therapeutic compounds that can mitigate its destructive actions.

SPEAKER ABSTRACTS

PANEL DISCUSSION: ETHICS IN GENOME ENGINEERING

ETHICS, POLICIES, AND GENE EDITING

Jeremy Sugarman

Johns Hopkins University, U.S.

New technologies make it possible to somewhat easily edit the human genome. Some of these tools are already being used experimentally to see if they can help with the treatment of diseases such as HIV infection. Others hope that these tools could be used in the future to prevent genetic diseases that are passed on to future generations. However, whether it is safe and ethically appropriate to do so is unclear. This introductory talk will describe the ethical implications associated with the potential uses of gene editing and recent policies aimed at addressing them.

NEW TOOLS FOR STEM CELL RESEARCH

SINGLE-CELL RNA-SEQ ANALYSIS MAPS DEVELOPMENT OF HUMAN GERMLINE CELLS AND GONADAL NICHE INTERACTIONS

Fuchou Tang

Peking University, China

Human fetal germ cells (FGCs) are precursors to sperm and eggs and are crucial for maintenance of the species. However, the developmental trajectories and heterogeneity of human FGCs remain largely unknown. We performed single-cell RNA-seq analysis of over 2,000 FGCs and their gonadal niche cells in female and male human embryos spanning several critical developmental stages. We found that female FGCs undergo four distinct sequential phases characterized by mitosis, retinoic acid signaling, meiotic prophase, and oogenesis. Male FGCs develop through stages of migration, mitosis, and cell-cycle arrest. Individual embryos of both sexes simultaneously contain several subpopulations, highlighting the asynchronous and heterogeneous nature of FGC development. Moreover, we observed reciprocal signaling interactions between FGCs and their gonadal niche cells, including activation of BMP signaling pathway in FGCs and Notch signaling pathway in gonadal niche cells. Our work provides key insights into the crucial features of human FGCs during their highly ordered mitotic, meiotic, and gametogenetic processes in vivo.

A CONTINUOUS MOLECULAR ATLAS OF REPROGRAMMING TO IPSCS BY HIGH-THROUGHPUT SINGLE CELL RNA-SEQ

Jian Shu[1], Geoff Schiebinger[1], Marcin Tabaka[1], Brian Cleary[1], Vidya Subramanian[1], Aryeh Solomon[1], Siyan Liu[1], Stacie Lin[3], Peter Berube[1], Lia Lee[1], Jenny Chen[1], Justin Brumbaugh[4], Philippe Rigollet[3], Konrad Hochedlinger[4], Rudolf Jaenisch[5], Aviv Regev[1], Eric Lander[1]

[1]Broad Institute of MIT and Harvard, [2]Wellesley College, [3]MIT, [4]Massachusetts General Hospital, [5]Whitehead Institute for Biomedical Research

Reprogramming somatic cells to iPSCs is a low efficiency process, with only a small fraction of cells capable of reaching pluripotency. Single cell RNA-seq can study the heterogeneity during reprogramming, however previous single cell RNA-seq analyses were based on gene expression profiles from limited genes or cells. Here we report a continuous high-resolution molecular reprogramming roadmap to iPSCs by analyzing approx. 70,000 single cells, with a computational analysis method developed to analyze this large scale dataset. Through progression analysis of each time points, it was discovered that early stage reprogramming is homogenous and that bifurcation begins after withdrawal of exogenous transcription factors. Cells that failed to become iPSCs expressed activation of certain lineage specification genes. Our data provides a high-resolution roadmap of reprogramming and the methods developed here can be used for future analysis of complex cellular programming and other reprogramming processes.

ESTABLISHMENT OF CULTURES OF MAMMALIAN EXPANDED POTENTIAL STEM CELLS

Pentao Liu[1], Jian Yang[1], David Ryan[1], Wei Wang[1], Jason Tsang[1], Guocheng Lan[2], and Xiangang Zhou[2]

[1]Wellcome Trust Sanger Institute, U.K., [2]Cancer Research UK Cambridge Institute, University of Cambridge, Li Ka Shing Centre, U.K.

Mouse embryonic stem cells derived from the epiblast contribute to the somatic lineages and the germline upon reintroduction to the blastocyst but are excluded from the extraembryonic tissues that are derived from the trophectoderm (TE) and the primitive endoderm (PrE). By inhibiting signal pathways implicated in the earliest embryo development, we have recently established cultures of expanded potential stem cells (EPSCs) from individual 8-cell blastomeres, by direct conversion of mouse embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). A single EPSC

SPEAKER ABSTRACTS

can contribute to both the embryo proper and the TE lineages in chimera assay. Bona fide trophoblast stem cell (TSC) lines and extraembryonic endoderm stem (XEN) cells could be directly derived from EPSCs in vitro. Molecular analyses of the epigenome and single-cell transcriptome revealed enrichment for blastomere-specific signature and a dynamic DNA methylome in EPSCs. The knowledge of mouse EPSCs has enabled establishing expanded potential stem cells of other mammalian species where in vitro pluripotent stem cells are not currently available. Mammalian EPSCs thus may provide a new source of cells for stem cell research and for regenerative medicine.

CHARACTERISATION OF DISTINCT STATES OF HUMAN NAIVE PLURIPOTENCY GENERATED BY REPROGRAMMING IDENTIFIES KLF4 AS A CONDUIT FOR PRIMED TO NAIVE CONVERSION

Xiaodong Liu[1], Christian Nefzger[1], Fernando Rossello[1], Joseph Chen[1], Anja Knaupp[1], Jaber Firas[1], Ethan Ford[2], Jahnvi Pflueger[2], Jacob Paynter[1], Hun Chy[3], Carmel O'Brien[3], Cheng Huang[4], Ketan Mishra[1], Margeaux Hodgson-Garms[1], Natasha Jansz[5], Sarah Williams[6], Marnie Blewitt[5], Susan Nilsson[3], Ralf Schittenhelm[4], Andrew Laslett[3], Ryan Lister[2], and Jose Polo[1]

[1]Department of Anatomy and Developmental Biology, Monash University, Australia, [2]ARC Center of Excellence in Plant Energy Biology, The University of Western Australia, Australia, [3]Australian Regenerative Medicine Institute, Monash University, and Manufacturing, CSIRO, Australia, [4]Department of Biochemistry and Molecular Biology, Monash University, Australia, [5]The Walter and Eliza Hall Institute of Medical Research, Australia, [6]Department of Anatomy and Developmental Biology, Monash University, Australia

The extent of naive characteristics of recently reported naive human pluripotent stem cells (hPSCs) obtained in different naive-permissive media, is unclear. Moreover, these naive hPSCs were mainly derived by conversion from primed hPSCs or by direct derivation from human embryos rather than by somatic cell reprogramming. Here, we derived genetically matched human naive hPSCs by direct reprogramming of fibroblasts as well as primed-to-naive conversion using different naive conditions (NHSM, RSeT, 5iLAF and t2iLGöY). Comprehensive characterisation showed that naive hPSCs obtained in these different conditions represent a spectrum of naive characteristics irrespective of whether they were derived by conversion or reprograming. Importantly, only t2iLGöY hPSCs displayed a similar transcriptome to human cells from the inner cell mass, karyotypic stability and require re-priming for trilineage differentiation. Furthermore, our analyses identified KLF4 as a key reprogramming factor which enables conversion of primed hPSCs into naive t2iLGöY hPSCs. These findings underscore the role that reprogramming factors can play for the derivation of bona fide naive hPSCs and provide a molecular and functional reference for all the analysed conditions, which will help accelerate the downstream applications of naive hiPSCs.

GENERATION OF A DUAL RECOMBINASE SYSTEM FOR TRACING ENDOGENOUS CARDIAC STEM CELLS

Bin Zhou

Institute for Nutritional Sciences, SIBS, CAS, China

There is a debate over the myogenic potential of Kit+ cardiac stem cells in adult heart after myocardial infarction (MI). Genetic lineage tracing studies reveal that Kit+ cardiac stem cells contribute to cardiomyocytes either minimally or substantially. Since Kit is expressed in both myocytes and non-myocytes, it remains unclear if Kit+ non-myocytes differentiate into any new myocyte after MI. Here we generated a new tracing system based on dual recombinases that specifically label Kit+ non-myocytes without myocyte contamination. We found that Kit+ non-myocytes did not generate any new myocytes after MI. Apart from Kit+ cells, it remains elusive if there are other stem cells in the adult heart. We further utilized dual recombinase system to distinctly label myocytes and all types of non-myocytes that may include putative cardiac stem cells. Dual fate mapping studies showed that non-myocytes significantly contribute to myocytes in the embryonic heart. The detection of non-myocyte to myocyte transition (NMT) in the embryonic stage suggested that this tracing system could be also employed to test the existence of NMT in adult heart. In addition, this study also provides a new means to explore the existence of endogenous stem cells in multiple organs or tissues without depending on known markers of putative stem cells.

SPEAKER ABSTRACTS

SUNDAY, 12 NOVEMBER

ROAD TO THE CLINIC – SESSION I

HEART REGENERATION WITH HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

Michael Laflamme

Toronto General Hospital Research Institute, Canada

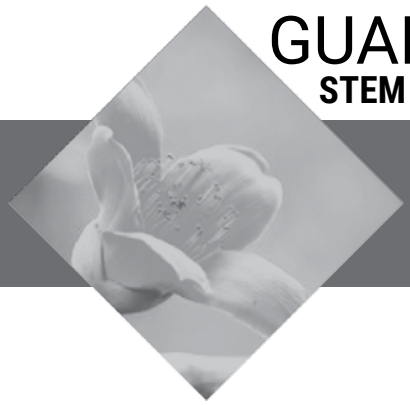
Human pluripotent stem cells (hPSCs) have a number of potential advantages for use in myocardial infarct (MI) repair, including a tremendous capacity for expansion in the undifferentiated state and the ability to differentiate into phenotypically unambiguous cardiomyocytes. Our group has contributed to the development of efficient, reliable protocols to generate large quantities of hPSC-derived cardiomyocytes (hPSC-CMs), and we have shown that the transplantation of hPSC-CMs can partially "remuscularize" the infarct scar with new, electrically-integrated myocardium in small and large animal models of myocardial infarction. This is a sine qua non for true heart regeneration; however, a number of important challenges remain to the successful development of hPSC-based solutions for heart failure. In this presentation, I will describe our team's recent efforts to 1) establish large-scale hPSC-CM manufacturing, 2) understand and improve the electrical function of hPSC-CM graft myocardium, and 3) test the safety and efficacy of hPSC-CM transplantation in a highly relevant porcine MI model.

CLINICAL AND PRECLINICAL IMAGING REVEALS THE DYNAMIC BEHAVIOR OF THE EXOGENOUS STEM CELLS TRANSPLANTED AND ENDOGENOUS STEM CELLS IN THE NERVOUS SYSTEM

Jianhong Zhu

Fudan University Huashan Hospital, China

Investigations of stem cell therapy have required non-invasive analysis of the dynamic distribution, survival and integration of exogenous transplanted stem cells and behavior of endogenous stem cells. Recently, new technologies have been specifically developed for the detection and quantification of neural stem cells in the living human brain. These technologies rely on the use of magnetic resonance imaging, available in preclinical animal research and clinical trials. Here, we report the feasibility of functional tracking of iPS cells-induced neural progenitor cells in monkey central nervous system. We labeled the cells with Superparamagnetic nanoparticles, then transplanted the injury region of the spinal cord or brain. MRI analysis was performed 1, 7, 14, 21 and 30 days, as well as 3 months following transplantation. The therapeutic efficacy of iPS cells-derived neural progenitor cells was concomitantly tested through functional recovery tests of injury model animals. In clinical research, the adult neural stem cells were implanted stereotactically around the region of brain damage of patients with open brain trauma. Imaging was obtained by gradient reflection echo with an 3.0T MR imager at 24 hours and every 7 days follow¬ing transplantation for ten weeks. We observed a susceptibility change with powerful signal damping on T2-weighted MRI. The injection sites were visible as circular dark tissue areas on the first day after implantation. The hypointense signal at each injection point faded thereafter. One week after implantation, the change in signal was consistent with the cells accumulating and proliferating around the lesion. The signal around the periphery of the lesion intensified during the second and third weeks, suggesting that the NSCs migrated from the primary sites of injection and to the border zone of the damaged tissue. The endogenous neural stem cells reside in the adult brain and are capable of generating new neural cells throughout the life span. The neural stem cells spectra demonstrated a unique profile, including a prominent peak at the frequency of 1.28 parts per million (ppm) by proton magnetic resonance spectroscopy (H-MRS). Based on singular value decomposition (SVD) signal processing, we detected the 1.28-ppm marker of



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neural stem cells in the monkey and human brains with MRS and quantified the abundance of the 1.28 ppm metabolite in the cortical and hippocampal voxels, normalized over the amplitude of the creatine peak. This data demonstrates the emerging non-invasive methods available to assess the dynamic behavior of the exogenous stem cells and endogenous stem cells in preclinical and clinical research.

This study was supported by grants (2013CB967400, 2012CB966300, 81271003) from the National Nature Science Foundation and Ministry of Science and Technology of China

THE SELECTIVE DERIVATION OF SOMATOTROPHS FROM HUMAN EMBRYONIC STEM CELLS

Jinghua Piao and Viviane Tabar

Memorial Sloan Kettering Cancer Center, New York, NY, U.S.

Growth hormone (GH) deficiency is caused by genetic factors, trauma, tumors and other causes. Current therapy consists of the administration of growth hormone, a very expensive treatment that lacks dynamic regulation by the hypothalamus. Our aim is to develop human somatotroph cell therapy which will provide the possibility to integrate into the host hypothalamic-pituitary axis. We have previously derived pituitary placode from human ES cells capable of differentiation into diverse pituitary lineages. Our current strategy involved initial enrichment for Pit1+ precursors via modulation of the Wnt/β-catenin signaling pathway. However Pit1+ precursors include somatotrophs, lactotrophs and thyrotrophs. We develop a successful strategy to bias the differentiation to somatotrophs, with upregulation of GH1 and suppression of pre-opiomelanocortin (POMC) which is normally secreted by corticotrophs. Somatotrophs were subsequently expanded, leading to the development of a majority of GH positive cells in the cultures. GH was detected in the supernatant and its secretion was increased by growth hormone–releasing hormone (GHRH) stimulation. The somatotrophs can be further enriched by flow cytometry sorting. In vivo data in animal models of dwarfism (Ames dwarf mice (PROP1df) is ongoing. Our data establish a GMP ready protocol for the derivation of human somatotrophs and the design of therapeutic strategies.

The work is supported by NIH 5R21CA176700.

ORGANOIDS AND DISEASE MODELING

DISRUPTION OF GRIN2B IMPAIRS NMDA-INDUCED CELL DIFFERENTIATION IN HUMAN NEURONS

Carl Ernst

McGill, Canada

NMDA receptors (NMDAR) are glutamate binding, multi-subunit ion channels in the central nervous system. Deletion or loss-of-function mutations in one copy of GRIN2B, a subunit of the NMDA receptor, cause a wide range of neurodevelopmental disorders including intellectual disability and autism. We developed clonal models of GRIN2B deletion and loss-of-function mutations in human neurons using gene-editing tools on an isogenic background, and neurons from a patient with a missense mutation in the glutamate binding domain. Whole genome transcriptome analysis revealed extensive increases in genes associated with cell proliferation in all genetic models, suggesting delayed differentiation of these cells. We identified 4-10 fold increases in KI67 and MET proteins, two representative markers of cell proliferation state. Genetic repair of the patient missense reversed these effects. Pharmacological block of NMDAR in healthy neurons recapitulated mutation models, including dose-dependent decreases in GRIN2B, consistent with a feed-forward model of NM-DA-mediated neuronal differentiation. These results suggest that loss of NMDA signalling maintains a proliferative state in neurons and that intellectual disability caused by GRIN2B mutations may be due, at least in part, to NMDA-induced cell differentiation deficits, implying the GRIN2B signalling may have functions beyond the synapse.

MODELING OF ALZHEIMER'S DISEASE USING IPSC TECHNOLOGY

Ling Li

CHA University, South Korea

Alzheimer's disease (AD) is the most common neurodegenerative disease which is pathologically characterized by the formation of amyloid-beta (Aβ) plaques and neurofibrillary tangles. Recent studies have demonstrated that excessive accumulation of Aβ peptides can increase hyperphosphorylation of tau, leading to the formation of neurofibrillary tangles. In this study, we have generated several induced pluripotent stem cell (iPSC) lines from familial and sporadic AD patients, and then differentiated them

SPEAKER ABSTRACTS

into cortical neurons. Extracellular deposition of Aβ levels were dramatically increased in the neurons differentiated from iPSC lines generated from the patients carrying presenilin-1 (PS1)-S170F mutation, amyloid precursor protein (APP)-V715M mutation or sporadic AD. Furthermore, some of these AD iPSC-derived neurons exhibited high expression levels of phosphorylated tau, especially in AT8 (Ser202/Thr205), which are also detected in the soma and neurites by immunocytochemistry. We next investigated the mitochondrial dynamics in AD iPSC-derived neurons, which exhibited abnormal patterns of mitochondria velocity using Mito-tracker. We also found that the levels of Mfn1 (membrane proteins mitofusin 1) and Mfn2 (membrane proteins mitofusin 2) were significantly reduced in AD iPSC-derived neurons. We also observed that LC3b and ubiquitin is highly increased in AD iPSC-derived neurons, indicating that the autophagy system is also defective. Taken together, we have characterized the patholo¬gical features of AD patients carrying mutations for PS1-S170F or APP-V715M using iPSC technology for the first time, which will serve as useful resources for studying AD pathogenesis and drug screening in the future.

NOVEL STEM CELL TYPES FOR VERSATILE BIOLOGICAL APPLICATIONS

Wei Li

Institute of Zoology, CAS, China

Haploids and double haploids are important resources for studying recessive traits and have large impacts on crop breeding, but natural haploids are rare in animals. Mammalian haploids are restricted to gametes and are occasionally found in tumors with massive chromosome loss. Here we report the successful generation of haploid embryonic stem (ES) cells in mice and rats with both androgenetic and parthenogenetic origins. The androgenetic haploid ES (ahES) and parthenogenetic haploid ES (phES) cell lines maintain haploidy and stable growth over 30 passages, possess the ability to differentiate into all three germ layers in vitro and in vivo, and can contribute to the germline of chimaeras when injected into blastocysts. Although epigenetically distinct from gametes, the ahES cells and phES cells can functionally replace sperms and oocytes, respectively, to produce viable and fertile animals. The gamete-like features make haploid ES cells a convenient tool for genetic screening and genetic modification, as well as for developmental studies. For example, after proper imprinting modifications, the mouse phES cells can efficiently produce viable fertile offspring upon intracytoplasmic injection into MII oocytes, which thus establish a novel strategy to generate bi-maternal mammals efficiently, and to uncover the function of genomic imprinting robustly. Moreover, we can produce mammalian interspecific allodiploid ES cells from the evolutionarily distantly related mammalian species by fusion of haploid ES cells, which can hardly be achieved due to reproductive isolation. The allodiploid ES cells combine the genomes of different species in the same cellular environment, containing the same trans-regulatory factors, thus could serve as a convenient system to identify functional differences of gene expression regulatory elements between species. They are also powerful tools for the identification of genes with different functions between distal-related species, and the mechanisms underlying the choice of X chromosome for inactivation and X-inactivation escaping. Together, our findings suggest the haploid and allodiploid ES cells serve as novel tools for biological research.

ROAD TO THE CLINIC – SESSION II

CLINICAL GRADE HESCS AND DERIVATIVES FOR THERAPY

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For practical application of human embryonic stem cell (hESC)-derived progenies for therapy, clinical grade cells and comprehensively preclinical evaluation in primates are essential. We have established completely xeno-free clinical-grade hESC lines following good manufacturing practice (GMP), thereby we consider these lines “clinical-grade”. In addition to the primary capacity for pluripotency, these cell lines were efficiently differentiated into various types of clinical-grade progenies. These cells were all recognized by the National Institutes for Food and Drug Control of China for further eligible accreditation. As a proof of concept demonstration, we evaluate the safety and efficacy by transplanting clinical-grade hESC-derived DA

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neurons into brains of MPTP induced monkey models of Parkinson's disease. While most monkeys exhibited variable but apparent behavioral improvement, no tumors occur based on MRI imaging and serological analysis. The transplanted cells survive, migrate and differentiate into mature DA neurons that express TH. In recipient PD monkeys that receive DA neuron transplantation, even slight increase of DA in striatum clearly correlates significant functional improvement. These results collectively demonstrate that although improved cell transplantation procedures are needed for ideal outcome, clinical-grade hPESCs prove to be a reliable source of cells for treating neural degenerative diseases such as PD. These results also provide strong support to China's first embryonic stem cell-based Phase I/IIa clinical study on PD.

THE STEM CELL DARK ECONOMY: IMPLICATIONS FOR THE FIELD

Douglas Sipp

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Therapeutic uses of stem cells and their derivatives in regenerative medicine and tissue engineering have attracted a great deal of scientific interest, media attention, and public funding. While precise global figures on public spending in stem cell R&D are difficult to obtain, clearly a number of major economies, including the United States, China and Japan have spent billions of dollars each in this field. Despite that large investment, however, approved stem cell therapeutics remain scarce, and to date there are no pluripotent stem cell-based medicinal products on any market in the world. However, a penumbral market sector has emerged over the past decade, which aggressively promotes unproven uses of supposed stem cell-based interventions for a great many serious medical conditions. Recent work has begun to show the extent of this industry, which surprisingly operates unimpeded in supposedly well-regulated nations such as Australia, Japan and the US. However, many fundamental details about this shadowy stem cell economy remain unknown. I will discuss how the industry has developed, the current state of knowledge, and the large gaps in our understanding of this "dark economy."

SUBRETINAL TRANSPLANTATION OF HESC-RPE: CLINiCAL TRIAL IN THE TREATMENT OF WET AGE-RELATED MACULAR DEGENERATION (WAMD) – ONE YEAR FOLLOW-UP

Zheng Qin Yin[1], Yong Liu[1], Shiying Li[1], Haiwei Xu[1], Qiyou Li[1], Tongtao Zhao[1], Yi Wang[1], Chen Qian[2], and Qi Zhou[3]

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Retinal pigment epithelium (RPE) transplantation is a particularly promising treatment of retinal degenerative diseases affecting the RPE-photoreceptor complex, and recently the stem cell therapy has been used clinically to treat the AMD and Stargardt's disease. The xeno-free clinical-grade human embryonic stem cell line (CTS hESC) was induced to RPE cells by spontaneous differentiation method. A series of safety assessments were finished for potential bacteria, mycoplasma, murine viruses according to standard protocols. The characteristics of this CTS hESC derived RPE-specific attributes including purity, differentiation and phagocytosis by quantitative PCR, FACS and immunohistochemistry staining. The purified CTS hESC derived RPE at passage 3 were used for clinical purpose. After registered with http://www.chictr.org.cn/ (ChiCTR-OCB-15006423), we performed clinical study of subretinal transplantation of CTS hESC derived RPE in treatment of wet Age-related Macular Degeneration (wAMD). The first three patients underwent vitrectomy and removal of choroidal neovascularization (CNV) membrane successfully. During the one year follow-up, there were no rejection showed in the clinical tests. Interestingly all patients had improved their vision in ETDRS test. The F-VEP recording of the patients confirmed their vision improvement and the patients' previous feeling of central vision blurry due to CNV disappeared. As we know this is the first study of subretinal transplantation of hESC derived RPE in the treatment of wet Age-related Macular Degeneration, which opened a new approach for the treatment of retinal degeneration diseases by the human embryonic stem cells in clinics.

Acknowledgements: This study supported by National Basic Research Program of China No.2013CB967002.

POSTER ABSTRACTS

Poster 02

DIRECTED EVOLUTION OF REPROGRAMMING FACTORS BY PHENOTYPIC SELECTION AND SEQUENCING (DERBY-SEQ)

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The programming of cell states in vitro mediated by exogenously provided transcription factor (TF) cocktails holds great promise for disease modeling, drug testing and regenerative biomedicine. However, for most cell types existing procedures are slow, inefficient, poorly reproducible and restricted to a limited set of donor cells. We have developed a high throughput screening and cell selection method (Directed Evolution of Reprogramming factors BY phenotypic selection and Sequencing (DERBY-Seq)) to identify artificially evolved TFs. The method involves, pooled library screening and selection of engineered Sox transcription factors (eSox TF) with enhanced abilities to generate induced pluripotent stem cells (iPSCs). The eSTF library entails ~8000 Sox variants randomized at three sites selected by structural analysis. We screened this library in biological triplicates for the conversion of mouse embryonic fibro-blasts into GFP-positive iPSCs in the presence of Oct4, cMYC and Klf4. We used fluorescent assisted cell sorting to separate GFP-positive and GFP-negative cells and genotyped eSox's by next-generation amplicon sequencing. This way we identified dozens of eSox candidates enhancing iPSC generation. Validation of eSox candidates from the GFP-positive and candidates from the GFP negative pools revealed true positives and true negatives, respectively, highlighting the robustness of our screening platform. Our top-performing eSox outperforms wild-type Sox2. We conclude that our eSox TF screening platform can provide a generally applicable tool to enhance lineage programming and differentiation of various cell types

Keywords: engineered Sox transcription factors (eSox TF), Directed Evolution of Reprogramming factors BY phenotypic selection and Sequencing (DERBY-Seq)

Poster 03

SEAMLESS GENE CORRECTION OF LDLR IN FAMILIAL HYPERCHOLESTEROLEMIA INDUCED PLURIPOTENT STEM CELLS MEDIATED BY CRISPR/CAS9 AND PIGGYBAC

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Familial hypercholesterolemia (FH) is an autosomal disorder, characterized by elevated circulating lipoproteins and high risk of premature coronary heart disease. Mutations in low-density lipoprotein receptor gene (LDLR) is one of the major causes. Induced pluripotent stem cell (iPSC) technology helps us generate cell model with patient-specific genetic background, while correction of mutations in patient-specific iPSCs offers the applied prospect of autologous transplantation to treat inherent disorders. In this study, we aimed to correct LDLR mutation in iPSCs reprogrammed from a FH patient, using CRIS-PR/Cas9 and piggyBac. Patient-specific iPSCs were generated from a FH patient with heterozygous duplication of "TGCTGGC" (c.2108_2114dup, p.Ala705fsX14) in exon 14 of LDLR. CRISPR/Cas9 targeting the mutation site and piggyBac were employed to correct the mutation via homologous recombination. Corrected iPSCs were subjected to karyotype analysis, and a series of tests of pluripotency, then differentiated into hepatocyte-like cells (iHeps) to assess the LDLR function in vitro and in vivo.As a result, we designed two sgRNAs which showed high cutting efficiency in the target site. Co-delivery of CRISPR/Cas9 and piggyBac in FH iPSCs facilitates the integration of donor constructs in the target site with high efficiency. Then, the drug selection cassette was removed by hyPBase expression vec-tors. After two rounds of genetic manipulation, we achieved a seamless genetic correction in iPSCs, which showed retain full pluripotency and normal karyo-types. Western blot showed a recovery of LDLR level in corrected iPSCs. In addition, the corrected iPSCs can be differentiated into functional iHeps efficiently, and these iHeps showed the restored function of LDLR, characterized by recovery of LDL receptor expression levels and the capacity of LDL uptake. Our study highlights the feasibility of using CRISRP/Cas9 and piggyBac to generate seamless, genetically corrected patient-specific iPSCs, and functional iHeps. These genetically corrected cells are not only ideal models for disease modeling, but also the potential source for autologous cell replacement therapies.

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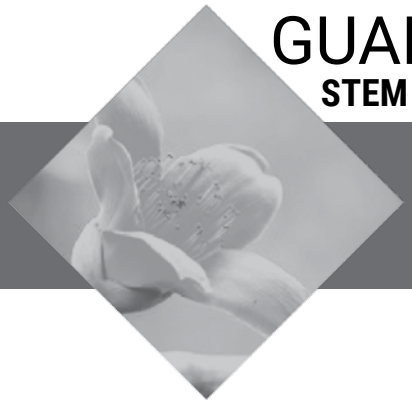
Keywords: Gene correction, Induced pluripotent stem cells, Familial hypercholesterolemia



POSTER ABSTRACTS

Poster 04

GENERATION OF MONOCYTE DERIVED DENDRITIC CELLS USING XENO-FREE CLINICAL GRADE HUMAN PLATELET LYSATE

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Dendritic cells (DCs), a major antigen-presenting cell type, have been used widely for vaccine development in cancer immunotherapy. Among all the sources of DCs, those derived from CD14+ monocytes (Mo-DCs) are favored for therapeutic use because they: 1) have strong T cell immunity inducing ability, 2) are relatively easy to generate, and 3) do not require cytokine pretreatment. Clinical manufacturing of Mo-DCs has traditionally relied on the use of either fetal bovine serum (FBS) which poses potential risks for viral and prion transmission as well as for adverse immunological reactions, or human AB serum (hABS) which is collected from a small number of donors and has considerable lot-to-lot variability. To address these issues, we have developed a meth-od for Mo-DC production using a xeno-free cell culture supplement: PLUSTM human platelet lysate. PLUSTM is manufactured in large lots under Good Manufacturing Practice (GMP) standards using platelet units obtained from AABB-accredited blood banks. We undertook this study to characterize the maturation state and functionality of PLUSTM-generated Mo-DCs in comparison to those generated using FBS or hABS. To perform the experiments, freshly isolated CD14+ monocytes (n=3) were differentiated into immature Mo-DCs by 4-days incubation in RPMI 1640 containing IL-4 (400 U/mL), GM-CSF (1000 U/mL) and either 5-10% FBS, 2.5-5% hABS, or 2.5-5% PLUS™, followed by 2-days maturation in FBS, hABS, or PLUSTM supplemented media with either TNF-α, IFN-γ or lipopolysaccharide. Flow cytometry analysis demonstrated that PLUSTM-generated Mo-DCs had a mature phenotype, with equivalently negative CD14 expression and equivalently positive CD209, CD83, CD86 and HLA-DR expression as FBS and hABS generated Mo-DCs. Phagocytosis of FITC-dextran was similar for Mo-DCs generated under all culture conditions. In a 6 day co-culture study, PLUS™-generated Mo-DCs stimulated allogeneic T cell proliferation as efficiently as FBS and hABS. Antigen expressions, dextran phagocytosis, and T cell stimulation were consistent between all maturation path-ways and were independent of FBS, hABS, or PLUSTM concentration. These studies demonstrate that PLUS human platelet lysate can successfully replace FBS or hABS for ex vivo production of clinical-grade Mo-DCs for immunotherapy applications.

Keywords: dendritic cells, clinical cell manufacturing, xenogeneic-free, human platelet lysate

Poster 05

XENO-FREE HUMAN PLATELET LYSATE FOR MANUFACTURING HUMAN BONE MARROW AND ADIPOSE DERIVED MESENCHYMAL STEM CELLS

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The rapidly growing interest of using Mesenchymal Stem Cells (MSCs) in regenerative medicine has increased the urgency for identifying a safe, effective, and xenogeneic-free alternative to fetal bovine serum (FBS) for clinical cell manufacturing. Among the options to replace FBS, human platelet lysate (hPL) has recently emerged as a promising candidate. Compass Biomedical's PLUS™ hPL is produced under Good Manufacturing Practice (GMP) standards using expired platelet units from AABB-accredited blood banks. Growth factor levels in PLUS™ are consistent across all lots with average concentrations of 9,939±682, 641±257, 3,175±309 and 156±18 pg/mL for PDGF-BB, VEGF, EGF, and bFGF, respectively. In this study, we accessed the capacity of PLUS™ to re-place FBS for the isolation, ex vivo expansion, and cryopreservation of human bone marrow derived MSCs (hBM-MSCs) and human adipose derived stromal cells (hASCs). To perform these experiments, hBM-MSCs and hASCs were isolated from fresh human bone marrow (n=9 donors) and fresh human lipoaspirates (n=6 donors), respectively, using different concentrations of PLUS™ (2.5%, 5% and 10%) and FBS (10%) supplemented medium. After isolation, the cells were expanded for additional 4 passages in appropriate PLUS™ or FBS medium. We found that doubling time for both cell types was significantly shorter in 5% PLUS™ versus 10% FBS. Based on flow cytometry analysis, hBM-MSCs and hASCs cultivated in PLUS™ of all concentrations were >95% positive for stem cell markers (CD73, CD90, CD105) and ≤2% positive for hematopoietic markers (CD45, CD34, CD14, CD20), which was equivalent to FBS cultivated cells. At passage 4, PLUS™-cultivated hBM-MSCs and hASCs demonstrated equivalent immunosuppressive capacity to FBS-cultivated cells as measured by a T cell proliferation assay. Cryopreserved hBM-MSCs and hASCs at passage 3 using PLUS and dimethyl sulfoxide (DMSO) have shown comparable cell viability (>90%) and immunosuppressive capacity post-thaw when compared to FBS-cryopreserved cells. These studies demonstrate that PLUS™ hPL is a safe and reliable supplement to use in clinical applications for the isolation, expansion, and cryopreservation of hBM-MSCs and hASCs without impacting their stem cell phenotype or immunosuppressive capacity.

Keywords: mesenchymal stem cell, cell manufacturing, xenogeneic-free, human platelet lysate

POSTER ABSTRACTS

Poster 06

ROBUST GENERATION OF INDUCED NEURAL STEM CELLS FROM HUMAN SOMATIC CELLS BY DEFINED FACTORS

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Recent studies have shown direct reprogramming of human somatic cells into human induced neural stem cells (hiNSCs) using diverse combinations of transcription factors. However, it's extremely low conversion efficiency precludes the clinical translation of iNSC technology. Here, we report that the ectopic of four iNSC factors (Brn4, Sox2, Klf4, and cMyc) together with small molecule cocktail could robustly convert human fibroblasts into hiNSCs (>80 fold than previously described other protocols) within 7 days. The directly converted hiNSCs share the key cellular, molecular, and functional features with control NSCs differentiated from human embryonic stem cells. Our novel approach for generating hiNSCs will be the useful platform for unveiling the underlying conversion mechanism and also for modeling various neuronal diseases such as Alzheimer's and Parkinson's diseases.

Keywords: neural stem cell, reprogramming

Poster 07

DEVELOP OUTCOME MEASURES FOR CELL-BASED THERAPY OF MYOTONIC DYSTROPHY TYPE 1

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Myotonic Dystrophy Type 1 (DM1) is a fatal disease from progressive muscular wasting. Regenerative medicine using muscle cell transplantation has emerged as a promising therapeutic modality, especially with the advancement of iPS technology and therapeutic gene-editing (Genome Therapy). We have been able to correct the mutation in disease/patient-specific DM1 iPS cells, which can potentially provide unlimited muscle precursor cells for cell transplantation. One other application of iPS cells is to generate myocytes for drug screening. Small molecules have been found to have the potential in ameliorating the muscle dysfunction observed in DM1. However, there is an unmet need to identify in vitro outcome measures to demonstrate the therapeutic effect before moving such therapies to in vivo clinical trials. In this study, we examined the muscle regeneration (myotube formation) in normal and DM1 myoblasts in vitro to establish outcome measures for therapeutic monitoring. We have noted normal proliferation of DM1 myoblasts but abnormal nuclear aggregation during the early stage myotube formation as well as late sage myofibril degeneration. These two outcome measures were validated via rescue of abnormal DM 1 myotube formation with normal myoblasts. We concluded that abnormal nuclear aggregation and myofibril degeneration offer easy and sensitive outcome measures to monitor therapeutic effects when using skeletal muscle stem cells for muscle regeneration.

Keywords: muscular dystrophy, pluripotent stem cell, cell transplantation, outcome measure, myotube formation

Poster 08

NEURAL DYSFUNCTIONS IMPROVEMENT BY MSCS SPONTANEOUS MIGRATION IN RAT NTDS AFTER INTRA-AMNIOTIC ADMINISTRATION

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Neural tube defects (NTDs) rank among the most common categories of congenital anomalies and available treatments have limited efficacy. Fetal surgery was the only prenatal treatment for NTDs. It has been practiced in clinic over a decade, but the neurological functional recovery was still not satisfactory. That was mainly due to the fetal surgery only repaired the muscle and skin structural defects but not the neural function. Our previous results have revealed that deficiencies of sensory, motor and parasympathetic neurons are primary anomalies coexisted with the spinal malformation in fetal rats with neural tube defects. Therefore, we have suggested nerve regeneration is the critical for NTDs therapy and successfully established a technique of intrauterine stem cell transplantation combined with fetal surgery, microsurgery and microinjection by mesenchymal stem cells (MSCs) transplantation into rat fetal spinal column. And we have found the transplanted MSCs survived, migrated and differentiated into various neurons in the defective spinal cord. But the prenatal surgery for local transplantation of stem cells was traumatic to fetal spinal cord and pregnant rats, and was only performed at late pregnancy, at the developmental stage the irreversible damages are eventually formed. Here, with the NTDs rat fetal model ex vivo and in vivo, we introduced a safe and effective transplantation approach that delivered MSCs into amniotic cavity of early embryos to treat NTDs. We

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found transplanted MSCs spontaneously migrated and specifically engrafted into the malformed tissues of early NTDs embryos cultured ex vivo. The highest engraftment rate (21.95%) of MSCs was obtained in the embryos with serious NTDs, while the engraftment rate was only 0.034±0.014% in normal embryos. The time-lapse analysis further confirmed the capability of MSCs to spontaneously migrate into malformed neural tube. And with the RNA-seq technique, we identified many chemokines especially the HGF/c-Met signaling was associated with the regulation of MSCs homing to defective neural tube. Otherwise, the increased expression of chemokines was also observed in the MSCs transplanted amniotic fluid by protein microassay technique. The spontaneous migration of MSCs into the defect spinal cord was also demonstrated by intra-amniotic injection in vivo. The engrafted MSCs in the embryos could differentiate into neurons, glias, epidermis cell and myocytes depending on the niches they survived. Meanwhile, the transplanted MSCs could enhance the expression of neuroprotective factors in the damaged neural tube and amniotic fluid, which could improve the microenvironment of embryo development. Finally, skin lesion analysis and electrophysiological test demonstrated the as compared with the NTDs fetuses with no MSCs injection, the skin lesion area in the fetuses with intra-amniotic MSCs transplantation was reduced 29.94%, and a short latency and a higher amplitude of MEPs were also achieved in these fetuses. We concluded that intra-amniotic MSCs transplantation could not only achieve the effect of skin repair similar to fetal surgery, but also resulted in a clinically relevant improvement in neurological function in the NTDs embryos via the neural/epithelial regeneration and neuroprotection. Moreover, besides the defective neural tube, the transplanted MSCs also migrated into other abnormal tissues such as abnormal craniofacial region and hypoplastic heart. This suggested that intra-amniotic MSCs trans-plantation may serve as a promising therapeutic intervention to treat multiple malformations. The intra-amniotic injection approach is easy to operate and could allow delivery of stem cells before the formation of irreversible damage. And the early embryonic microenvironment in which tissues and organs rap-idly grow is conducive to differentiation of MSCs into mature cells. In addition, the immature immune system of embryos would not reject the allogeneic stem cells. This treatment method might not fully repair all the congenital deformities, but would result in partial recovery. It could be used as an effective adjuvant treatment for the late corrective surgery, and has good prospects for clinical use.

Keywords: Prenatal treatment, intra-amniotic cell transplantation, spontaneous migration, neural functional recovery, epithelial regeneration

Poster 10

DIRECT INDUCTION OF FUNCTIONAL NEURONAL CELLS FROM FIBROBLAST-LIKE CELLS DERIVED FROM ADULT HUMAN RETINA

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Obtaining and manipulating neuronal cells are critical for neural biology basic mechanism studies and translational applications. Recent advances in protocol development and mechanism dissections have made direct induction of neuronal cells from other somatic cells (iN) a promising strategy for such pur-poses. In this study, we established a protocol to expand a population of fibroblast-like cells from adult human retinal tissues, which can be reprogrammed into iNs by forced expression of neurogenic transcription factors. Interestingly, the combination of Ascl1, Brn2, Myt1l, and NeuroD1 transcription factors, which has been demonstrated to be sufficient to reprogram human embryonic and dermal fibroblasts into iNs, failed to reprogram the fibroblast-like cells from human retinas into iNs. Instead, supplementing Ascl1 with Pax6 sufficed to convert the cells into iNs, which exhibited a typical neuronal morphology, expressed neural marker genes, displayed active and passive neuronal membrane activities, and made synaptic communications with other neurons. Moreover, iNs converted from retina-derived fibroblast-like cells contained high ratios of γ-Aminobutyric acid- (GABA-) and tyrosine hydroxylase- (TH-) positive neurons. Thus, the present study proposes a protocol that makes use of discarded retinal tissues from eye banks for iN generation, and suggests that different sources of somatic cells require different iN induction recipes and may also affect the iN subtype outputs. Our study may also facilitate the future development of methods to convert resident cells in situ into retinal neurons for treating retinal degeneration disease purpose.

Keywords: Direct reprogramming, Induced neuronal cell, Retina

POSTER ABSTRACTS

Poster 15

SMALL MOLECULE-BASED LINEAGE SWITCH OF HUMAN ADIPOSE-DERIVED STEM CELLS INTO NEURAL STEM CELLS AND FUNCTIONAL GABAERGIC NEURONS

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Cellular reprogramming using small molecules (SMs) without genetic modification provides a promising strategy for generating target cells for cell-based therapy. Human adipose-derived stem cells (hADSCs) are a desirable cell source for clinical application due to their self-renewal capacity, easy obtainability and the lack of safety concerns, such as tumor formation. However, methods to convert hADSCs into neural cells, such as neural stem cells (NSCs), are inefficient, and few if any studies have achieved efficient reprogramming of hADSCs into functional neurons. Here, we developed highly efficient induction protocols to generate NSC-like cells (iNSCs), neuron-like cells (iNs) and GABAergic neuron-like cells (iGNs) from hADSCs via SMs without genetic manipulation. All induced cells adopted morphological, molecular and functional features of their bona fide counterparts. Electrophysiological data demonstrated that iNs and iGNs exhibited electrophysiological properties of neurons and formed neural networks in vitro. Microarray analysis further confirmed that iNSCs and iGNs underwent cellular reprogramming toward a neural fate. Together, these studies provide rapid, reproducible and robust protocols for efficient generation of functional iNSCs, iNs and iGNs from hADSCs, which have utility for modeling disease pathophysiology and providing cell-therapy sources of neurological disorders.

Keywords: adipose-derived stem cells, lineage switch, neuron, nervous

Poster 17

DIFFERENTIATION EFFICIENCY OF HUMAN INDUCED PLURIPOTENT STEM CELL (IPSC) TO ENDOTHELIAL CELLS IN PATIENTS WITH END STAGE RENAL DISEASE (ESRD)

Ko Eun Jeong[1], Kyoung Woon Kim[2], Bo Mi Kim[2], Chul Woo Yang[1], and Byung Ha Chung[1]

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Human induced pluripotent stem cell-derived endothelial cells (hiPSC-ECs) could be promising for treatment of renal disease. However, it is unclear whether hiPSC could be differentiated to endothelial cell (EC) in ESRD patients. Therefore, we first sought to generate hiPSC from peripheral blood mononuclear cell (PBMC) of ESRD patient, then compared the efficiency of hiPSC lines differentiating into ECs with healthy control. The hiPSC-ECs were generated from differentiation of hiPSCs using vascular endothelial growth factor (VEGF) and bone morphogenetic protein-4 (BMP-4). At first, the expression of iPSC markers (NANOG, SSEA-4, and TRA-1-81) were assessed with confocal laser scanning microscopy, then hiPSC-ECs were purified based on positive expression of CD31. Subsequently, expression of endothelial markers (CD31, CD 34, and CD 133) were assessed with flow cytometric analysis. After 6 days in cell culture, stain with pluripotency markers (NANOG, SSEA-4, and TRA-1-81) on confocal image revealed iPSC were successfully generated in both healthy control and ESRD patient. Upon magnetic purification based on CD31+ expression, the hiPSC-EC population was observed to display typical endothelial surface markers in both groups (CD31, CD34, CD133, vWF, and Flt). However, hiPSC-ECs from ESRD patient showed much lower colonies of co-expression of CD31/CD34, CD31/CD133, and CD34/CD133 in FACS, compared to normal control. This was consistent with that the percentage of CD31 expression cell or co-expression of CD31/CD34 cells to total cells were much lower in ESRD group compared to that of healthy control. In conclusion, the efficiency of hiPSC differentiating into ECs in ESRD patient were diminished compared to healthy control.

Keywords: iPSC, ESRD, Endothelial cell

POSTER ABSTRACTS

Poster 18

COMPARISON OF 2-DIMENSIONAL AND 3-DIMENSIONAL HUMAN IPSC NEURAL INDUCTION METHODS FOR THE GENERATION OF NEURAL PROGENITOR CELLS (NPCS)

Andras Dinnyes[1], Abinaya Chandrasekaran[2], Hasan Avci[2], Anna Ochalek[2], Kinga Molnar[3], Lajos Laszlo[3], Tamas Bellak[2], Krisztina Pesti[4], Arpad Mike[5], and Julianna Kobolak[2]

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Human induced pluripotent stem cells (hiPSCs) derived from patient samples provide useful tools to model pathologies of the central nervous system following in vitro differentiation into disease relevant cell types. Our aim was to compare the efficiency of monolayer (2D) induction with the spheroid based (3D) induction method to generate neural precursor cells (NPCs), neurons and astrocytes. Neural differentiation was analysed for gene expression by qRT-PCR and on protein level by immunocytochemistry for NPC (SOX2, PAX6, NESTIN), neuronal (MAP2, TUBB3), cortical layer (TBR1, CUX1) and astrocyte markers (GFAP, AQP4). Electron microscopic investigation demonstrated that both methods resulted in morphologically similar neural rosettes. The evaluation of SOX1 and SOX9-expressing cell populations showed a higher percentage of SOX1 positive NPCs obtained with the 2D method. 3D induction yielded longer neurite lengths than 2D. Patch clamp analysis of electrophysiological activities revealed spontaneous and evoked synaptic activity in both of the 2D and 3D systems, and no significant differences were detected between the two methods and among hiPSC lines with different genetic background. In conclusion both 2D and 3D neural induction methods were suitable for the differentiation of hiPSCs into comparable, functional cortical neurons, suitable to be utilised further for in vitro disease modelling, drug development and developmental neurotoxicology.

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Keywords: human iPSC, neuronal progenitor cell, 3D organoids

Poster 19

DIFFERENTIAL DIFFERENTIATION OF DOPAMINERGIC CELLS WITH A PURINE RECYCLING DEFICIENCY

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Lesch-Nyhan syndrome (LNS) is a purine recycling disorder caused by mutations in HPRT1 and includes features such as dystonia and self-aggressive behaviour. Human studies have repeatedly implicated dopamine dysfunction in the disorder, but it remains unclear how mutations in HPRT1 lead to dysfunctions in the dopamine system. To address this question we made iPSC-derived forebrain neurons from 3 LNS patients and controls, and iPSC-derived midbrain neurons from 5 LNS patients and controls, all from fibroblasts. We deleted HPRT1 from two independent control cells lines and made forebrain and midbrain neurons simultaneously from these and isogenic control cells. We developed a novel methodology to produce midbrain neurons, which resulted in >90% cells becoming tyrosine hydroxylase positive. Metabolite and HPLC measurements showed a 4-fold reduction in dopamine in mature midbrain, and 5-fold increases in hypoxanthine in all cells with mutated HPRT1 suggesting these cell models recapitulates disease. We performed gene expression and metabolic profiling across all cell lines at two developmental timepoints, and we provide evidence that dopaminergic cells with HPRT1 dysfunction take on characteristics of glutamatergic cells in early development, including increases in VGLUT2 and decreased expression of TH. No alterations in glutamategic markers were observed in forebrain cells. This is the first large scale analysis of iPSC derived dopaminergic cells in HPRT mutant cells, and the data provide a novel link between purine recycling and altered cell fate determination.

Keywords: Dopamine, Crispr/Cas9

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THE HYPOXIA/HIF-1ALPHA PATHWAY REGULATES ENERGY METABOLISM DURING ENGINEERED CHONDROGENESIS

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Articular cartilage has very limited capacity for repair following injury or degeneration. The reparative stem cells or chondrocytes are readily located in a hypoxic microenvironment during cartilage repair. Hypoxia inducible factor-α (HIF-α) is identified as a key mediator for adaptation to low oxygen tension. However, the molecular mechanisms of hypoxia/HIF-α regulation on energy metabolism in chondrocytes remain unclear. Here, we examined the effects of hypoxia environment on chondrogenesis in the three dimensional (3D) culture system. The chondrocytes were isolated from the ribs of new-born mice and cultured under defined conditions in micromass culture or 3D culture. Hypoxia up-regulated HIF-1α and downstream target genes expression indicated by real-time PCR analysis. The chondrogenic differentiation under hypoxia was enhanced indicated by increased proteoglycan synthesis and chondrogenic marker genes expression. The increased proliferation and differentiation of chondrocytes in the 3D culture is accompanied by up-regulation of Glucose transporter 1 (Glut1) and Glut4 levels. At cellular level, 6-phosphofructokinase, liver type (PFKL) and PGK1 mRNA and protein were dramatically up-regulated in chondrocytes under hypoxia than that of normoxia revealed by PCR array analysis and Western blot respectively. In addition, the expression of genes involved in glycolytic metabolism, such as Hexokinase 2 (Hk2), Pyruvate dehydrogenase kinase, isozyme 1 (Pdk1), Lactate dehydrogenase a (Ldha) was upregulated in chondrocytes under hypoxia compared with that of normoxia. This was accompanied by enhanced glucose uptake and lactate production of chondrocytes. Deletion of HIF-1α in chondrocytes carrying loxp flanked HIF-1α allele eliminated the expression of the above genes associated with glycolysis. Interestingly, glutamine, the second source of carbon and first supplier of nitrogen for cell energy, was shown to be increased in chondrocytes under hypoxia than that of normoxia. This was accompanied by upregulation of glutaminase 2 (Gls2) gene expression, a key gene involved in catalyzing glutamate production from glutamine. The results indicate that HIF-1α functions as an important mediator to regulate energy metabolism of chondrocytes during engineered chondrogenesis.

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THE INTRACELLULAR REGION OF BETA-DYSTROGLYCAN IS REQUIRED FOR NORMAL CAVEOLIN-3 TRAFFICKING AND ION CHANNEL FUNCTIONS IN HIPSC-DERIVED CARDIOMYOCYTES

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The dystrophin glycoprotein complex (DGC) plays a key role in maintaining normal functions of skeletal muscle cells and cardiomyocytes. Mutations in the DGC can cause cardiomyopathy, the leading cause of advanced heart failure. However, the precise mechanisms leading to the deterioration of cardiac function remain poorly understood due to many aspects. Dystroglycan, a transmembrane protein linking the extracellular matrix and the cytoskeleton, is a fundamental component of DGC. As another important member of DGC, caveolin-3 involves in the regulations of electrophysiological activities and can inter-act with β-dystroglycan (a subunit of dystroglycan). Utilizing genome editing approaches and human induced pluripotent stem cells (hiPSCs), we were able to create several mutant hiPSC models (DMD-KO, DAG1-KO, DAG1-ΔL14 and DAG1-ΔCyto) to investigate the interactions between dystroglycan and cave-olin-3 in cardiomyocytes. Additionally, hiPSCs from a 9-year dystrophin-null patient (DMD-iPS) and controls have been established following transduction with reprogramming vectors. These would help us to understand the mechanisms and pathophysiology underlying disease progression. We then used a chemically defined protocol to differentiate these hiPSCs into cardiomyocytes. Western blots and immunofluorescence analysis confirmed that the DGC was disrupted in mutant hiPSC-derived cardiomyocytes. The immunofluorescence data indicated that most caveolin-3 proteins were located in Golgi apparatus of hiPSC-derived cardiomyocytes at early stages of differentiation and gradually transported to membranes during maturation in vitro. Interestingly, cave-olin-3 proteins from DAG1-ΔCyto and DMD-iPS mutants were trapped in the Golgi apparatus even cultured for 3 months. Meanwhile, the recovery of sodium currents from fast inactivation was impacted in

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the same mutant cells. This defect was only observed after 90 days' culture. Calcium image recordings displayed similar results that the 3-month old DAG1 or DMD-mutant cardiomyocytes needed a longer time for calcium reuptake compared to controls. Live-cell imaging system discovered that caveolin-3 trafficking was disturbed in those mutant cardiomyocytes. The restoration of dystroglycan in DAG1-mutant cardiomyocytes successfully rescued the aberrant localization and expression of caveolin-3. Collectively, our results indicate that the intracellular region of β-dystroglycan plays an essential role in forming the complex with dystrophin to maintain normal functions of sodium and calcium channels and trafficking of caveolin-3 in hiPSC-derived cardiomyocytes.

Keywords: β-dystroglycan, caveolin-3, cardiomyocyte, trafficking, ion channel

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HIGH EFFICIENCY CLONING OF HUMAN PLURIPOTENT STEM CELLS USING CLONER™ TO FACILITATE GENE-EDITING AND DISEASE MODELLING

Andrew Gaffney[1], Adam Hirst[1], Yolanda Chang[1], Alicia Zhang[1], Jessica Norberg[1], **Yu Sun**[2], Wing Chang[1], Melanie Kardel[1], Arwen Hunter[1], Arthur Sampaio[1], Terry Thomas[2], Allen Eaves[4], Stephen Szilvassy[1], Sharon Louis[1], and Vivian Lee[1]

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Recent advances in gene-editing techniques such as CRISPR, have led to more accessible and cost effective methods to generate variant human pluripotent stem cell (hPSC) lines for a wide range of research areas. However a major hurdle for gene-editing in hPSCs is the extremely low cloning efficiency of these cells (< 5%), making the generation of clonal cell lines an inefficient process. To address this hurdle we have developed a novel hPSC cloning supplement, CloneR™. To optimize the formulation of CloneR™, cells were seeded at a clonal density (25 cells/cm2) in multi-well plates precoated with Vitronectin-XF™ containing mTeSR™1 or TeSR™-E8™ and CloneR™ or 10 μM Y-27632. The cultures were expanded for 7 days and the resulting colonies in each well were stained for alkaline phosphatase and counted. Cloning efficiencies of 28.1 ± 6.3% and 24.3 ± 6.7% (mean ± SD, n=12) were achieved in mTeSR™1 and TeSR™-E8™ containing the CloneR™, respectively using four independent hPSC lines. This is a significant increase when compared to mTeSR™1 (4.9 ± 2.3%) and TeSR™-E8™ (1.6 ± 1.1%) supplemented with 10 μM Y-27632. CloneR™ was further validated using the more stringent method of single cell deposition. Specifically, hPSCs were dissociated to single cell suspensions and sorted into individual wells (1 cell/well) pre-coated with Vitronectin-XF™ and containing 100 μL of mTeSR™1 with CloneR™ or 10 μM Y-27632. Cloning efficiency was significantly higher across all cell lines tested (H1: 18.9%, H7: 15.8%, WLS-1C:17.9% and STiPS-M001: 27.4%) compared to control mTeSR™1 containing 10 μM Y-27632 (H1: 2.1%, H7: 3.2%, WLS-1C: 5.3% and STiPS-M001: 3.2%). Five H1 hESC and WLS-1C hiPSC subclones were manually picked and expanded in mTeSR™1 for 5 passages. All clonally established hPSC lines displayed similar morphology, expansion rates and proportions of undifferentiated cells compared to the non-clonal control hPSC lines. To demonstrate the utility of this supplement CRISPR/Cas9 and CloneR™ were used to edit KCNH2 (hERG) and efficiently derive a clonal heterozygous KCNH2 mutant hiPSC line. The dele-tion produced an early stop codon in the PAS domain located in the N-terminal region of the hERG channel and is expected to induce Long QT syndrome. Control and edited isogenic hiPSC lines were differentiated to cardiomyocytes using the STEMdiff™ Cardiomyocyte Differentiation Kit. Single cell cloning efficiency was 20% for a seeding density of 7 hiPSCs per cm2. The clonal control and edited isogenic hiPSC lines were differentiated to cardiomyocytes. The isogenic hiPSC-CMs expressed high levels of cardiac troponin T (>80%). Electrophysiological characterization was performed on hiPSC-CMs 28 days after differentiation. hiPSC-CMs with the heterozygous hERG deletion had prolonged field potential durations (FPDs) with an irregular excitability profile and beat period. The control hiPSC-CMs had shorter FPDs and a stable excitability profile. In summary, supplementing TeSR™ media with CloneR™ improves the single cell cloning efficiency of hPSCs which can advance gene-editing studies by facilitating the rapid and successful generation and establishment of new and clonal hPSC cell lines required for those studies.

Keywords: Gene-editing, hPSC, Efficiency

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STEMDIFF™ CEREBRAL ORGANOID KIT SUPPORTS EFFICIENT GENERATION OF BRAIN ORGANOIDS FROM HUMAN PLURIPOTENT STEM CELLS

Yu Sun[1], Leon H. Chew[1], Adam Añonuevo[1], Sam Lloyd-Burton[1], Andrew Gaffney[1], Allen C. Eaves[2], Terry E. Thomas[1], Sharon A. Louis[1], and Vivian M. Lee[1]

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2-D neural cultures derived from human pluripotent stem cells (hPSCs), including human embryonic and induced pluripotent stem cells (hESCs or hiPSCs), are useful models with which to study the nervous system, but they are limited in their capacity to fully recapitulate the complex organization of brain tissues. Lancaster et al. (Nature 2013) established a hPSC-based organoid culture system that models the major features of early human brain development. Based on the published media formulations, we developed the STEMdiff™ Cerebral Organoid Kit to enable generation of organoids in a simple and highly reproducible manner. This kit contains 2 basal media and 5 supplements, which are combined to prepare four separate complete media corresponding to the 4 stages of cerebral organoid formation. hPSCs maintained in mTeSR1™ were single-cell dissociated and cultured in Embryoid Body (EB) Formation Medium (day 1 - 5, Stage 1). The resulting EBs were then transferred to Induction Medium (day 6 - 7, Stage 2); next, they were expanded by embedding in Corning® Matrigel® and cultured in Expansion Medium (day 7 - 10, Stage 3). The expanded organoids were then cultured in Maturation Medium, with agitation, for extended periods of time (day 10 - 40+, Stage 4). Morphological analysis of organoids was performed on days 5, 7, 10 and 40, which are the endpoints of Stages 1 - 4 respectively. Organoids at Day 40 were analyzed by RT-qPCR or cryosectioned and processed for immunofluorescence (>3 organoids per analysis; 2 hESCs, n = 2 and 2 iPSCs, n = 2). We achieved high efficiencies across multiple cell lines (2 hESCs, n = 2 and 2 iPSCs, n = 2) for EB generation (100% success, n = 128/128), expansion (>95% exhibited extensive folding of neuroepithelia, n = 104/107) and maturation (>60% of organoids were >1 mm in diameter with dense cores , n = 62/94). In vivo, the human cortex consists of progenitor and neuronal populations that organize into distinct layers. The mature organoids generated here exhibited a similar architecture with neural progenitors (SOX2+, PAX6+) localized in apical regions surrounding a central ventricle. Adjacent to the apical progenitors, neuronal progenitors (TBR2+, Ki-67+) were found abutting neurons (CTIP2+, MAP2+, TBR1+), resembling the intermediate zone and cortical plate regions. Furthermore, we examined the transcriptome profile of day 40 organoids by RNA-Sequencing and compared our data to published data sets (Luo et al. 2016 Cell Reports). Our results indicate that the gene expression profile of the cerebral organoids generated using the STEMdiff™ kit aligns closely to published organoids.

Keywords: organoid, cerebral

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AN IPSC-BASED HIGH THROUGHPUT PLATFORM FOR DEVELOPMENT OF NOVEL THERAPEUTICS FOR TREATMENT OF ALZHEIMER'S DISEASE

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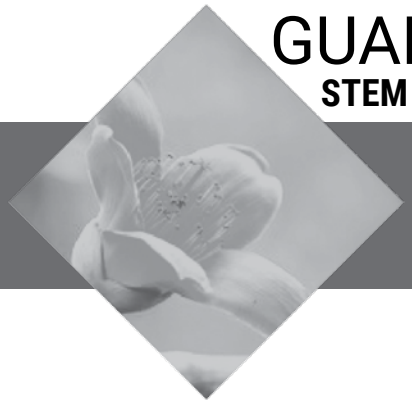
Alzheimer's disease (AD) is a complex neurodegenerative disease that affect millions of people worldwide and impose huge medical and economic burden on human society. Despite decades of efforts that have consumed tremendous resources, drug development for treatment of AD has been particularly ineffective. There are two major hurdles for AD drug development: one is lack of suitable cellular models for drug candidate screening and validation, and the other is lack of enough diversity in existing compound libraries. To overcome those hurdles, we have leveraged somatic cell reprogramming and genome editing technologies to create a novel cellular model that displays AD phenotype in vitro. In addition, we have streamlined the manufacturing process for large scale production of AD-relevant neuronal cells and established a high throughput platform for drug screening. Using this platform we have conducted a preliminary screening of natural chemical compounds and herb extracts derived from Traditional Chinese Medicine (TCM). Our results indicate that combinations of suitable cellular models and the diversity of natural compounds derived from TCM offers a golden opportunity for development of novel therapeutics for treatment of AD. This presentation will discuss the major characteristics of our platform and its application for AD drug development as well as the results from our preliminary screening.



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GENERATION OF ISOGENIC HUMAN G2019S PARKINSON'S MODEL

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Studying Parkinson's disease (PD) progression is difficult due to multifactorial nature of the disease causes and the lack of access to diseased dopaminergic (DA) neurons. Mutations in leucine rich repeat kinase 2 (LRRK2) is one of the known causes for PD. Various studies have shown LRRK2 mutation, G2019S, causes phenotypic defects in DA neuron. However, the exact mechanism is not clearly understood. Patient-derived induced pluripotent stem cells carrying G2019S mutation were used to study phenotypic signs of diseased DA neurons but does not account for the varied genetic background of the individual patient. Here, we generate an isogenic stem cell line of G2019S based on H9 human ES cell line using CRISPR technology. Sanger sequencing was performed to check the off-target effects of CRISPR gene editing technique. Validations of the stemness of isogenic lines were performed with various methods: immunofluorescence, RT-PCR, teratoma formation and karyotyping. Our results showed that the isogenic line retain the stemness and can be differentiated into various neurons. Functional analysis of DA neurons derived from this isogenic line will be carried out to examine the effects of the mutation and understand the mechanism of the PD disease.

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HLA GENE RESTRICTION OF NEURONAL REGENERATION FROM NEURAL STEM CELLS TRANSPLANT IN HUMANIZED MOUSE MODEL

Yuchao Feng[1], Renrong Wei[1], Jinglong Guo[2], Jun Zou[2], Zheng Hu[2], Shouhai Wu1, Qingfeng Xie[1], Yongguang Yang[2], Yang Xu[1]* and **Tongxiang Lin**[1]

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Neural stem cells and other stem cell derivates have long been investigated in regenerative medical trials for incurable brain diseases. However, the most clinical trials failed of negative results due to immunological incompatible. The human leukocyte antigen (HLA) system is a gene complex encoding major histocompatibility complex proteins in human. Here we report that regeneration of neurons from neural stem cell transplants of different HLA types arouse controversy results of neuronal differentiation in humanized mouse model. The in vitro neural stem cells for transplantations were derived from human induced pluripotent stem cells (iPSC) of the same HLA type embryos, proximate HLA type embryos with only one gene difference and the distant HLA type embryos with 2 more difference in detected region of the HLA genes. Further investigations showed that neural stem cell proliferation and neuronal differentiation from neural stem cells regulated by the HLA gene expression. While the same HLA gene expression neural stem cells could regenerate neural tissues with complete compatible, the derivates with proximate HLA typing might show immune impairment with some damaged neuron proliferation and differentiation, and the neural stem cells with unmatched HLA types might trigger immune attacks, might hardly be detected with development of neurons. Our data confirm HLA gene expression restriction of neuronal development from neural stem cell transplantations in humanized immunology mouse models and suggest that the regeneration treatment using allogenic neural cells have to consider immunogenic issue.

Acknowledgement: We are particularly grateful for the following grant support: The National Basic Research of major development projects (973 project, Lin Tongxiang, 2013CB9669002); Guangdong Provincial Science and Technology Department funded projects (Lin Tongxiang, 015A020212032); Guangdong Provincial Hospital project funds (Lin Tongxiang, YK2013B2N08).

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ANTIOXIDATIVE EFFECTS OF ARTEMISITENE IN NEURAL CELLS DERIVED FROM HUMAN IPSC

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While some Chinese medical herbal formulas are effective in clinical treatment, the formulas are too complex in components and perplexing in mechanisms to be optimized. To elucidate traditional Chinese herbs which have been proved efficient in clinical treatments for neural degenerative diseases, we have established a drug screening platform of in vitro neural stem cell based on human induced pluripotent stem cells (iPSCs). By using the human iPSCs based neuronal cell differentiation system, we mimic the neural cells damages in vitro with hydrogen peroxide to study the mechanisms of the neurodegerative diseases, and to analyze the antioxidant effects of Chinese herbal components. After screening the Chinese herbal components using the aforementioned platform, we found some potential effective components of Chinese formula. A small molecule, Artemisitene, from a Traditional Chinese Herb Artemisia annua Linn which is famous herbal medicine for malaria treatment but not for neurodegenerative disease, could significantly protect neural cells against oxidative agent H2O2 treatment. Artemisitene treatment significantly activated neuronal gene expressions, such as Tuj1 and TH, under oxidative damage. Further q-PCR analysis data showed that the herbal compound also significantly activated the expression of many neural stem cell genes, including Nestin, Pax6, Nurr1 and PITX3. The mechanism analysis data showed that it strongly reduced the levels of reactive oxygen species (ROS), synergized with active antioxidant signaling pathway genes, such as HO-1 and NQO1 and GPX2. We therefore recommend that artemisitene might be used as a candidate drug for antioxidant therapy in neural degenerative diseases. The drug study platform of in vitro neural cells based on iPSC technology might be further used for neural drug screening and mechanism studies of potential neurodegenerative drugs.

Acknowledgement: We are particularly grateful for the following grant supports: The Major State Basic Research Development Program of China (973 Program, Lin Tongxiang, 2013CB9669002); Guangdong Provincial Science and Technology Department Research Funds (Lin Tongxiang, 2015A020212032); Guangdong Provincial Hospital Research Funds (Lin Tongxiang, YK2013B2N08).

Keywords: iPSC, neural stem cells, dopaminergic neurons, antioxidant, reactive oxygen species (ROS) Artemisitene, Neurodegenerative diseases.

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HIGH-THROUGHPUT AND HIGH-SPEED DATA ACQUISITION OF COMPOUNDS RESPONSES ON CALCIUM OSCILLATION OF HUMAN IPSC-DERIVED CORTICAL NEURONS

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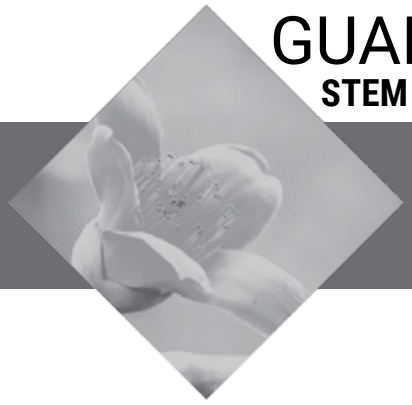
There is a surge of interest in high-throughput fluorescence measurement of calcium oscillations in neuronal cultures, both normal and disease models. However, progress has been limited by the long imaging acquisition time for an entire 96- or 384-well plate, and also by the heterogeneity of neurons derived from human iPSCs. With the advent of new camera technologies as well as availability of highly enriched, functionally mature human cortical neurons derived from iPSCs, these limitations can now be overcome. We utilized a newly developed fast data acquisition protocol (200 Hz for 96-well plate) on the FDSS/µCELL calcium imager to capture fluorescence measurements of calcium oscillations from human cortical neurons. These cortical glutamatergic neurons were seeded in rapid maturation medium in 96-well plates, loaded with the calcium indicator dye Cal-520, and calcium dynamics were recorded. We evaluated cultures at different stages of development (6–21 days in vitro) and at different neuronal densities by monitoring calcium activity over 10-minute periods with high data acquisition speed. Several different culture media and measurement buffers were also evaluated to determine the optimal condition for producing neuronal oscillation. Finally, we applied a set of excitatory and inhibitory agonists and antagonists to study the contribution of voltage-gated ion channels and AMPA, NMDA and GABA receptors to neuronal oscillation.

Keywords: neurons, cardiomyocytes, calcium



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HOMOGENEOUS MIDBRAIN ORGANOIDS FROM HUMAN PLURIPOTENT STEM CELLS

Taehwan Kwak, Jonghun Kim, Ji Hyun Kang, Sai Hali, Hyewon Chung, and Dong Wook Han

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Recent studies have successfully described the generation of organoid, a self-organized miniature organ-like structures differentiated from pluripotent stem cells under three-dimensional (3D) culture conditions. The organoids are able to recapitulate the physiological and structural features of in vivo organs such as liver, kidney, intestine, and even brain with distinct regional identities. Brain organoids consist of region-specific neuronal cells and organized with distinct layer structures of their corresponding in vivo brain regions. Notably, recent studies demonstrated that organoids mimicking human midbrains in terms of their microanatomy, molecular characteristics, and neuronal functionality could be generated from human pluripotent stem cells (PSCs) and neural stem cells (NSCs). However, heterogeneous cellular composition of PSC-derived midbrain organoid and absence of typical layer structure in NSC-derived midbrain organoid have been considered as major obstacles for clinical translation of recent midbrain organoid technology. Here, we described an advanced and robust technology for generating the functionally mature midbrain organoids showing highly homogeneous cellular composition. 3D bioimaging analysis clearly demonstrated that our midbrain organoids have highly stratified layer structure of midbrain DA neurons and progenitors. Our midbrain organoids can provide a more suitable and reliable platform not only for understanding midbrain specification during development but also for modeling midbrain-related neurodegenerative diseases such as Parkinson's disease.

Keywords: Organoid, Midbrain, Heterogeneity, Three-dimentional culture

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CEREBRAL ORGANOIDS AS A PLATFORM FOR AUTISM SPECTRUM DISORDER MODELING

Dong Wook Han, **Hali Sai**, Tae Hwan Kwak, Jonghun Kim, Seon In Hwang, and Kyu-ree Kang

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The generation of three-dimensional organoids which share the key structural and cellular features of various organs by inducing the self-organization of pluripotent stem cells (PSCs) might provide an advanced novel platform for understanding the organ development as well as modeling various diseases in vitro. Indeed, the recent studies demonstrated that cerebral organoids generated from human PSCs could recapitulate the micro anatomy and molecular characteristics of human cerebral cortex, suggesting their potential application for modeling CNS diseases. Autism spectrum disorder is a neurodevelopmental disorder, most cases lack a clear etiology. Here we use the cerebral organoid derived from iPSC to investigate neurodevelopmental alterations in patients with idiopathic ASD.

Keywords: Cerebral organoid, Autism spectrum disorder, CNS disease, neurodevelopment

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LIVER ORGANOID GENERATION FROM HUMAN PLURIPOTENT STEM CELLS USING THE STEP-WISE PROTOCOL

Jonghun Kim, Taehwan Kwak, Sai Hali, Ji Hyun Kang, and Dong Wook Han

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Organoid, a miniaturized three-dimensional (3D) organ-like structure which is self-organized from pluripotent stem cells (PSCs) have a great potential for both disease modeling and drug screening. Recent many studies described the generation of organoids recapitulating actual organ morphogenesis and development. In the case of liver, liver organoid from human PSCs have not been described, although liver organoids from in vivo liver tissues have been well documented. Recently, it was shown that human PSCs could be self-organized into liver organoids using ectopic expression of GATA6. However, the resultant organoids exhibited the heterogeneous structural characteristics compared with in vivo liver tissues. Indeed, early neuronal tissues were frequently observed in the liver organoid. Moreover, the organoids could not be maintained in vitro over two weeks, resulting in immature hepatic properties. Here we described the step-wise protocol for robustly generating 3D liver organoids which could stably be maintained over two months. Our organoids display very homogenous population in which no ectodermal cells or tissues were observed. Instead, they were completely full of ALB-positive hepatocytes. Our novel approach for generating 3D liver organoids might serve as a useful platform for understanding various liver diseases as well as drug discovery.

Keywords: Organoid, Liver, Pluripotent stem cells

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A SCALABLE APPROACH FOR THE GENERATION OF HUMAN PLURIPOTENT STEM CELL-DERIVED HEPATIC ORGANOIDS WITH SENSITIVE HEPATOTOXICITY FEATURES

Tobias Cantz[1,2,6], Zhen Dai[2,3], Robert Zweigerdt[4], Amar Deep Sharma[2,3], Michael Ott[2,5], and Malte Sgodda[1,2]

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The quest for physiologically-active human hepatocyte-like cells for in vitro research and drug screening is high. The recent progress in the field of pluripotent stem cell (PSC) -derived hepatic cells within the last decade brings those cells closer to applications in translational medicine. However, the classical two-dimensional (2D) cell culture systems are of limited use, because relevant cell-cell interactions based on cell polarity, which is a major prerequisite for proper hepatic cell metabolisms, are not provided. Here, we report a scalable 3D suspension culture system, in which PSC-derived hepatic cells can be maintained for up to three weeks with stable gene expression profiles and metabolic features in a suspension culture system ranging from a 1.5 ml up to a 15 mL. Adjustments of culture conditions and, most importantly, the size of the organoids resulted in the robust generation of hepatic organoids consisting of a quite homogenous cell population. Importantly, the generation of these hepatic organoids was highly reproducible and allowed, in contrast to hepatic PSC-derivatives in 2D-culture conditions, a sensitive assessment of acetaminophen-related toxicity, the most common source for drug-induced liver failure.

Keywords: hepatic differentiation, organoid culture, toxicity assays

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IDENTIFICATION AND CHARACTERIZATION OF FUNCTIONAL ENHANCER ELEMENTS IN MESCS BY FAIRE-STARR-SEQ

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Max Planck Institute for Molecular Genetics, Germany

Embryonic stem cells (ESCs) are characterized by the ability to differentiate into any given cell type and therefore display a versatile tool to study gene regulation with the possibility to investigate different states of differentiation and cellular identity. Gene expression networks determine cell type specificity and are tightly regulated by transcription factors (TF) and their targeted regulatory genomic elements (enhancers). To date enhancers are mainly identified by indirect measures of activity such as correlating histone modifications and rely on multiple cell type-specific data sets e.g. ChIP-seq for histone marks and open chromatin assays. However, these modifications have been shown not to directly/quantitatively correlate with enhancer activity. The question arises, which of the thousands of enhancers, as predicted by chromatin marks, are functionally active, how the enhancer usage changes during differentiation and what features are determining. To elucidate the regulatory landscape of murine ESCs, we apply a massively parallel reporter assay, termed STARR-seq (self-transcribing active regulatory region sequencing; Arnold et al., 2013) assessing genomic DNA fragments prepared from accessible mESC chromatin by FAIRE (Formaldehyde-Assisted Isolation of Regulatory Elements) for their putative enhancer function. Here, functional enhancers drive their own expression from a reporter construct and their activity can be directly assessed by targeted sequencing. Hence, the obtained data can be used to generate a genome wide quantitative map of functional mESC enhancers in native state and after induced differentiation. The comparison to parallel generated ChIP-seq data for histone modifications, data for open chromatin and TF motif search will show different enhancer subsets characterized by distinct features which might display different functional units of regulatory elements. Specifically, we want to investigate enhancers of developmental regulators in comparison to housekeeping or cell type-specific genes are associated with different distinct chromatin marks. Furthermore, the combined integration of these data sets from mESCs can be used to generate a computational model to predict functional enhancers and their activity in a given cell type, where the STARR-seq data can be used for validation and correction of this model. Ultimately, this approach will lead to a better understanding of the complexity and composition of regulatory elements, as well as their plasticity during differentiation, and will contribute to a more precise prediction of enhancer activity.

Keywords: enhancer, gene regulation, differentiation, STARR-seq

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NANOG REGULATES ESCS PLURIPOTENCY THROUGH INVOLMENT IN ALTERNATIVE SPLICING AND MRNA PROCESSING

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Nanog is one of the core transcriptional factors that plays an important role in regulation of self-renewal and pluripotency maintenance through its transcriptional activity. Here we reported an expanded Nanog interactome which includes 75 novel Nanog associating proteins that were enriched in mRNA splicing processing. We found Nanog knockout led to switch of transcripts for a subset of pluripotency related genes. And some of these genes were found to be component of Hdac1 related complex and switch of transcripts result in integration of Hdac1 into different complex and exert its histone deacetylation activity in combination with different components after Nanog KO. At last we showed that Nanog may regulate splicing by binding sequences adjacent to the splicing sites of its target genes. Collectively, our work present a larger integrated Nanog-centered pluripotency network and reveal the possible role of Nanog in regulating pluripotency through regulation of splicing process.

Keywords: Nanog, mESC, Splicing regulation

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TARGETED GENE DISRUPTION BY CRISPR/CAS9-MEDIATED HOMOLOGY INDEPENDENT DUAL-REPORTER KNOCK-IN

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Targeted gene disruption in diploid somatic cells has been a challenge hindering in-depth study on gene functions in human cells. Recently emerged CRIS-PR/Cas9 technology and homology-independent DNA knock-in approach has offered a unique opportunity to achieve biallelic gene disruption at high efficiency. By targeting upstream non-coding and coding exons of CtIP gene, we demonstrate that simultaneous knock-in of dual-repoter genes, through CR-ISPR/Cas9-induced homology independent DNA repair and followed by cell sorting, permit one-step generation of cells carrying biallelic gene disruption. Using three different types of donor designs and targeting strategies, we have generated various knock-in clones devoid of intact wild type alleles of CtIP. Quantitative RT-PCR and western blot analysis have verified substantial reduction of corresponding mRNA and proteins respectively. The reduced activities in HDR pathway further confirmed the loss-of-function effect introduced by insertional disruptions of CtIP gene. This study demonstrates a universal dual-reporter system which could be applied to introduce biallelic gene disruption at high efficiency in human cell lines, through CRISPR/Cas9-mediated homo-logy independent knock-in strategy.

Keywords: Gene disruption, Homology independent DNA knock-in

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ASSESSMENT OF THE DEVELOPMENTAL NEUROTOXICITY OF ENVIRONMENTAL POLLUTANTS WITH A MOUSE EMBRYONIC STEM CELL MODEL

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In recent years, the use of embryonic stem cells (ESCs) in toxicological applications has revolutionized the field. In this study, we investigated the overall toxicity of three typical environmental pollutants, Bisphenol A (BPA), Bisphenol F (BPF) and Bisphenol S (BPS), with a mouse embryonic stem cell system. Our results indicate no obvious cytotoxic effects for the three chemicals on self-renewing ESCs, as demonstrated by cell viability and oxidative stress measurements, in the range of concentrations used (from 100 nM to 10 μM). However, BPA, BPF and BPS, showed significant developmental toxicity when we mimicked embryonic development in vitro by differentiating ESCs via embryoid body (EB) formation. In fact, we proved that BPA/BPF/BPS significantly down regulated ectoderm specification, especially neural ectoderm related gene such as Pax6, Nestin, Sox1 and Sox3, up to 20-day exposure. The neurotoxic effects of BPA/BPF/BPS were also clearly revealed in our two additional differentiation procedures specifically directed towards neural ectoderm and/or neural progenitor cells, via

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EB formation or in monolayer conditions. Contrary to the common understanding that BPA and its derivatives, BPF and BPS, cause toxicity by interfering with the estrogen receptor (ER) pathway, our Fulvestrant based antagonistic experiments revealed the neurotoxic effects of the three chemicals were not related with the ER signaling cascade. Moreover, RNA-Seq analyses of BPA/BPF/BPS treated samples, harvested at different time points during the global, and neural progenitor cell specific, EB-based differentiations, implied multiple molecular mechanisms of toxicity, including disruption of the axon guidance and Wnt signaling pathway. Taken together, our study confirmed that BPA/BPF/BPS are developmental toxicants affecting gene expression during neural differentiation. We also demonstrated that our stem cell toxicology system could serve as a noteworthy improvement over traditional cell toxicity assays.

Keywords: Developmental toxicity, Embryonic stem cells, BPA/BPF/BPS, Neural development

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REGULATION OF MESENCHYMAL STEM CELL SENESCENCE BY HISTONE DEMETHYLASES KDM3A AND KDM4C-DEPENDENT HETEROCHROMATIN REMODELING

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Cellular senescence is a process in which cells experience the gradual loss of proliferation and differentiation potential. Bone Marrow Stromal Cells (BMSCs) are extremely important adult stem cells for tissue homeostasis, regeneration and repair. The regenerative function of BMSCs declines in aged people. Previous work has shown that cellular senescence is accompanied by extensive spatial rearrangement of heterochromatin. However, the question of whether heterochromatin reorganization underlies BMSC aging and the mechanisms involved in initiating and maintaining this unique epigenetic state remain unclear. Here, we show that BMSC senescence is accompanied by a dynamic heterochromatin reorganization process. Moreover, we have identified two conserved histone H3 Lys 9 demethylases KDM3A and KDM4C, which cooperatively mediate the heterochromatin remodeling and homeostasis during MSC senescence. Mechanistically, KDM3A and KDM4C transcriptionally activate centromere packaging and chromosome condensation genes, and function as scaffold to facilitate the formation of heterochromatin complex. Aberrant expression of KDM3A or KDM4C disrupts the heterochromatin homeostasis and leads to heterochromatin disorganization. Conversely, deficiency of KDM3A or KDM4C results in exaggerated DNA damage response and progressive cellular senescence. Moreover, MSCs and bone tissues derived from Kdm3a knockout mice exhibit defective chromosome organization and exacerbated DNA damage response. Importantly, a marked downregulation of KDM3A and KDM4C associated with a decrease in H3K9me3/2, HP1 and chromosome organization genes is found in MSCs derived from old human individuals. In conclusion, our study have revealed a previously undefined role of histone demethylase in controlling MSC senescence. Moreover, the findings of our study provide novel insights into the chromatin reorganization that governs stem cell senescence and aging process.

Keywords: cellular senescence, histone demethylase

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IN VITRO AND IN VIVO BIOCOMPATIBILITY OF DECELLULARIZED SKELETAL MUSCLE SCAFFOLDS

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In decellularized tissues the stroma as well as original complex microstructure of the extracellular matrix (ECM) remain well preserved. Combination of acellular scaffolds with cells represents a great tool for production of new biological constructs in tissue engineering. The tibialis anterior obtained from C57Bl/6 mice was decellularized by osmotic shock followed by SDS extraction and peracetic acid to sterilize the bioscaffold; DNA was removed with DNase. After a thorough washing with PBS buffer the scaffolds were collected for chemical and microscopic characterization or for recellularization experiments. Light microscopy of paraffin-embedded sections proved absence of cell nuclei and cytoplasmic components in decellularized muscle. Transmission electron microscopy of the scaffolds revealed well preserved general microarchitecture including basal laminas and transversely striated collagen fibrils. Immunohisto-chemical analysis confirmed preservation of proteoglycans and adhesive glycoproteins such as laminins and fibronectin in decellularized scaffolds. Decellularized scaffolds were implanted to the tibialis anterior muscle of recipient mice. Histological analysis performed after 1, 2, and 3 weeks confirmed scaffold recellularization of the graft accompanied by mild infiltration of mononuclear cells that ceased at 4 weeks post implantation. The scaffolds recellularized with myoblasts were cultured for 3, 6, 9 and

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12 days in vitro. Histological examination confirmed biocompatibility of the scaffolds as these were successfully reseeded with cells demonstrating the ability of cells to survive, adhere, grow and migrate through this ECM without affecting the scaffold structure. Murine myoblasts were aligned with the scaffold ECM, they had physiological morphology with a well-established actin cytoskeleton and a centrally located nucleus. Or findings indicate preservation of the 3-dimensional microarchitecture and ECM composition in scaffolds obtained after decellularization of the skeletal muscle. The scaffolds are biocompatible in vivo and in vitro, allow cell attachment and preserve a host environment for guiding and spatially organizing cells after recellularization. Our data confirm the decellularized muscle scaffolds can be considered as a promising alternative for construction of muscle tissue replacements.

Acknowledgements: The work was supported by the Grant Agency of the Czech Republic 15-09161S and PROGRES Q40/06.

Keywords: decellularization, skeletal muscle

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PERIPHERAL CHIMERISM OF BONE MARROW-DERIVED STEM CELLS AND REGENERATION OF NON-HEMATOPOIETIC TISSUES

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Bone marrow-derived cells represent a heterogeneous cell population containing haematopoietic stem and progenitor cells. These cells have been identified as potential candidates for use in cell therapy for the regeneration of damaged tissues caused by trauma, degenerative diseases, ischemia and inflammation or cancer treatment. In our study, we examined a model using whole-body irradiation and the transplantation of bone marrow or haematopoietic stem cells (HSCs) to study the repair of haematopoiesis, extramedullary haematopoiesis and the migration of GFP+ transplanted cells into non-haematopoietic tissues. We investigated the repair of damage to the bone marrow, peripheral blood, muscle, spleen and thymus and assessed the ability of this treatment to induce the entry of bone marrow cells or GFP+lin-Sca-1+ cells into non-haematopoietic tissues. The transplantation of bone marrow cells or GFP+lin-Sca-1+ cells from green fluorescent protein (GFP) transgenic mice successfully repopulated haematopoiesis and the haematopoietic niche in haematopoietic tissues, specifically the bone marrow (BM), spleen and thymus. The trans-planted GFP+ cells also entered the gastrointestinal tract (GIT) and muscle tissues following whole-body irradiation. Our results demonstrate that whole-body also induced myeloablation and chimerism in tissues, and induced the entry of transplanted cells into the small intestine, liver and muscle tissues. This result demonstrates that grafted bone marrow cells or GFP+lin-Sca-1+ cells are not transient in the GIT. Thus, these transplanted cells could be used for the long-term treatment of various pathologies or as a one-time treatment option if myeloablation-induced chimerism alone is not sufficient to induce the entry of transplanted cells into nonhaematopoietic tissues.

Acknowledgements: This work was supported by grant GACR No 15-09161S and PROGRES Q40/06.

Keywords: bone marrow, chimerism

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CONTROLLING PLURIPOTENT STEM CELLS FATE AT SINGLE CELL LEVEL THROUGH BIO-NANO INTERFACE

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Design of biologically inspired cell-specific engineered Bio-nano Interfaces have attracted considerable attentions in tissue engineering and re¬generative medicine fields as well as pharmaceutical drug screening system. Embryonic stem cells (ES cell) and induced pluripotent stem cells (iPS) which have characteristics such as self-renewal and pluripotency, are considered to hold great promise in regenerative medicine and drug design for pharmacolo¬gical evaluation systems. However, pluripotency is highly dynamic and evolves at different stages. Most of the studies reported that proliferation of undif-ferentiated state and induced differentiation of somatic cells from ES and iPS cells have been based on cell-cell aggregated colony culture system. In con¬ventional cell-cell aggregated colony culture system, stimulating factors fail to directly and homogeneously interact with all cells at the same time, and there are many disadvantages for using in clinical applications. To overcome these problems, we established a novel uniform single cell level culture system for controlling ES/iPS cells functions. Based on the analysis on molecular mechanism of ES/iPS cell-cell aggregation, we will introduce a novel technique for controlling pluripotent stem cells fate at single cell level culture system by utilizing bio-mimetic nano-interface. under defined single cell level culture conditions

Keywords: Single cell level, Pluripotent stem cells, bio-nano interface, directed-differentiation

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DEVELOPMENT OF A NOVEL HIGH EFFICIENT DIRECTED–DIFFERENTIATION TECHNIQUE OF NEURAL PROGENITOR CELLS FROM MOUSE EMBRYONIC STEM CELLS UNDER DEFINED SINGLE CELL LEVEL CULTURE CONDITIONS

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Embryonic stem cells (ES Cells) are pluripotent stem cells which isolated from the inner cell mass of the early embryo in the blastocyst stage. ES cells have self-renewal ability and pluripotency, with great potential to differentiate into all three germ line cells in vivo and in vitro culture system. Thus, embryonic stem cells are considered one of the cell sources for the clinical application of neurodegenerative diseases in cell transplantation and neural system cells re¬generation. However, the conventional directed-differentiation method has low induction efficiency and heavily injuring purification process. In this study, we developed a novel induction technique for undifferentiated ES cells to neural progenitor cells with high efficiency and without any purification step. In the study, we used defined conditional retinoic acids stimulating interaction and single cell level homogeneous culture system. After cultured for three days with the undifferentiated culture medium of mouse embryonic stem cells, the culture medium was stimulated with retinoic acid to stimulate the pluripotent stem cells to the neural stem cells for two days. The expression of marker gene of E-cadherin, Oct3 / 4 of embryos stem cells and N-cadherin and Nestin of neural stem cells were detected by RT-PCR. Further experimental results from immunofluorescence staining flow cytometry data will also be presented.

Keywords: directed differentiation, single cell level culture, ES cells, neural progenitor cell

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DEVELOPMENT OF HIGHLY EFFICIENT STEPWISE INDUCTION SYSTEM FOR HEMATOPOIETIC STEM CELLS FROM SINGLE CELL LEVEL CULTURE OF MOUSE EMBRYONIC STEM CELLS

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Hematopoietic stem cells transplantation is one of the prior successful stem cell transplantation in clinical application with wide range of life-threatening malignant and non-malignant hematopoietic disorders. According to the World Marrow Donor Association (WMDA), stem cell products provided for unrelated transplantation worldwide had increased to 20,604 In 2014. Transplantable and functionally definitive hematopoietic stem cells efficiently differentiated from embryonic stem cells and inducted pluripotent stem cells are an unlimited cell source for effectively treating many kinds of blood diseases. However, conventional cell-cell aggregated colony culture system faced many drawbacks in clinical applications. Such as: 1) Stimulating factors fails to directly and homogeneously interact with all cells; 2) Damage from repeatedly enzyme treatment; 3) Sorting process is necessary for purification of objected cells from final differentiated cellular population; 4) and also very low induction rate to objected cells... etc. For overcoming these problems, we will introduce a novel homogeneous culture system at single cell level in pluripotent stage. Single cell level stimulation and analysis of ES cells will deepen our understanding on different pluripotent stages and developmental stability. Our approach is to differentiate hematopoietic stem cell from embryonic stem cells utilizing single-cell level embryonic stem cells culture system. We will report the examination results of the differentiation efficiency of colony and single cell level culture system under defined stimulation factors interactions. The different types of functionally somatic cells from embryonic stem cells and induced pluripotent stem cells are important for regenerative medicine, drug screening and tissue engineering.

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PRC1 COMPONENTS MEDIATE DUAL FUNCTIONS IN TRANSCRIPTIONAL REGULATION VIA INTERACTING WITH OCT4 OR NANOG

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Polycomb group (PcG) complex plays an important role in gene repression. The complex with different subunits exerts different function, while the exact component of certain complex are not clear. Some members also showed gene activation activity in recent studies, little is known about the insight mech-anism. By using Immunoprecipitation-mass spectrometry (bioSAIP followed by MS identification) analysis, numerous newly found proteins were pulled down by Ring1A, Ring1B and RYBP antibody, which exerted negative regulation of transcription or epigenetic modification function. The master transcription factor in mESC, Oct4 and Nanog, mediated distinct development genes' repression. However, another polycomb member, PCGF6 displays gene activate activity in mESC. Meanwhile, by interacting with Oct4, Nanog and RBBP5, PCGF6 in different transcriptional factor complex shows distinct function significantly, such as protein associated function, lipid associated metabolism and assembly, transcriptional regulation and through super-enhancer activity. Furthermore, many proteins in Ring1A, Ring1B, Rybp and PCGF6 interactome participante in the development, reprogramming and differentiation process. Be-sides, high correlation of abnormal expression of RYBP and PCGF6 with types of cancer were also observed. Thus, PcG shows dual functions in transcription-al regulation via forming different complex with Oct4 and Nanog, which reveals a new insight for the function of PcG.

Keywords: Polycomb group, Oct4, dual functions

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PRC2 SPECIFIES ECTODERM LINEAGES AND MAINTAINS PLURIPOTENCY IN PRIMED BUT NOT NAÏVE ESCS

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Polycomb repressive complex 2 (PRC2) and the epigenetic mark that it deposits, H3K27me3, are evolutionarily conserved and play critical roles in development and cancer. However, their roles in cell fate decisions in early embryonic development remain poorly understood. Here, we report that knock-out of PRC2 genes in human embryonic stem cells (hESCs) causes pluripotency loss and spontaneous differentiation toward a mesoendoderm fate, owing to de-repression of BMP signaling. Moreover, hESCs with deletion of EZH1 or EZH2 fail to differentiate into ectoderm lineages. We further show that PRC2-deficient mouse ESCs also release Bmp4 but retain their pluripotency. However, when converted into a primed state, they undergo spontaneous differenti-ation similar to that of hESCs. In contrast, PRC2 is dispensable for pluripotency when hESCs are converted into the naïve state. Our studies reveal both lin-eage- and pluripotent state-specific roles of PRC2 in cell fate decisions.

Keywords: PRC2, Spontaneous differentiation, BMP signalling, Primed pluripotency, Naive pluripotency

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DECODING TOTIPOTENCY WITH NOVEL LINCRNAS – A BIOINFORMATIC STUDY

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The enormous value of stem cell-based therapy has attracted broad attention and prompted its applications in clinical regenerative medicine. Totipotent cell, which is capable of developing into a complete organism from a single cell, is expected as an important tool in regenerative medicine and disease modeling. However, to date, in vitro totipotent cell model has not been well established in mammal. Thus, it is foreseeable that an in vitro cultured cell model of mammalian totipotent stem cells is of paramount interests to both biomedical and clinical research. Long intergenic non-coding RNA (lincRNA) has been identified to play the critical roles in circuitry controlling and maintaining the pluripotency of embryonic stem (ES) cells. Recent studies suggest lincRNAs bind to chromatin regulatory proteins and primarily regulate pluripotent gene expressions. Thus, lincRNA is expected as a novel important tool to induce cell reprogramming by altering the cell epigenetic landscape. However, lincRNAs active in totipotent stage have been so far rarely studied. Here, we initially discovered ~6000 novel lincRNAs from mouse pluripotent stem cells by bioinformatics data mining. Subsequently we performed a bioinformatics pipeline to identify the functional annotations of these novel lincRNAs. To our surprising, a gene module which is specifically active in mouse totipotent stage was obtained from single cell RNA-seq (scRNA-seq) of ~850 mouse ES cells. The gene module contains ~300 novel lincRNAs and ~700 protein cod-ing genes including totipotent markers. Besides, the gene module has distinct epigenetic features compared to pluripotent genes. In summary, our results discovered a gene module active in totipotency including ~300 novel lincRNAs. They are potential candidates to alter the epigenet-ic landscape of mouse pluripotent stem cells, and eventually promote the establishment of mouse totipotent stem cell lines in vitro.

Keywords: stem cell, lincRNA, totipotency

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NAC1 IS DISPENSABLE FOR ESC MAINTENANCE BUT REGULATES NEURAL DIFFERENTIATION AND SOMATIC CELL REPROGRAMMING

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NAC1 (Nucleus Accumbens-associated protein 1) belongs to the Bric-a-brac Tramtrac Broad complex/Pox virus and Zinc finger (BTB/POZ) family of transcription factors. It is a ubiquitously expressed protein originally identified in the nucleus accumbens of the rat brain as a cocaine-inducible gene. In embryonic stem cells (ESCs), NAC1 is a common interacting partner of, and upstream modulator, for many pluripotency factors, including NANOG, OCT4 and SOX2, and epigenetic regulators. However, its mechanistic actions in pluripotency are not well defined. We have begun to investigate the role of NAC1 in the maintenance and establishment of pluripotency and demonstrated that Nac1 was surprisingly dispensable for early embryo development. In this study, we derived Nac1 knockout mESCs, and proved they could undergo normal self-renewal and maintain pluripotency, although their embryoid body (EB) based differentiation was skewed towards endoderm and throphectoderm. They were also defective in generating functional neural progenitor cells. As far as nuclear reprogramming was concerned, NAC1 was required for the establishment of pluripotency. It was essential for proper expression of E-cadherin by dual regulatory mechanism: it facilitated NANOG binding to the E-cadherin promoter and fine-tuned its expression; most importantly, it down-regulated the E-cadherin repressor ZEB1, directly via transcriptional repression and indirectly via post-transcriptional activation of the miR-200 miRNAs. Our study thus uncovered a previously unappreciated role for the pluripotency regulator NAC1 in promoting efficient somatic cell reprogramming.

Keywords: NAC1, embryonic stem cells, somatic cell reprogramming, Neural differentiation

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CHROMATIN ACCESSIBILITY DYNAMICS DURING REPROGRAMMING

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Cell fate decisions remain poorly understood at the chromatin level. Here we report a dynamic logic for chromatin remodeling during reprogramming. Global mapping of accessible chromatin loci in MEFs expressing Oct4-Sox2-Klf4 by ATAC-seq reveals dynamic changes from open to closed (OC) and closed to open (CO), with a burst of OC in the beginning and a climax CO at the end. We then show that factors/conditions known to impede reprogramming prevent OSK-driven OC and skew the overall OC-CO dynamics. Mechanistically, while the CO loci are enriched with and opened by Oct4-Sox2-Klf4 directly, the OC loci are devoid of them suggesting an indirect OC process. Indeed, we show that Sap30 is activated early and plays a key role in the OC process. These results reveal a chromatin accessibility logic during reprogramming and identify Sap30 as one of the first responders critical for the prerequisite silencing of somatic genes.

Keywords: reprogramming, chromatin dynamics

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AN EPIGENETIC CODE CONTROLS THE CLASS-SPECIFIC EXPRESSION OF TRANSPOSABLE ELEMENTS IN MOUSE EMBRYONIC STEM CELLS

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Transposable elements (TEs) comprise nearly half of mammalian genomes, and they constitute essential motors of genetic variation, adaption and evolution through their capability of autonomous and non-autonomous duplication or deletion. However, TEs mobility can lead to deleterious effect on genome sta-bility. Accordingly, hosts have evolved tight epigenetic mechanisms to control their mobility appropriately. Here, systematically surveyed epigenetic profiles and show that TEs harbor unique TE-family-specific epigenetic profiles in mouse embryo stem cells (ESCs), several epigenetic marks including H3K9me3, H4K20me3,

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H3K27me1, H3.3, H3K27ac, 5mC, 5hmC, H4R3me2 and H3K56ac are strongly enriched on TEs. We identify the epigenetic factors Rnf2 (polycomb-member RING1B), the 5mC-associated Uhrf1, the H3K9me2 associated Rrp8, the H4R3me2 associated Prmt5, the H3K4 methylation associated Ash2l and the histone acetyltransferase Kat5 as major epigenetic hubs to control TE suppression. RNA-seq analysis reveals knockdown of those epigenetic factors induced widespread changes in TE-family expression. Our study suggests that a major function of the epigenetic system as a whole is to regulate the expression of TEs and we illuminate the comprehensive epigenetic code by which ESCs repress TEs within the genome.

Keywords: Transposable elements, Epigenetics

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YY1 POSITIVELY REGULATES TRANSCRIPTION BY **TARGETING PROMOTERS AND SUPER-ENHANCERS THROUGH THE BAF COMPLEX IN STEM CELLS**

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Yin Yang 1 (YY1) regulates early embryogenesis and adult tissue formation. However, the exact role of YY1 in stem cell regulation is not clear. Although YY1 has a Polycomb group proteins (PcG)-dependent role in mammalian cells, PcG-independent functions of YY1 have also been reported with the underlying mechanism remaining to be defined. Here, we report YY1 has Oct4-dependent but PcG-independent transcriptional activity in mouse embryonic stem cells (mESCs), which is mainly through the BAF complex. The interaction of YY1 with the BAF complex can be detected both at promoters to induce transcription activation and at super-enhancer elements to further stimulate transcription. A similar transcription activation effect of YY1 that is independent of PcG-dependent repression function is also observed in unipotent cells. This study thus clarifies the role of YY1 in controlling stem cell pluripotency through its association with Oct4 and the BAF complex, integrating YY1 into the core pluripotency network.

Keywords: YY1, BAF complex

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DIRECT CONVERSION OF MOUSE FIBROBLASTS INTO CHOLANGIOCYTE PROGENITOR CELLS

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Disorders of the biliary epithelium, cholangiocytes induce severe and irreversible liver diseases. The limited accessibility of bile duct precludes modeling of several cholangiocyte-mediated diseases. For this reason, novel concept for obtaining functional cholangiocytes with high purity are needed. Previous re-port has shown that the transcription factor combination, Hnf1β and Foxa3 could convert mouse fibroblasts into bipotential hepatic stem celllike cells, termed iHepSCs (induced Hepatic Stem Cells). However, conversion efficiency of fibroblasts to iHepSCs is quite low, and iHepSCs exhibit limited differentiation potential toward cholangiocytes—impeding the clinical usage of iHepSCs. Here we present that a new combination of transcription factors could pro-mote the robust generation of iHepSCs. Interestingly, extended in vitro culture of iHepSCs induces a Notch signalingderived secondary conversion into cholangiocyte progenitorlike cells which show the dramatically enhanced differentiation efficiency into mature cholangiocytes. Our study provides a novel two-step approach for obtaining cholangiocyte progenitorlike cells using defined factors.

Keywords: Direct conversion, Hepatic stem cells, Cholangiocyte progenitor cells

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PRECONDITIONING OF MESENCHYMAL STEM CELLS (MSC): AN IN VITRO STUDY TO EVALUATE THE REPARATIVE AND MOLECULAR PROPERTIES OF MSC IN HYPOXIC BRAIN INJURY MODEL

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Over the past two decades, regenerative therapies using stem cell technologies have been developed for various neurological diseases. Although very promising, it is still under development so as not to show significant treatment effects in clinical settings. AIMS: To evaluate the ability of mesenchymal stem cells (MSC) to regenerate and survive in hypoxic pathological microenvironments. To develop the cell labelling techniques that are safe and reliable for clinical testing. To Identification and validation of surrogate markers of recovery. Phase-I: Isolation and characterization of allogenic MSCs from Cord blood following two levels of preconditioning with Hypoxia and growth factors. Phase II: Gene profiling of MSC before and after preconditioning. Comparison of gene profile of NSC derived from MSC after preconditioning and the nor-mal brain samples harvested from the cadavers. Phase III: Dedifferentiation of MSC into NSC and invitro evaluation of MAP & tran-synaptic conductions. This is an ongoing study and phase 1 results are presented here. CD marker profile suggests the successful differentiation to MSC from Cord blood. MSC viability was not affected even after two levels of preconditioning. There are no morphological changes observed in both control and test samples after preconditioning with hypoxic environment and growth factors (VEGF, BDNF & IGF). Our study suggests that the MSC preconditioning sustains the cell survival and biological functions of MSCs in vivo. Stem cell preconditioning can be applied prior to transplantation towards the safe long-term efficacy of stem cell–based therapy. Further investigations are required to understand the structural and biological properties of stem cell aggregates in order to enhance cell survival and trophic functions.

Keywords: Mesenchymal Stem cells, Preconditioning, Hypoxia

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CONVERSION OF HUMAN SPERMATOGONIAL STEM CELLS INTO FUNCTIONAL DOPAMINERGIC NEURONS AND TRANSPLANTATION FOR RECOVERY IN MOUSE PRIMATE MODEL OF PARKINSON'S DISEASE

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Recent progress in the generation of induced dopaminergic neurons (DANs) derived from different types of stem cells or reprogrammed somatic cells holds tremendous potential for the treatment of Parkinson's disease(PD). However, a major limitation is still the lack of a reliable source for cell replacement therapy in the neurological disorder. On the other hand, these current protocols for in vitro differentiation or cell reprogramming to generate DANs are laborious and time-consuming and expensive. Here, we present an improved system for direct neural conversion of human spermatogonial stem cells (hSSCs) to DANs. The induction system combined olfactory ensheathing cell conditioned culture medium (OECCM) and a set of defined cell extrinsic factors and small molecules. The converted neurons not only acquired neuronal morphological features, but also expressed various DANs critical markers such as Tuj-1, TH, Nurr1 and DAT, as demonstrated by inverted microscope, immunostaining, western-blot and real-time PCR. In addition, transcriptome analysis revealed that the differentiated DANs closely resembled wild ones but distinct from hSSCs. More importantly, the hSSCs-derived DANs exhibited sophisticated func-tional properties including synapse formation, dopamine release, electrical activity, and neuronal specific Ca2+ signaling changes. Furthermore, these pro-induced hSSCs after transplantation into mouse striatum efficiently survived and differentiated into DANs, and alleviated animal symptoms of animal model of Parkinson's disease. Excitingly no tumor was found post-injection. Overall, to our knowledge, this is first report of generation of safe DANs and may suggest an alternative strategy suitable for clinical cell therapy for treatment of PD.

Keywords: human, Spematogonial stem cells

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GENOME-WIDE ANALYSIS REVEALS CIS-REGULATORY LOGIC OF REPROGRAMMING COMPETENT AND INCOMPETENT POU TRANSCRIPTION FACTORS

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Despite strong sequence conservation only Oct4 is able to induce pluripotency when forcibly expressed in somatic cells but the paralogous factors Oct6, Brn1, Brn2 direct neural differentiation. To uncover the molecular underpinning of this process, we used ChIP-seq to map Oct4 binding and monitored gene expression in a time dependent manner in comparison to Oct6 and three rationally designed Oct4 mutants. Expression profiles suggest that reprogramming takes the same trajectory in the early phase for all TFs and starts to diverge only at late stages. Oct4 utilises the SoxOct heterodimer motif to direct the ex-pression of pluripotency in a progressive fashion. However, initially, Oct4 also utilizes gene proximal homodimer promoting MORE sequences to transiently drive the expression of a small gene set critical to effectively kick-start the reprogramming process. Oct4 is able to target chromatin closed in MEFs and in-duces immediate opening. The four other POU factors show dramatically different binding profiles demonstrating the profound consequence of subtle alterations to the sequences of the DNA binding POU domain. Oct6 and mutant POUs favour the MORE sequence throughout reprogramming. Nevertheless, if binding to a subset of functionally critical SoxOct elements occurs, reprogramming competency is retained suggesting that the majority of acquired MORE sites act as genomic scavengers without affecting the regulatory outcome. Surprisingly, reprogramming competency does not appear to be a function of the effective targeting of pluripotency enhancers at early stages but of the ability to resist clearance as reprograming proceeds. The binding profile of Oct6 strongly diverges from Oct4 throughout reprogramming with a bias towards neural genes and in particular Oct4 enhancers earmarked by the Sox-Oct sequence cannot be targeted. Together, the pluripotency program relies on dynamically utilised cis-elements and versatile molecular interfaces of POU proteins enabling their correct interpretation.

Keywords: Somatic Cell reprogramming, POU proteins, Synthetic Transcription Factors

Poster 67

A HOXC DEPENDENT SKIN ZIP CODE CONTROLS REGIONAL ADULT STEM CELL REGENERATION

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Stem cell regeneration ability is distinct in different body regions, providing great model to explore key regeneration regulators. Here, using an unbiased multi-step screening approach, we identified a single gene cluster, Hoxc, with expression associated with the regional activation of hair follicle stem cells (HFSC). Hoxc genes are only highly expressed in regions where HFSCs exhibit long-term regenerative ability. Lentivirus mediated in vivo over-expression in epithelial cells excludes the intrinsic ability of Hoxc gene in promoting HFSC activation. A chromosome 15 inversion results in ectopic expression of multiple Hoxc genes in dermis and results in regional activation of otherwise dormant HFSCs. Using CRISPER/Cas9 mediated gene knockouts and lentivirus mediated gene over expression in functional studies, we conclude niche expressed Hoxc genes function redundantly to determine the regenerative capacity of HFSCs. The region-specific expression pattern of Hoxc genes is controlled by antagonistic switch between PcG-dependent repression and enhancer activation epigenetic modifications. Circularized Chromosome Conformation Capture (4C) results reveal the chromosome 15 inversion leads to ectopic interaction of Hoxc cluster with an active regulatory chromatin domain and increased Hoxc genes expression. Dermis expressed Hoxc genes switch on the epithelial HFSCs regeneration through activating Wnt signaling. Together, these data delineate an altered epigenetic landscape in dermis from different skin regions that controls the differential expression pattern of Hoxc genes, which encode the positional identity of adult HFSCs and regulate their regenerative ability.

Keywords: stem cell regeneration, position difference, Hoxc

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