Stem Cells:
BASIC BIOLOGY TO DISEASE THERAPIES

Arranged by

Hongkui Deng, Peking University, China
Andrew Elefanty, Murdoch Children’s Research Institute, Australia
Gordon Keller, McEwen Centre for Regenerative Medicine, Canada
Duanqing Pei, Guangzhou Institute of Biomedicine & Health, CAS, China
Kathrin Plath, UCLA School of Medicine, USA
Cover design by: John Bart, International Society for Stem Cell Research.
CSHA & ISSCR would like to thank the following supporters:

GOLD

Beckman Coulter Commercial Enterprise (China) Co., Ltd.
12F, UC Tower,
500 Fu Shan Road, Pudong,
Shanghai 200122, P. R. China

Phone: General Line: 86-21-38651000, Product Enquiry Hotline: 400 821 8899
English Website: www.beckmancoulter.com,
Chinese Website: www.beckmancoulter.com.cn

Beckman Coulter Life Sciences is dedicated to improving the health of people around the world. The company’s global leadership and world-class service and support delivers sophisticated instrument systems, reagents and services to life science researchers in academic and commercial laboratories, enabling new discoveries in biology-based research and development. A leader in centrifugation and flow cytometry, Beckman Coulter has long been an innovator in life sciences instruments and solutions used at the forefront of important areas of investigation, including genomics, proteomics and cellular research. For more information, please visit www.beckmancoulter.com.

SILVER

BD Biosciences
30 Tuas Avenue 2
Singapore 369461

Phone: +65 66908571
www.BD.com

BD is a leading medical technology company that partners with customers and stakeholders to address many of the world’s most pressing and evolving health needs. Our innovative solutions are focused on improving medication management and patient safety; supporting infection prevention practices; equipping surgical and interventional procedures; improving drug delivery; aiding anesthesiology and respiratory care; advancing cellular research and applications; enhancing the diagnosis of infectious diseases and cancers; and supporting the management of diabetes. We are more than 45,000 associates in 50 countries who strive to fulfill our purpose of “Helping all people live healthy lives” by advancing the quality, accessibility, safety and affordability of healthcare around the world. In 2015, BD welcomed CareFusion
and its products into the BD family of solutions. For more information on BD, please visit www.bd.com.

Shenzhen Cell Inspire Biotechnology Co., Ltd
Room 115-124,127-130, Incubator A,
TaoHuayuan Science and Technology Innovation Park
Shenzhen, Guangdong, China
Phone: 86-18682315223
http://www.cib.cc

Cell Inspire Biotechnology (CIB) is a biotechnology company focusing on development of stem cell products for applications in basic and clinical research. We currently offer more than 40 human iPSC lines, iPSC-derived neural stem cells, neurons, hepatocytes, cardiomyocytes as well as clinical grade mesenchymal stem cells. We also provide services for iPSC generation and genome editing, large scale NSC and MSC production for pre-clinical studies, and bioassay development using iPSC-derived primary cells.

NIKON INSTRUMENTS (SHANGHAI) CO. LTD.
26F Hang Seng Bank Tower
1000 Lu Jia Zui Ring Road
Pudong New district, Shanghai 200120, China
Phone: 021-6841-2050
http://www.nikon-instruments.com.cn

Nikon is a world-renowned brand, a market leader in optical instrumentation, with its over 90 year’s expertise, Nikon offers a diverse lineup of microscopes for a broad spectrum of applications, from education and lab training to clinical and research use. To meet the increasing demand for Nikon products in Chinese market, NIKON Instruments (Shanghai) Co., Ltd. was established, and opened its own branch companies at Beijing and Guangzhou, Chengdu and Wuhan. As a part of Nikon Corporation, we wish to make better cooperation with our honorable users in China. Nikon’s product Biostation CT dedicated for iPS research is specially designed for unlimited long term cell incubation, high throughput and image acquisition.
Thermo Fisher Scientific (China) Co., Ltd.
Building 7, No.27, Xin Jinqiao Road
Shanghai, China

Phone: (86)-21-6865 4588 ext 5655
Web Site: www.thermofisher.cn

Thermo Fisher Scientific has been operating in China for over 30 years. Headquartered in Shanghai, Thermo Fisher Scientific China has over 3800 employees and 8 manufacturing sites in Shanghai, Beijing, Suzhou and Guangzhou. The 6 application centers nationwide keep bringing cutting edge technology and products with application development and trainings service to local customers. Our mission is to enable our customers to make the world healthier, cleaner and safer. For more information, please visit www.thermofisher.cn

BRONZE

Carl Zeiss Shanghai Co., Ltd.
No.60 Meiyue Road,
China (Shanghai) Pilot Free Trade Zone, 200131

Phone: +86 21 2082 1188
http://www.zeiss.com.cn/microscopy/

ZEISS is one of the world leading manufacturers of microscopes. ZEISS manufactures a diverse range of fluorescence optical sectioning systems as well as high-resolution electron microscopes. Microscope systems by Zeiss are much more than just hardware. A dedicated and well-trained sales force, an extensive support infrastructure and a responsive service team enable customers to use their ZEISS instruments to their full potential.

CORNING Life Sciences
3/F Liming Building
111 Guiqing Road
Shanghai 200233, China

Phone: 400-077-0082
http://www.corning.com/lifesciences/china

Corning is developing technologies that change the way researchers work, proprietary Corning® Epic® Technology and services for label-free detection, a range of optimized synthetic surfaces for cell culture and assay, biological surfaces, single-use products and closed system solutions for more efficient scale-up and downstream bioprocess, automation solutions, Next-Generation Sequencing solutions to simplify sample
preparation and library construction, bringing new efficiencies and improved results to scientists

**ExCell Biology**
5F, Building 18
481 Guiping Road
Shanghai 200233
China

Phone: 136-0169-2807
http://www.excellbio.com

ExCell Biology, Inc., founded in July, 2006, is an innovative Hi-biotech enterprise specialized in researching, manufacturing and distributing and is a subsidiary of Genetimes. It provides cytobiological and immunobiological products for Life Science Research and drug development, including fetal bovine serum and newborn calf serum, ELISA kits, reliable reagents for molecular biology and stem cell research, consumables and instruments. ExCell Bio products are widely cited in journals such as Nature, Immunity, PNAS.

**Lonza Pharma&Biotech - Bioscience Solutions**
 Lonza China
Room 1710, No.369 Kai Xuan Rd,
Shanghai 200051, China

Phone: +86 21 6340 3488
www.lonza.com

Lonza supports stem cell research from the laboratory to clinical application. Discover how Lonza’s L7™ hPSC Reprogramming and Culture System, CytoSMART™ Live Cell Imaging and Monitoring System, can benefit your projects. Learn more about our products: Primary cells and specialized media, Nucleofector™ Technology, hPSC Reprogramming Kits, PSC Medium, hPSC Matrix, hPC Passaging Solution, hPSC Cryosolution, 3D RAFT™ Culture System.
At the heart of Nissan Chemical's operations you'll find general chemical products and a lot more. Today we are moving ahead with a keen focus on our major growth engines. These encompass performance materials (electronic, inorganic, and organic materials) that harness ultrafine particle control and functional polymer design technologies as well as life sciences products, including agricultural chemicals and pharmaceuticals employing fine organic synthesis and biological evaluation technologies.

We, Ruich Allway Instrument Technology Co., Ltd., founded in 2005, as a major authorized agent of Olympus microscope corporation in the field of researching area in Beijing, is a high-tech enterprise. We focus on the instruments of professional research, education and related field. Since its foundation, we keep living up to the belief of providing the outstanding products and services, so we will be your best & trusted partner!

PeproTech is the trusted source for the development and manufacturing of high quality cytokine products for the life-science and cell therapy markets. Over the past 26 years the company has grown into a global enterprise with state-of-the-art manufacturing facilities in the US, and offices around the world.
R&D Systems was founded in 1976 in Minneapolis, MN, USA and is a leading supplier of cell biology reagents with about 40 years of experience serving the research community. Through recent acquisitions of Tocris Bioscience, BostonBiochem and Novus Biologicals, R&D Systems has enhanced substantially our capability to serve the researchers with brand new one-stop solutions. In the field of Stem cell research, we can offers comprehensive optimized cell media, antibodies and assay kits for research on stem cell proliferation, differentiation and verification and further investigation.

Selleck provides signaling inhibitors, modulators and compound libraries with terrific validation, customer reviews, product citations, tech support and prompt delivery. Selleck actively tracks the latest science so our customers can rely on us to be the leading supplier of the newest inhibitors.

STEMCELL Technologies Inc. is a Canadian biotechnology company that helps power leading-edge life science research globally. We offer highly specialized cell culture media, cell separation products, instruments, ancillary products and educational tools that support scientists performing stem cell, immunology, cancer, regenerative
medicine and cellular therapy research. As Scientists Helping Scientists™, STEMCELL is driven by a passion for quality, performance and innovation.
The CytoFLEX flow cytometer is designed to deliver superior performance, flexibility, and ease of operation for research applications. Innovation in the design of both the hardware features and new CytExpert software provides intuitive tools for researchers. Simplified system setup, data acquisition, analysis, and export of experimental results are integrated into a complete workflow solution.

The CytoFLEX system features a compact footprint, integrated detection optics and lasers, and a simplified, highly reliable fluidics system. In addition, the flow cell design is integrated into the optical path, to greatly improve system performance, stability and signal resolution.

The instrument is designed to be compact and fits easily on a bench top or a standard biohood. Twenty one (21) standard configurations are available to provide the ultimate in application flexibility with up to three lasers and 15 parameters.

The CytoFLEX flow cytometer is easily installable and upgradeable. All lower configuration systems have full upgrade capability to higher configurations enabling future application expansion.

Together, the CytoFLEX system and CytExpert software brings high performance flow cytometry to a variety of researchers with simple to complex needs.

Please contact your Beckman Coulter representative for more information.
Thermo Scientific™ Heracell VIOS CO₂ 培养箱

Thermo Scientific™ Heracell™ VIOS 系列作为新一代先进培养箱的代表，专门用于培养尖端研究、制药和临床应用中的干细胞和原代细胞等敏感样品。

我们最新的细胞培养箱采用全面的控制方法，能满足最严苛最关键的使用领域的要求。通过将现有成熟可靠的技术与污染控制和生长条件均一体化的最新技术相结合，现在，您可以轻松快速地获得更可靠的结果，以达成您的目标。
BD Horizon Brilliant™ Blue 515 brings innovation to the blue laser in the form of a novel format that is resolved in the FITC channel. Developed using technology from Amersham, BD Horizon Brilliant Blue 515 is up to seven times brighter than FITC and offers significantly better resolution, with less spillover into the PE channel.

BD Horizon Brilliant Blue conjugated antibodies give researchers new avenues to resolve dim cell populations, take advantage of better panel design, and have more confidence in their results. This unique blue laser dye is just another way BD Biosciences continues to innovate and deliver improvements to the world of multicolor flow cytometry so that you can get more out of your research.

To find out about brighter ways to use blue and obtain free samples, visit bd.biosciences.com/go/newblue.
Joint CSH Asia / ISSCR Conference

STEM CELLS: BASIC BIOLOGY TO DISEASE THERAPY

Monday, October 19 – Friday, October 23, 2015

Monday  7:00 pm  Welcome Remarks

Keynote Speaker

Tuesday  9:00 am  1 Pluripotent Stem Cells
Tuesday  12:15 pm  Meet the Experts Lunch
Tuesday  2:00 pm  2 Genome Editing I
Tuesday  4:45 pm  Panel Discussion
Tuesday  7:00 pm  3 Poster Session
Tuesday  8:30 pm  Chinese Tea and Beer Tasting
Wednesday  9:00 am  4 Stem Cells of the Mesodermal Lineage
Wednesday  2:00 pm  Visit to Old Suzhou
Wednesday  7:00 pm  5 Genome Editing II
Thursday  9:15 am  6 Stem Cells and Fate Decisions
Thursday  2:00 pm  7 Road to the Clinic
Thursday  4:30 pm  Keynote Speaker
Thursday  5:30 pm  Cocktails and Banquet
Friday  7:00 pm  Departure

Exhibit Hours:
Monday: 7:00pm –9:00pm
Tuesday: 10:30am – 4:00am, 7:00pm – 9:00pm
Wednesday: 10:30am – 12:00, 7:00pm – 9:00pm
Thursday: 10:30am – 12:00

Oral presentation sessions are located in the Watson Auditorium.
Poster session and Chinese Tea & Beer Tasting Party are in the Poster Hall.
Cocktail social hour is held in the Grand Ballroom Foyer.
Old Suzhou visit departs from the hotel lobby and requires additional fee.

Mealtimes locations and times are as follows:
Breakfast  Octagon  7:00am - 9:00am
(Only provided for guests who stay in Worldhotel Grand Dushulake Suzhou .)
Lunch  Grand Ballroom  12:00pm - 1:30pm
Dinner  Grand Ballroom  6:00pm - 7:30pm
Banquet  Grand Ballroom  7:00pm

More information will be available at CSHA office.
(Maps of the hotel and the exhibition are at the end of this abstract book)
Abstracts are the responsibility of the author(s) and publication of an abstract does not imply endorsement by Cold Spring Harbor Asia of the studies reported in the abstract.

These abstracts should not be cited in bibliographies. Material herein should be treated as personal communications and should be cited as such only with the consent of the author.

Please note that ANY photography or video/audio recording of oral presentations or individual posters is strictly prohibited except with the advance permission of the author(s), the organizers, and Cold Spring Harbor Asia.
PROGRAM

MONDAY, October 19—7:00 PM

Welcome Remarks

Hongkui Deng, Peking University
Nancy Witty, International Society for Stem Cell Research

KEYNOTE SPEAKER

Progression from the embryonic stem cell ground state
Tüzer Kalkan, Austin Smith [40’]
Presenter affiliation: University of Cambridge, Cambridge, United Kingdom. 1

TUESDAY, October 20—9:00 AM

SESSION 1 PLURIPOTENT STEM CELLS

Chairperson: Austin Smith, University of Cambridge, Cambridge, United Kingdom

Small molecule-induced cell fate reprogramming
Hongkui Deng [20’]
Presenter affiliation: Peking University, Beijing, China. 2

Decoding microRNA function in the maintenance and exit of naive pluripotency
Yangming Wang [10’]
Presenter affiliation: Peking University, Beijing, China. 3

Regulation of 2-cell specific gene expression in mouse ES cells
Hitoshi Niwa, Yoko Futatsugi [20’]
Presenter affiliation: RIKEN Center for Developmental Biology (CDB), Kobe, Japan; Institute of Molecular Embryology and Genetics, Kumamoto University, Kumamoto, Japan. 4

Coffee break
Transprogramming into hepatic lineage by defined factors
Kyung Tae Lim, Jonghun Kim, Seung Chan Lee, Seon In Hwang, Yong-Han Paik, Yimeng Gao, Lijian Hui, Dong Wook Han [20']
Presenter affiliation: Konkuk University School of Medicine, Seoul, South Korea.

Deficiency of microRNA mir-34a expands cell fate potential in pluripotent stem cells
Chao-Po Lin, Yong Jin Choi, Davide Risso, Sean Chen, Meng How Tan, Jin B. Li, Yalei Wu, Chaifu Chen, Zhenyu Xuan, Todd Macfarlan, Weiqun Peng, Sang Yong Kim, Terence P. Speed, Lin He [10']
Presenter affiliation: University of California at Berkeley, Berkeley, California.

BAF170/miRNA-302 interaction in human embryonic stem cells and its importance in differentiation
Trevor K. Archer, Staton Wade, James Ward, Lee Langer [10']
Presenter affiliation: NIH/NIEHS, RTP, North Carolina.

Cell fate decisions during somatic cell reprogramming
Duanqing Pei [20']
Presenter affiliation: Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, China.

TUESDAY, October 20—12:15 PM
Meet the Experts Lunch

TUESDAY, October 20—2:00 PM

SESSION 2 GENOME EDITING I

Chairperson: Hongkui Deng, Peking University, Beijing, China

Establishing monkey models for human diseases via precision gene editing
Weizhi Ji [20']
Presenter affiliation: Institute of Translational Medicine, Kunming University of Science and Technology, Kunming, China.
A genome-wide CRISPR screen to identify genes important for human definitive endoderm lineage commitment
Qing V. Li, Danwei Huangfu [10']
Presenter affiliation: Sloan Kettering Institute, New York, New York. 10

Cellular models of autism-associated de novo mutations in human embryonic stem cells
Xi Shi, Neville Sanjana, Congyi Lu, Alexandria Nikish, Jen Pan, Feng Zhang [10']
Presenter affiliation: Broad Institute of MIT and Harvard, Cambridge, Massachusetts. 11

Arrayed mutant haploid stem cell libraries facilitate genetic screen for the "Exit-from-Pluripotency" factors
Guang Liu, Xue Wang, Yufang Liu, Meili Zhang, Yue Huang [10']
Presenter affiliation: Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China; State Key Laboratory of Medical Molecular Biology, Chinese Academy of Medical Sciences, Beijing, China. 12

Coffee Break

TUESDAY, October 20—4:45 PM

PANEL DISCUSSION

Modeling Human Development and Disease—New Horizons

Moderator

Andrew Elefanty, Murdoch Childrens Research Institute, Parkville, Australia

Panelists

Olivia Kelly, ViaCyte, Inc., San Diego, California, USA
Weizhi Ji, Kunming University of Science and Technology, China
Darrell Kotton, Boston University School of Medicine, Massachusetts, USA
Danwei Huangfu, Sloan Kettering Institute, New York, New York, USA

This panel discussion will focus on themes of disease modeling and also cell and tissue transplantation to treat disease. Current challenges and opportunities faced by researchers as the stem cell field progresses toward the clinic will be explored.
SESSION 3  POSTER SESSION

A novel, multifactorial approach for human iPS cell differentiation and reprogramming using an automated cell culture system
T Guo, M Waston, N Devaraju, S Boutet, L Szpankowski, Qing Chang
Presenter affiliation: Fluidigm (Shanghai) Instrument Technology Co., Ltd., Shanghai, China.

Transdifferentiation of adipose tissue-derived mesenchymal stem cells into epidermal cells in vitro
Deyun Chen, Haojie Hao, Weidong Han, Xiaobing Fu
Presenter affiliation: Chinese PLA General Hospital, Beijing, China.

Simple conversion of primed human iPS cells to naïve-state iPS cells
Shunsuke Yoshida, Brad Hamilton, Robert Annand, Naoki Nishishita, Mitsuru Inamura, Yingju (Miliya) Chen
Presenter affiliation: Reprocell Co., Yokohama, Japan.

KUF4-mediated suppression of adipogenesis of adipose derived stromal cells
Jihae Han, Hye Yeon Choi, Ahmed A. Dayem, Kyeongseok Kim, Gwangmo Yang, Sohee Lim, Jihye Won, Subbroto K. Saha, Sang baek Choi, Yingfu Yin, Ssang-Goo Cho
Presenter affiliation: Department of Animal Biotechnology, Animal Resources Research Center, 120 Neungdong-ro, Gwangjin-gu, Seoul, 143-701, South Korea.

Human iPSC-derived neuronal cell sub-populations that are suitable for drug development and the study of divergent neurological disorders
Brad Hamilton, Harumi Kogami, Yuichi Okuda, Kaishu Shiina, Ryu Yamanaka, Paul Cizdziel, Yutaka Shindo, Yu Ching (Zachary) Lin, Shunsuke Yoshida, Kotaro Oka, Mitsuru Inamura
Presenter affiliation: Reprocell Co., Yokohama, Japan.
Reprogramming of endothelial progenitor cells (EPCs) derived from human blood using a self-replicative RNA (srRNA) vector and microRNA augmentation
Sarah Eminli-Meissner, Jung-II Moon, Kevin Yi, Fedir Kiskin, Baraa Kwieder, Paul Cizdziel, C-Hong Chang, Amer Rana, Brad Hamilton
Presenter affiliation: Reprocell Co., Tokyo, Japan.

Mesenchymal stem cells or acellular derivative from mesenchymal stem cells promote liver regeneration in individuals with steatosis undergoing liver resection surgery
Javiera P. Bahamondes Azcuy, Fernando F. Ezquer, Flavia A. Bruna, David E. Contador, Marcelo E. Ezquer
Presenter affiliation: Centro de Medicina Regenerativa, Santiago, Chile.

Developing of spatial-temporal-controlled gene knockout method using CRISPR system in axolotl to study spinal cord and limb regeneration
Ji-Feng Fei, Dunja Knapp, Maritta Schuez, Prayag Murawala, Yan Zou, David Drechsel, Elly M. Tanaka
Presenter affiliation: DFG Center for Regenerative Therapies Dresden, Dresden, Germany.

Selenium enhances wound healing effects and hair follicle regeneration of AF-MSC conditioned medium
Wei-Wei Gao, Junghyun Park, Eun Kyung Jun, Seungkwon You
Presenter affiliation: Korea University, Seoul, South Korea.

Application of Bio-nano interface for controlling stem cell fate
Bayar Hexig
Presenter affiliation: Inner Mongolia University, Huhhot, China.

Highly efficient and reproducible differentiation of human pluripotent stem cells to pancreatic progenitors using a novel serum-free medium
Michael J. Riedel, Stephanie Lam, Yvonne Luu, Simon Hilcove, Terry E. Thomas, Allen C. Eaves, Sharon A. Louis
Presenter affiliation: STEMCELL Technologies, Vancouver, Canada.

Generating induced pluripotent stem cells with ReproRNA™-OKSGM, a non-integrating self-replicating RNA vector
Wing Y. Chang, Arwen Hunter, Alvin Ng, Annie Chen, Erik Hadley, Simon A. Hilcove, Terry E. Thomas, Allen C. Eaves, Sharon A. Louis
Presenter affiliation: STEMCELL Technologies, Vancouver, Canada.
PneumaCult™—An integrated culture medium system for in vitro human airway modeling
Juan Hou, Michael J. Riedel, Terry E. Thomas, Allen C. Eaves, Sharon A. Louis
Presenter affiliation: STEMCELL Technologies, Inc., Vancouver, Canada.

Efficient establishment and long-term maintenance of 3-dimensional mouse intestinal organoids using a novel defined and serum-free medium
Juan Hou, Ryan K. Conder, Michael J. Riedel, Terry E. Thomas, Allen C. Eaves, Sharon A. Louis
Presenter affiliation: STEMCELL Technologies Inc, Vancouver, Canada.

CHD1L may promote neuronal differentiation in human embryonic stem cells by up-regulating PAX6
Dandan Dou, Xingwu Wu, Zili Li, Yan Zhao, Lingfeng Dong, Liang Hu
Presenter affiliation: Central South University, Changsha, China; National Engineering Research Center of Human Stem Cells, Changsha, China.

Netrin regulate glioblastoma cell proliferation and stemness
Yizhou Hu, Irene Ylivinkka, Jorma Keski-Oja, Marko Hyytiäinen
Presenter affiliation: Departments of Virology and Pathology, Helsinki, Finland; Helsinki University Hospital, Helsinki, Finland.

Direct conversion of somatic cells into functional integration-free hepatocytes-like cells
Seon In Hwang, Jonghun Kim, Kyung Tae Lim, Seung Chan Lee, Suy Hyun Park, Yong Han Paik, Dong Wook Han
Presenter affiliation: School of Medicine, Konkuk University, Seoul, South Korea.

Enhancement of cell growth and self-renewal of pluripotent stem cells and improvement of neuroprotective properties in vivo by dihydroxyflavone treatment
Dawoon Han, Kyeongseok Kim, Sang baek Choi, Sohee Lim, Jihae Han, Hye Yeon Choi, Ahmed A. Dayem, Gwangmo Yang, Jihye Won, Subbroto K. Saha, Yingfu Yin, Ssang-Goo Cho
Presenter affiliation: Department of Animal Biotechnology, Animal Resources Research Center, Seoul, South Korea.
Molecular mechanism of alternative lengthening of telomeres in early embryos and its role in somatic cell reprogramming
Rongrong Le
Presenter affiliation: Tongji University, Shanghai, China.

Heterogeneity of CD34 and CD38 expression in acute B lymphoblastic leukemia cells is reversible and not hierarchically organized
Peng Li, Bing Xu, Zhiwu Jiang, Manman Deng
Presenter affiliation: Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, China; Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, China.

Enhanced direct conversion of fibroblasts into hepatocyte-like cells via accelerated conversion kinetics
Kyung Tae Lim, Seung Chan Lee, Eun-Sook Park, Kyun-Hwan Kim, Dong Wook Han
Presenter affiliation: Department of Stem Cell Biology, Seoul, South Korea.

Identification and establishment of mouse ectoderm-like stem cells
Chang Liu, Ran Wang, Jun Chen, Guangdun Peng, Patrick Tam, Naihe Jing
Presenter affiliation: Chinese Academy of Sciences, Shanghai, China.

Low immunogenicity of neural progenitor cells differentiated from induced pluripotent stem cells derived from less immunogenic somatic cells
Pengfei Liu, Shubin Chen, Xiang Li, Guangjin Pan, Jinglei Cai, Duanqing Pei
Presenter affiliation: Key Laboratory of Regenerative Biology, Guangzhou, China; Department of Regenerative Medicine, Changchun, China.

From DNA to nucleosomes—Paternal specific targeting of an H3.3 chaperone controls integrity and segregation of paternal chromosomes in mouse early embryos
Zichuan Liu, Mark E. Gill, Mathieu Tardat, Antoine Peters
Presenter affiliation: Friedrich Miescher institute for Biomedical Research, Basel, Switzerland.
Metabolic implications of retinoid-sequestering blue fluorescent lipid bodies
Thangaselvam Muthusamy, Radhika Menon, Odity Mukherjee, Megha Bangalore, Mitradas Panicker
Presenter affiliation: National Center for Biological Sciences, Bangalore, India.

Overexpression of reprogramming factor in AF-MSC accelerates DP cell activity and promotes hair follicle regeneration
Junghyun Park, Eun Kyung Jun, Daryeon Son, Seungkwon You
Presenter affiliation: Korea university, Seoul, South Korea.

Small molecule-directed high efficient conversion of pluripotent human embryonic stem cells into functional human neuronal or cardiomyocyte cell therapy derivatives for regenerative medicine
Xuejun H. Parsons
Presenter affiliation: XiAn JiaoTong University, Suzhou, China.

Does allogeneic multipotent mesenchymal stromal cells transplantation promote the progression of precancerous lesions?
Anita Plaza-Flores, Martha Arango, Flavia Bruna, Iris Espinoza, Paulette Conget
Presenter affiliation: Universidad Austral de Chile, Valdivia, Chile.

Quantitative integration of epigenetic variation and transcription factor binding by MAmotif toolkit unveiled a novel promoter regulatory module for human embryonic stem cells
Zhaohui Gong, Jiawei Wang, Yijing Zhang, Zhen Shao
Presenter affiliation: Shanghai Institute of Biological Sciences, Shanghai, China.

Inhibition of hepatic stellate cell activation by bone marrow derived stem cells in a 3D culture model
Ervina J. Sitanggang, Sri W. Jusman, Radiana D. Antarianto
Presenter affiliation: Universitas Indonesia, Jakarta, Indonesia; Universitas HKBP Nommensen, Medan, Indonesia.

Preameloblast-derived factors mediate osteoblast differentiation of human bone marrow mesenchymal stem cells via Runx2-Osterix-BSP signaling
Chul Son, Han-Wool Choung, Su-Jin Park, Hee-Bum Yang, You-Mi Seo, Joo-Cheol Park
Presenter affiliation: School of Dentistry, Seoul National University, Seoul, South Korea.
LPS-preconditioned mesenchymal stem cells modify macrophage polarization for resolution of chronic inflammation via exosome-shuttled let-7b
Dongdong Ti, Haojie Hao, Xiaobing Fu, Weidong Han
Presenter affiliation: Chinese PLA General Hospital, Beijing, China.

GMP culture media development for therapeutical stem cells
Mohan C. Vemuri
Presenter affiliation: Thermo Fisher Scientific, Frederick, Maryland.

Directing iPSC differentiation
Mohan C. Vemuri
Presenter affiliation: Thermo Fisher Scientific, Frederick, Maryland.

The Mediator subunit MED23 couples H2B mono-ubiquitination to transcriptional control and cell fate determination
Gang Wang, Xiao Yao
Presenter affiliation: State Key Laboratory of Cell Biology, Inst of Biochemistry and Cell Biology, Shanghai, China.

Multiplex high-throughput analysis of IDH-gene mutations
Wei Wang, Anna Bertoni, Andrey Korshunov, Janna Kirchhof, Katja Beck, Peter Lichter, Bernhard Radlwimmer
Presenter affiliation: German Cancer Research Center, Heidelberg, Germany; National Center for Tumor Diseases, Heidelberg, Germany.

Exosomes released from human iPSC-derived MSCs promote bone repair and regeneration by activating PI3K-Akt signaling pathway
Jieyuan Zhang, Xiaolin Liu, Xin Niu, Zongping Xie, Yang Wang
Presenter affiliation: Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China.

Echinacoside, Acteoside and 2-acetylacteoside from Cistanches herba promotes adult NSCs proliferation in vitro
Huimin Lin, Jing Han, Weibing Duan, Yan Zhu, Yu Wang
Presenter affiliation: Tianjin University of Traditional Chinese Medicine, Tianjin, China.

Heart regeneration using engineered ECM mimicking carriers to transplant cardiac progenitor cells in large animal models
Shuo Tian, Qihai Liu, Ienglam Lei, Xiaoping Li, Liangxue Lai, Peter X. Ma, Zhong Wang
Presenter affiliation: University of Michigan Medical School, Ann Arbor, Michigan.
Early steps in hematopoietic differentiation
Jennifer Yang, Yoshiaki Tanaka, Montrell D. Seay, In-Hyun Park, Yuval Kluger, Xinghua Pan, Sherman M. Weissman
Presenter affiliation: Yale University School of Medicine, New Haven, Connecticut.

Dhcr7 is required for murine normal palatogenesis
Wen-lin Xiao, Dai-zun Zhang, Yao-xiang Xu
Presenter affiliation: The Affiliated Hospital of Qingdao University, Qingdao, China.

miR-125b-Bcl-2-caspase axis participates in erythroblast enucleation
Xiaoyan Xie, Fang Fang, Minyi Qu, Quan Zeng, Junnian Zhou, Jiafei Xi, Wen Yue, Xuetao Pei
Presenter affiliation: Beijing Institute of Transfusion Medicine, Beijing, China; South China Research Center for Stem Cell & Regenerative Medicine, Guangzhou, China.

Origin of hair follicle stem cells and bulge niche
Zijian Xu, Wenjie Wang, Kaiju Jiang, Zhou Yu, Huanwei Huang, Bin Zhou, Ting Chen
Presenter affiliation: National Institute of Biological Sciences, Beijing, China.

Stem cell based drug screening to develop pulmonary arterial hypertension targeted therapy
Jun Yang, Shiqiang Gong, Qingxia Wei, Daniel Ortmann, Mariaestela Ortiz, Roger A. Pedersen, Nick W. Morrell
Presenter affiliation: Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing, China; University of Cambridge, Cambridge, United Kingdom.

Mechanism study on the effect of KUS nanoparticles in induction of the neuronal differentiation in comparison to retinoic-acid induced neuronal differentiation
Ahmed A. Dayem, Yingfu Yin, Gwangmo Yang, Jihae Han, Hye Yeon Choi, Kyeongseok Kim, Sohee Lim, Jihye Won, Subbroto K. Saha, Sang baek Choi, Ssang-Goo Cho
Presenter affiliation: Animal Resources Research Center, Konkuk University, Seoul, South Korea.

TUESDAY, October 20—8:30 PM
Chinese Tea and Beer Tasting
SESSION 4 STEM CELLS OF THE MESODERMAL LINEAGE

Chairperson: Andrew Elefanty, Murdoch Childrens Research Institute, Parkville, Australia

Modeling human development and disease with pluripotent stem cells
Gordon Keller [20’]
Presenter affiliation: University Health Network, Toronto, Canada. 58

T cell mediated acute inflammation is required for muscle regeneration
Xin Fu, Jun Xiao, Sheng Li, Hongyan Wang, Ping Hu [10’]
Presenter affiliation: Chinese Academy of Sciences, Shanghai, China. 59

Non-coding regulatory genome in blood stem cell development and diseases
Jian Xu [20’]
Presenter affiliation: UT Southwestern Medical Center, Dallas, Texas. 60

Coffee break

Microenvironmental regulation of normal and malignant hematopoietic progenitors
Jin-A Kim, Ga-Young Lee, Il-Hoan Oh [20’]
Presenter affiliation: Catholic University of Korea, Seoul, South Korea. 61

SSB1/NABP2 and SSB2/NABP1 have essential and overlapping roles in maintaining hematopoietic stem and progenitor cells
Wei Shi, Therese Vu, Glen Boyle, Fares Al-Ejeh, Didier Boucher, Steven Lane, Kum Kum Khanna [10’]
Presenter affiliation: QIMR Berghofer Medical Research Institute, Brisbane, Australia. 62

Etv2-mediated specification of mesoderm cells into hemangiogenic fate
Haiyong Zhao, Kyunghee Choi [10’]
Presenter affiliation: Washington University in St. Louis, School of Medicine, St. Louis, Missouri. 63
Conversion of T lymphoid-primed progenitors into the myeloid lineage in the absence of Irf4 during embryogenesis

Feng Liu [10']
Presenter affiliation: Institute of Zoology, Chinese Academy of Sciences, Beijing, China.

WEDNESDAY, October 21—2:00 PM

Visit to Old Suzhou

WEDNESDAY, October 21—7:00 PM

SESSION 5  GENOME EDITING II

Chairperson:  Sally Temple, Neural Stem Cell Institute, Rensselaer, New York, USA

Generation and application of pluripotent stem cells—From bench to bedside
Qi Zhou [20']
Presenter affiliation: State Key Laboratory of Stem Cell and Reproductive Biology, Beijing, China.

Cnot3 promotes differentiation gene mRNA deadenylation and degradation to maintain the pluripotent state in ESCs and epiblast
Xiaofeng Zheng, Pengyi Yang, Brad Lackford, Brian Bennett, David Fargo, Raja Jothi, Guang Hu [10']
Presenter affiliation: National Institute of Environmental Health Sciences, RTP, North Carolina.

Opposing roles for the lncRNA Haunt and its genomic locus in regulating HOXA activation during ESC differentiation
Yafei Yin, Pixi Yan, Jinlong Lu, Xiaohua Shen [10']
Presenter affiliation: Tsinghua University, Beijing, China.

Genome editing in human pluripotent stem cells for understanding the mechanisms of pancreatic development and diabetes
Danwei Huangfu [20']
Presenter affiliation: Sloan Kettering Institute, New York, New York.
SESSION 6  STEM CELLS AND CELL FATE DECISIONS

Chairperson:  Gordon Keller, University Health Network, Toronto, Canada

Study familial hypertrophic cardiomyopathy using patient specific induced pluripotent stem cells
Lei Yang, Lu Han, Yang Li, Kimimasa Tobita, Glenna Bett, Randall Rasmusson  [10’]
Presenter affiliation: University of Pittsburgh, Pittsburgh, Pennsylvania.

A balancing act between stratification and EMT in human thymic epithelial cells
Matteo Pluchinotta, Melissa Maggioni, Tiphaine M. Arlabosse, Paola Bonfanti, Roxana M. Wasnick, Christèle Gonneau, Ariane Rochat, Yann Barrandon  [10’]
Presenter affiliation: Ecole Polytechnique Fédérale Lausanne, Lausanne, Switzerland; Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland.

Tissue regeneration in liver—Plasticity or stem cells?
Markus Grompe, Bin Li, Willscott Naugler, Branden Tarlow  [20’]
Presenter affiliation: Oregon Health & Science University, Portland, Oregon.

Coffee break

Endodermal differentiation of pluripotent stem cells to understand development and regenerate in vivo organ function
Darrell Kotton  [20’]
Presenter affiliation: Boston University School of Medicine, Boston, Massachusetts.

The role of TET proteins in hESC pluripotency and differentiation
Nipun Verma, Danwei Huangfu  [10’]
Presenter affiliation: Sloan-Kettering Institute, New York, New York; Weill Graduate School of Medical Sciences at Cornell University, New York, New York.
Primate-specific endogenous retrovirus-driven transcription defines naive-like stem cells
Presenter affiliation: Max-Delbrück-Center for Molecular Medicine, Robert-Rössle-Strasse 10, 13125 Berlin, Berlin, Germany.

Oocyte factors suppress mitochondrial polynucleotide phosphorylase to remodel the metabolome and enhance reprogramming
Shyh-Chang Ng
Presenter affiliation: Genome Institute of Singapore, Singapore.

THURSDAY, October 22—2:00 PM

SESSION 7 ROAD TO THE CLINIC

Chairperson: Marcus Grompe, Oregon Health and Science University, Portland, Oregon, USA

Development of an encapsulated stem cell-based therapy for diabetes
Olivia G. Kelly
Presenter affiliation: ViaCyte, Inc., San Diego, California.

Embryonic stem cell-derived basal forebrain cholinergic neurons ameliorate the cognitive symptoms associated with Alzheimer’s disease in mouse models
Chunmei Yue, Wei Yue, Yuanyuan Li, Ting Zhang, Man Jiang, Naihe Jing
Presenter affiliation: State Key Laboratory of Cell Biology, Institute of Biochemistry and Cell Biology, Shanghai, China.

Human neural stem cells—Early clinical studies in spinal cord injury and age related macular degeneration
Ann Tsukamoto, Nobuko Uchida, Alexandra Capela, Stephen Huhn
Presenter affiliation: StemCells, Inc., Newark, California.
Clinical trial—hESC-RPE transplantation into the sub retina of wet AMD patients
Zheng Qin Yin [20’]
Presenter affiliation: Southwest Hospital, Third Military Medical University, China

Coffee break
Paul Simmons [20’]
Presenter affiliation: Mesoblast, Ltd., Melbourne, Australia.

THURSDAY, October 22—4:30 PM

KEYNOTE SPEAKER

Neural stem cells, their role in development and therapy development
Sally Temple [40’]
Presenter affiliation: Neural Stem Cell Institute, Rensselaer, New York.

THURSDAY, October 22—5:30 PM

COCKTAILS and BANQUET
AUTHOR INDEX

Al-Ejeh, Fares, 62
Annand, Robert, 15
Antariano, Radiana D., 42
Arango, Martha, 40
Archer, Trevor K., 7
Arlabosse, Tiphaine M., 70
Bahamondes Azcuy, Javiera P., 19
Bangalore, Megha, 37
Barrandon, Yann, 70
Beck, Katja, 48
Bennett, Brian, 66
Bertoni, Anna, 48
Besser, Daniel, 74
Bet, Glenna, 69
Bonfanti, Paola, 70
Boucher, Didier, 62
Boutet, S, 13
Boyle, Glen, 62
Bruna, Flavia, 19, 40
Cai, Huiqiang, 74
Cai, Jinglei, 35
Capela, Alexandra, 78
Chang, C-Hong, 18
Chang, Qing, 13
Chang, Wing Y., 24
Chen, Annie, 24
Chen, Chaifu, 6
Chen, Deyun, 14
Chen, Jun, 34
Chen, Sean, 6
Chen, Shubin, 35
Chen, Ting, 55
Chen, Wei, 74
Chen, Yingju (Miliya), 15
Cho, Ssang-Goo, 16, 30, 57
Choi, Hye Yeon, 16, 30, 57
Choi, Kyunghhee, 63
Choi, Sang baek, 16, 30, 57
Choi, Yong Jin, 6
Choung, Han-Wool, 43
Cizdziel, Paul, 17, 18
Conder, Ryan K., 26
Conget, Paulette, 40
Contador, David E., 19
Dayem, Ahmed A., 16, 30, 57
Deng, Hongkui, 2
Deng, Manman, 32
Devaraju, N, 13
Dong, Lingfeng, 27
Dou, Dandan, 27
Drechsel, David, 20
Duan, Weibing, 50
Eaves, Allen C., 23, 24, 25, 26
Eminli-Meissner, Sarah, 18
Espinoza, Iris, 40
Ezquer, Fernando F., 19
Ezquer, Marcelo E., 19
Fang, Fang, 54
Fargo, David, 66
Fei, Ji-Feng, 20
Fu, Xiaobing, 14, 44
Fu, Xin, 59
Fuchs, Nina V., 74
Futatsugi, Yoko, 4
Gao, Wei-Wei, 21
Gao, Yimeng, 5
Ghanbarian, Avazeh T., 74
Gill, Mark E., 36
Gong, Shiqiang, 56
Gong, Zhaohui, 41
Gonneau, Christèle, 70
Grompe, Markus, 71
Guo, T, 13
Hadley, Erik, 24
Hamilton, Brad, 15, 17, 18
Han, Dawoon, 30
Han, Dong Wook, 5, 29, 33
Han, Jihae, 16, 30, 57
Han, Jing, 50
Han, Lu, 69
Han, Weidong, 14, 44
Hao, Haojie, 14, 44
He, Lin, 6
Hexig, Bayar, 22
Hilcove, Simon, 23, 24
Hou, Juan, 25, 26
Hu, Guang, 66
Hu, Liang, 27
Hu, Ping, 59
Hu, Yizhou, 28
Huang, Huanwei, 55
Huang, Yue, 12
Huangfu, Danwei, 10, 68, 73
Huh, Stephen, 78
Hui, Lijian, 5
Hunter, Arwen, 24
Hurst, Laurence D., 74
Hwang, Seon In, 5, 29
Hyytiäinen, Marko, 28
Inamura, Mitsuru, 15, 17
Ivics, Zoltán, 74
Izsvák, Zsuzsanna, 74
Ji, Weizhi, 9
Jiang, Kajiu, 55
Jiang, Man, 77
Jiang, Zhiwu, 32
Jing, Naihe, 34, 77
Jothi, Raja, 66
Jun, Eun Kyung, 21, 38
Jusman, Sri W., 42
Kalkan, Tüzer, 1
Keller, Gordon, 58
Kelly, Olivia G., 76
Keski-Oja, Jorma, 28
Khanna, Kum Kum, 62
Kim, Jin-A, 61
Kim, Jonghun, 5, 29
Kim, Kyeongseok, 16, 30, 57
Kim, Kyun-Hwan, 33
Kim, Sang Yong, 6
Kirchhof, Janna, 48
Kiskin, Fedir, 18
Kluger, Yuval, 52
Knapp, Dunja, 20
Kogami, Harumi, 17
Korshunov, Andrey, 48
Kotton, Darrell, 72
Kwieder, Baraa, 18
Lackford, Brad, 66
Lai, Liangxue, 51
Lam, Stephanie, 23
Lane, Steven, 62
Langer, Lee, 7
Le, Rongrong, 31
Lee, Ga-Young, 61
Lee, Seung Chan, 5, 29, 33
Lei, Weiling, 51
Li, Bin, 71
Li, Jin B., 6
Li, Peng, 32
Li, Qin V., 10
Li, Sheng, 59
Li, Xiang, 35
Li, Xiaoping, 51
Li, Yang, 69
Li, Yuanyuan, 77
Li, Zili, 27
Lichter, Peter, 48
Lim, Kyung Tae, 5, 29, 33
Lim, Sohee, 16, 30, 57
Lin, Chao-Po, 6
Lin, Huimin, 50
Lin, Yu Ching (Zachary), 17
Liu, Chang, 34
Liu, Feng, 64
Liu, Guang, 12
Liu, Pengfei, 35
Liu, Qihai, 51
Liu, Xiaolin, 49
Liu, Yufang, 12
Liu, Zichuan, 36
Lorincz, Matthew C., 74
Louis, Sharon A., 23, 24, 25, 26
Lu, Congyi, 11
Lu, Jinlong, 67
Luu, Yvonne, 23
Ma, Peter X., 51
Macfarlan, Todd, 6
Maggioni, Melissa, 70
Menon, Radhika, 37
Moon, Jung-II, 18
Morrell, Nick W., 56
Mukherjee, Odity, 37
Murawala, Prayag, 20
Muthusamy, Thangaselvam, 37
Naugler, Willscott, 71
Ng, Alvin, 24
Ng, Shyh-Chang, 75
Nikish, Alexandria, 11
Nishishita, Naoki, 15
Niu, Xin, 49
Niwa, Hitoshi, 4
Oh, Il-Hoan, 61
Oka, Kotaro, 17
Okuda, Yuichi, 17
Ortiz, Mariaestela, 56
Ortmann, Daniel, 56
Paik, Yong-Han, 5, 29
Pan, Guangjin, 35
Pan, Jen, 11
Pan, Xinghua, 52
Panicker, Mitradas, 37
Park, Eun-Sook, 33
Park, In-Hyun, 52
Park, Joo-Cheol, 43
Park, Junghyun, 21, 38
Park, Su Hyun, 29
Park, Su-Jin, 43
Parsons, Xuejun H., 39
Pedersen, Roger A., 56
Pei, Duanqing, 8, 35
Pei, Xuetao, 54
Peng, Guangdun, 34
Peng, WeiQun, 6
Peters, Antoine, 36
Plaza-Flores, Anita, 40
Pluchinotta, Matteo, 70
Prigione, Alessandro, 74
Qu, Minyi, 54
Radjwimmer, Bernhard, 48
Rana, Amer, 18
Raskó, Tamás, 74
Rasmusson, Randall, 69
Riedel, Michael J., 23, 25, 26
Risso, Davide, 6
Rochat, Ariane, 70
Saha, Subbroto K., 16, 30, 57
Sanjana, Neville, 11
Schuez, Maritta, 20
Schumann, Gerald G., 74
Seay, Montrell D., 52
Seo, You-Mi, 43
Shao, Zhen, 41
Shen, Xiaohua, 67
Shi, Wei, 62
Shi, Xi, 11
Shiina, Kaishu, 17
Shindo, Yutaka, 17
Singh, Manvendra, 74
Sitanggang, Ervina J., 42
Smith, Austin, 1
Son, Chul, 43
Son, Daryeon, 38
Speed, Terence P., 6
Szpankowski, L., 13
Szoetnik, Attila, 74
Tam, Patrick, 34
Tan, Meng How, 6
Tanaka, Elly M., 20
Tanaka, Yoshiaki, 52
Tardat, Mathieu, 36
Tarlow, Branden, 71
Temple, Sally, 79
Thomson, Terry E., 23, 24, 25, 26
Ti, Dongdong, 44
Tian, Shuo, 51
Tobita, Kimimasa, 69
Tsukamoto, Ann, 78
Uchida, Nobuko, 78
Vemuri, Mohan C., 45, 46
Verma, Nipun, 73
Vu, Therese, 62
Wade, Staton, 7
Wang, Xue, 12
Wang, Gang, 47
Wang, Hongyan, 59
Wang, Jiawei, 41
Wang, Jichang, 74
Wang, Ran, 34
Wang, Wei, 48
xxiii
Wang, Wenjie, 55
Wang, Yang, 49
Wang, Yangming, 3
Wang, Yu, 50
Wang, Zhong, 51
Ward, James, 7
Wasnick, Roxana M., 70
Waston, M, 13
Wei, Qingxia, 56
Wei, Sherman M., 52
Won, Jihye, 16, 30, 57
Wu, Xingwu, 27
Wu, Yalei, 6
Xi, Jiafei, 54
Xiao, Jun, 59
Xiao, Wen-lin, 53
Xie, Gangcai, 74
Xie, Xiaoyan, 54
Xie, Zongping, 49
Xu, Bing, 32
Xu, Jian, 60
Xu, Yao-xiang, 53
Xu, Zijian, 55
Xuan, Zhenyu, 6
Yamanaka, Ryu, 17
Yan, Pixi, 67
Yang, Gwangmo, 16, 30, 57
Yang, Hee-Bum, 43
Yang, Jennifer, 52
Yang, Jun, 56
Yang, Lei, 69
Yang, Pengyi, 66
Yao, Xiao, 47
Yi, Kevin, 18
Yin, Yafei, 67
Yin, Yingfu, 16, 30, 57
Ylivinkka, Irene, 28
Yoshida, Shunsuke, 15, 17
You, Seungkwan, 21, 38
Yu, Zhou, 55
Yue, Chunmei, 77
Yue, Wei, 77
Yue, Wen, 54

Zeng, Quan, 54
Zhang, Dai-zun, 53
Naïve pluripotency is the emergent potential of individual cells to produce all lineages of the mature organism in response to extrinsic cues. In rodents this initiation phase at the foundation of mammalian development can be captured and propagated in the form of embryonic stem (ES) cells. In the appropriate culture environment shielded from differentiation cues ES cells exhibit robust self-renewal, are highly homogeneous and are equipotent – a so-called ground state. When released from ground state culture conditions ES cells rapidly enter into multilineage specification and differentiation. We are employing both genome-wide screens and candidate approaches to characterise the multiplex molecular machinery that extinguishes the naive pluripotency programme and drives developmental transition to a formative phase of pluripotency.
Pluripotent stem cells can be induced from somatic cells, providing an unlimited cell resource, with potential for studying disease and use in regenerative medicine. We demonstrated that pluripotent stem cells can be generated from mouse somatic cells using a combination of seven small-molecule compounds. The chemically induced pluripotent stem cells resemble embryonic stem cells in terms of their gene expression profiles, epigenetic status, and potential for differentiation and germline transmission. This chemical reprogramming strategy has potential use in generating functional desirable cell types for clinical applications.

We recently demonstrated that mouse fibroblasts can be directly converted into neuronal cells using only a cocktail of small molecules, with a yield of up to >90% being TUJ1-positive after 16 days of induction. After a further maturation stage, these chemically induced neurons (CiNs) possessed neuron-specific expression patterns, generated action potentials, and formed functional synapses. Mechanistically, we found that a BET family bromodomain inhibitor, I-BET151, disrupted the fibroblast-specific program, while the neurogenesis inducer ISX9 was necessary to activate neuron-specific genes. Overall, our findings provide a “proof of principle” for chemically induced direct reprogramming of somatic cell fates across germ layers without genetic manipulation, through disruption of cell-specific programs and induction of an alternative fate.
Multiple types of pluripotent stem cells (PSCs) can be derived under distinct culture conditions to represent different stages of early mammalian embryos. Naive PSCs such as mouse embryonic stem cells (ESCs) cultured in 2i/lif conditions are thought to be similar to naive epiblast cells and have the most unrestricted developmental potential comparing to other PSCs, while primed PSCs such as mouse epiblast-like stem cells (EpiLCs) or epiblast stem cells (EpiSCs) are similar to post-implantation embryos and have less unrestricted developmental potential. The molecular mechanisms controlling the maintenance and dismantling of naive pluripotency are poorly understood. I will present our efforts in decoding microRNA (miRNA) function during the maintenance and dismantling of naive pluripotency. Previously we and others showed that the miR-290/302 family of miRNAs plays critical roles in promoting cell cycle progression, blocking the silencing of pluripotency by differentiation-inducing miRNAs, and enhancing the glycolysis metabolism in mouse ESCs and during reprogramming (Cao et al., 2015; Guo et al., 2015; Wang et al., 2013). Unexpectedly, we recently found that multiple miRNAs from the miR-290/302 cluster are also essential for the exit of naive pluripotency during the naive to primed pluripotency transition (ESCs to EpiLCs). I will present our current understanding of the mechanism on how pluripotency-promoting miRNAs become a destructive force of naive pluripotency. Furthermore, we identified several other miRNAs that help maintain ESCs at the naive pluripotent state in differentiation conditions when overexpressed. Based on these findings, we are currently developing methods to derive naive human PSCs by manipulating the expression of key miRNAs. Our study reveals miRNAs as an important class of regulators in the regulation of naive pluripotency and provide potential targets for the derivation of naive PSCs in non-rodent species.

References:

REGULATION OF 2-CELL SPECIFIC GENE EXPRESSION IN MOUSE ES CELLS

Hitoshi Niwa1,2, Yoko Futatsugi1

1RIKEN Center for Developmental Biology (CDB), Laboratory for Pluripotent Stem Cell Studies, Kobe, Japan, 2Institute of Molecular Embryology and Genetics, Kumamoto University, Department of Pluripotent Stem Cell Biology, Kumamoto, Japan

Mouse ES cells show remarkable heterogeneity of gene expression in conventional culture condition. Pluripotency-associated transcription factors such as Nanog, Klf4 and Tbx3 are expressed heterogeneously in different patterns (Niwa et al, Nature, 2009). It was reported that the genes expressed in 2-cell embryos also show unique pattern of heterogeneous expression in mouse ES cells. Among them, so called 2-cell specific genes, Zscan4c is confirmed to be essential for the maintenance of ES cell population in long-term culture. Zscan4c, as well as other 2-cell specific genes, is expressed in 5% of ES cells cultured in FCS-containing medium. Knock-down of Zscan4c causes the crisis of ES cell population after 1 month due to shortening of telomere (Zalzman et al, Nature, 2010). Artificial expression of Zscan4c contributes to the maintenance of full pluripotency in long-term culture (Amano et al, Nat Commun, 2013). Therefore, its particular expression pattern may couple with its functional significance.

How such unique expression pattern of Zscan4c is achieved in ES cell population? We recently reported the involvement of the transcription factor Nr0b1 in the regulation of Zscan4c-expression (Fujii et al, Sci Rep, 2015). Knockout of Nr0b1 caused increase in ES cell population expressing a Zscan4c reporter from 5% to 20%. Inducible expression of Nr0b1 resulted in reciprocal regulation of Zscan4c-positive population, indicating that Nr0b1 acts as a direct repressor of Zscan4c. We also revealed that Nr5a2 could act as a partner of Nr0b1 to mediate the repressive function.

We also analyzed the dynamics of Zscan4-positive cells using live imaging. When the expression of Zscan4c reporter was monitored at single cell level, we found that the activation of Zscan4c had an interesting correlation with the cell-cycle length. The length of the cell-cycle ranged from 10 to 22 hours in conventional culture condition and it tended to elongate along the proliferation. ES cells dividing with 20 hours of cell-cycle length had the highest incidence of Zscan4c expression. Once Zscan4c was transiently expressed, the cell-cycle length in the next division tended to be shortened. Since the telomere length showed negative correlation with the cell-cycle length, either elongation of cell-cycle or shortening of telomere may trigger the expression of Zscan4c to promote telomere elongation. Detailed molecular mechanism behind this phenomenon is under investigation.
TRANSPROGRAMMING INTO HEPATIC LINEAGE BY DEFINED FACTORS

Kyung Tae Lim¹, Jonghun Kim¹, Seung Chan Lee¹, Seon In Hwang¹, Yong-Han Paik², Yimeng Gao³, Lijian Hui³, Dong Wook Han¹

¹Konkuk University School of Medicine, Stem Cell Biology, Seoul, South Korea, ²Sungkyunkwan University School of Medicine, Medicine, Seoul, South Korea, ³Shanghai Institutes for Biological Sciences, State Key Laboratory of Cell Biology, Shanghai, China

Recent studies have shown that defined hepatic factors could lead to the direct conversion of fibroblasts into induced hepatocytes-like cells (iHeps). However, the underlying mechanism of the hepatic lineage conversion is largely unknown. Here we report that direct conversion into iHeps is a stepwise transition involving erasure of somatic memory, mesenchymal-to-epithelial transition (MET), and induction of hepatic cell fate in a sequential manner. Through screening for additional factors that could potentially enhance the conversion kinetics, we found that activation of MET process enhanced the kinetics of the MET and hepatic programs, resulting in remarkably improved generation of iHeps (>87 fold). We also demonstrate that combined treatment with small molecules could replace the additional factor and thus lead to the highly efficient conversion of mouse and human fibroblasts into iHeps. The novel reprogramming cocktail defined herein offers a method for obtaining a sufficient number of iHeps for both mechanistic and clinical studies.
Mouse embryonic stem cells (ESCs) and induced pluripotent stem cells possess pluripotent cell fate potential, efficiently contributing to all embryonic cell types, but rarely to extra-embryonic lineages. Here, we identify a microRNA, \textit{miR-34a}, whose deficiency in mouse pluripotent stem cells expands their developmental potential. In a variety of functional assays \textit{in vitro} and \textit{in vivo}, \textit{miR-34a}-deficient pluripotent stem cells exhibit totipotent-like cell fate potential both at the population level and at the single cell level, giving rise to both embryonic and extra-embryonic cell lineages. The expression profiles of \textit{miR-34a}–/– pluripotent stem cells are characterized by a strong induction of MuERV-L (MERVL) family endogenous retroviruses (ERVs), a unique molecular hallmark of totipotent 2-cell stage blastomeres and totipotent-like ESCs. We demonstrate that \textit{miR-34a} represses MERVL expression through transcriptional regulation, at least in part by directly repressing the transcription factor GATA-binding protein 2 (Gata2). Consistent with the strong correlation between MERVL activation and totipotent-like fate potential, the \textit{miR-34a}/Gata2 pathway that represses MERVL expression also restricts the acquisition of totipotency in pluripotent stem cell culture. Taken together, our findings provide novel insights into the molecular basis for the transition between pluripotency and totipotency in culture, and highlight the functional importance of miRNAs in regulating this cell fate plasticity.
The human ESC SWI/SNF chromatin-remodeling complex consists of the catalytic ATPase Brg1 and several additional core subunits. Notably, knockdown of specific subunits, including BAF170, can result in severely impaired hESC pluripotency. In addition, BAF170 is absent from the mouse ESC SWI/SNF complex, which contains two copies of the highly homologous BAF155. This difference suggests that BAF170 may contribute to the dissimilarities between these two cell types.

Our results suggest the existence of a feedback loop between BAF170 and the powerful pluripotency regulator mir-302. In support of the functional significance of this interaction, a BAF170-overexpressing hESC line (BAF170-OE) generated in our laboratory was found to be impaired in endodermal differentiation and to exhibit a different response than parental hESCs following retinoic acid-induced differentiation.

RNA-seq data for hESCs following BAF170-knockdown and mir-302-inhibition are currently being analyzed for effects at the gene and enhancer levels, and we predict that these data will lead a more precise understanding of BAF170’s role in hESCs and its interaction with mir-302. Moreover, RNA-seq data obtained from hESCs following BAF155 knockdown will provide insights into the general mechanisms by which SWI/SNF subunit composition translates into cell fate decisions.
Somatic cell reprogramming is emerging as an ideal system for the analysis of mechanisms involved in cell fate decisions. With clearly defined starting cells and the finished iPSCs, it has becoming possible to define the molecular events associated with the various fate changes. We initially reported that a mesenchymal to epithelial transition or MET initiates somatic cell reprogramming and have also identified factors critical for this starting step. Subsequently, we refined this mechanism by demonstrating a sequential EMT-MET process for optimal reprogramming. Therefore, the switching between mesenchymal and epithelial fates appears to underlie the cell fate decisions during somatic cell reprogramming. We then focus on the molecular mechanisms that specify the mesenchymal and epithelial fates and factors that can facilitate or inhibit the transitions. We will discuss the newly identified factors for these fate decisions. We believe that a comprehensive analysis for the EMT-MET process may help us better understand not only reprogramming but also other cell fate changes in both normal development and diseases.
ESTABLISHING MONKEY MODELS FOR HUMAN DISEASES VIA PRECISION GENE EDITING

Weizhi Ji

Institute of Translational Medicine, Kunming University of Science and Technology, Yunnan Key Laboratory of Primate Biomedical Research, Kunming, China

Monkeys are an ideal animal model for human disease as the closest phylogenetic relatives to humans. These animals are often the best—and sometimes the only—available model for studying a variety of human health issues, ranging from diseases and disorders to potential therapies and preventive strategies. Precision gene editing, including ZFN, TALENs and CRISPR, paves ways to establish transgenic animal models for human diseases. Building on our past successful study on rhesus and cynomolgus monkeys with CRISPR and TALENs, several nervous system diseases, such as PD, DMD, and RTT have been generated in monkeys with either TALENs or CRISPR. The results implied that the phenotype variations can be seen in transgenic monkeys which have a greater potential to produce human-like phenotypes than mice. We believe that efficient gene-editing techniques in monkeys will definitely provide more powerful ways to study complex nervous system diseases.
A GENOME-WIDE CRISPR SCREEN TO IDENTIFY GENES IMPORTANT FOR HUMAN DEFINITIVE ENDODERM LINEAGE COMMITMENT

Qing V Li, Danwei Huangfu

Sloan Kettering Institute, Developmental Biology Program, New York, NY

Endodermal organs and cells, such as liver and pancreatic β cells, all originate from a common precursor called definitive endoderm (DE). Dissecting the molecular mechanisms for DE lineage commitment from pluripotent stem cells will have profound implications for understanding early human development. Human embryonic stem cells (hESCs) have the capability of unlimited self-renewal and differentiation into all three germ layers. Activation of the WNT and TGFβ signaling pathways induce hESCs to exit pluripotency and differentiation to mesoendoderm. Subsequent TGFβ signaling specifies definitive endoderm differentiations, which is manifested by the expression of SOX17 and FOXA2. Here, we used a genome-wide CRISPR knockout screen approach to identify the genes that play a role in the process of DE differentiation. In order to faithfully report DE differentiation efficiency, we employed our previously established iCRISPR gene-editing platform to generate a SOX17GFP hESC reporter line. Taking advantage of E8 feeder-free culture system, we also established a reproducible feeder-free DE differentiation protocol and successfully adapted it with the lentiviral Genome-scale CRISPR Knock-Out (GECKO) library to screen genes responsible for either inhibiting or promoting DE differentiation. A pilot genome-wide screen was conducted by using half of the GECKO library (3 sgRNA/gene) in order to evaluate the feasibility and sensitivity of the screen. Pooled mutant hESCs were selected and differentiated into DE with an efficiency of 60%. Sorted SOX17GFP positive and negative cells were subject to HiSeq to calculate the abundance of each sgRNA. Using a Z-score method, we were able to verify that the SOX17 and GATA6 sgRNA sequences were enriched in the SOX17GFP negative cell population, suggesting the feasibility of the screen. However, some of the known positive regulators like EOMES were not recovered from HiSeq, suggesting that a more complex library and a higher coverage of deep sequencing would be needed to improve the sensitivity of the screen. Nevertheless, ongoing experiments are seeking to individually validate the top hits discovered from the pilot screen. Based on our first-hand experience from the pilot screen, we will repeat the screen with the full GECKO library (6 sgRNA/gene) plus additional positive control spike-in probes in order to refine the screen. Successfully completing the screen will not only identify novel regulators of human definitive endoderm lineage commitment, but also set the ground foundation for using genetic screens to study human embryonic development to other lineages or later developmental stages.
CELLULAR MODELS OF AUTISM-ASSOCIATED DE NOVO MUTATIONS IN HUMAN EMBRYONIC STEM CELLS

Xi Shi1, Neville Sanjana2,3,4,5, Congyi Lu2,3, Alexandria Nikish4, Jen Pan1, Feng Zhang2,3,4,5

1Broad Institute of MIT and Harvard, Stanley Center for Psychiatric Research, Cambridge, MA, 2Massachusetts Institute of Technology, McGovern Institute for Brain Research, Cambridge, MA, 3Massachusetts Institute of Technology, Department of Brain and Cognitive Sciences, Cambridge, MA, 4Massachusetts Institute of Technology, Department of Biological Engineering, Cambridge, MA, 5Broad Institute of MIT and Harvard, Cambridge, MA

Abstract: Autism spectrum disorder (ASD), with early onset prior to age three and morbidity as high as 1-2.6%, is one of the leading causes of disability in childhood and a source of severe suffering for families. Recent whole exome sequencing (WES) studies revealed that a number of highly disruptive (nonsense) de novo mutations confer significant susceptibility to ASD. These genetic findings provide novel clues about the pathophysiology of ASD. Among them, de novo loss of function (LOF) mutations in CHD8, SCN2A, KATNAL2, DYRK1A and SHANK3 have been reported to be highly associated with ASD risk. In order to study these genes and understand how they influence neural development, we introduced de novo LOF mutations at specific locations within genes implicated in ASD into human embryonic stem cells (hESCs) using the CRISPR-Cas9 system. Wild-type (WT) and engineered hESCs (ASD model cells) were then differentiated into cortical-like neurons in vitro. These directly differentiated neurons form synapses and exhibit robust electrophysiological properties. We then studied the induced human neurons carrying CHD8 (chromodomain helicase DNA-binding protein 8) nonsense mutations (CHD8+/−) (human patients are heterozygous; homozygous deletion is lethal in mice). Whole-cell patch-clamp and high-throughput non-invasive multi-electrode array (MEA) showed comparable resting membrane potential, but significantly lower neural activity in the CHD8+/− induced neurons compared to WT (spike rate: ~0.5Hz Vs. 1.5Hz). Such reduced neural activity has also been reported in certain autistic patients and ASD mouse models. These data suggest that 50% reduction of CHD8 causes a functional deficit in cortical-like neurons. Our data show that using hESCs to model ASD in an isogenic background, in combination with the recently developed method of neuron differentiation, provides a novel approach to study gene function in human neurons. Given the relative efficiency of generating these models, it will be possible to scale up this approach and test hundreds if not thousands of associated loci, offering a remarkable opportunity to study the genetics of human disease.
ARRAYED MUTANT HAPLOID STEM CELL LIBRARIES FACILITATE GENETIC SCREEN FOR THE "EXIT-FROM-PLURIPOTENCY" FACTORS

Guang Liu*1,2, Xue Wang*1, Yufang Liu1, Meili Zhang1,2, Yue Huang1,2

1Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China, 2State Key Laboratory of Medical Molecular Biology, Chinese Academy of Medical Sciences, Beijing, China

Mammalian embryonic stem cells (ESCs) retain unlimited self-renewal potential in culture and can be induced to differentiate into all somatic cell types under appropriate culture conditions. Now, it has been well defined how ES cells establish and sustain pluripotency during self-renewal, but the process by which ES cells exit from self-renewal remains largely unknown. Recently, mammalian haploid ESC lines were successfully established in mouse, rat and monkey, which have displayed unique potentials in functional genetic studies. Here, we conducted a forward genetic screen in haploid mouse ESCs to identify factors which were essential for the “exit from pluripotency”. Firstly, we generated a genome-wide mixed mutant library by using haploid ES cells and PiggyBac (PB) transposon, and based on this mixed library, we created an arrayed library, which can eliminate the interplay between different mutant clones and facilitate recessive genetic screen. Secondly, we utilized the arrayed library to screen mutant clones which showed resistance to differentiation under differentiation induced culture conditions. After screened 2,208 individual mutant clones, we got 181 differentiation-defect ESC clones. The PB transposon insertion sites in these clones were identified by Splinkerette PCR and sequencing. Sixty-nine out of them were mapped to encoding genes. Among them, several known critical “exit from pluripotency” genes, such as Tcf3, were included. Finally, to establish causality between the transposon insertion and the mutant phenotype, we removed PB transposon mutagen by re-expressing PBase in these mutant ESC clones. We observed that the revertant clones regained the ability of differentiation. Moreover, the functional role of these genes in the exit-from-pluripotency were verified in diploid ESCs via CRISPR/Cas9 mediated gene knockout.
A NOVEL, MULTIFACTORIAL APPROACH FOR HUMAN IPS CELL DIFFERENTIATION AND REPROGRAMMING USING AN AUTOMATED CELL CULTURE SYSTEM

T Guo, M Waston, N Devaraju, S Boutet, L Szpankowski, Qing Chang

Fluidigm(Shanghai)InstrumentTechnologyCo.,Ltd., marketing, Shanghai, China

A major challenge in the stem cell field is to define the optimal condition for cell expansion, differentiation and reprogramming. Because multiple intracellular and extracellular signaling pathways are involved in each cellular process, a combinatorial approach to screen multiple factors is highly desirable. To facilitate the exploratory processes, we have developed CallistoTM, an automated cell-culture system for cell manipulation and environmental control. The system consists of an integrated fluidic circuit (IFC), an electropneumatic controller instrument, experimental designer software and automated run-time control software. Each IFC has 32 culture microchambers and 16 reagent inlets. Each microchamber can be dosed separately with different combinations and ratios of the 16 reagents at various predefined time points. It enables long-term cell culture (more than three weeks) with three-day hands-off operation. Using this system, we have developed a novel nonintegrating method for direct conversion of human BJ fibroblasts to neurons. We also demonstrated that human iPSCs can differentiate to neural progenitor cells or nociceptor neurons on the IFC after treatment with small molecules. Finally, we have developed a protocol for RNA transfection that allows efficient delivery of mRNAs and siRNAs into hiPSCs. In summary, the automated microfluidic platform employs precise control of the microenvironment of cells, facilitates studies of multifactorial combinations and enables development of robust, reproducible and chemically defined cell culture and manipulation.
Objective
To investigate the characteristics of adipose tissue-derived mesenchymal stem cells (ASCs) and collagen-chitosan scaffolds. To explore the potential epidermal differentiation of ASCs in collagen-chitosan scaffolds with all-trans retinoic acid and bone morphogenetic protein 4.

Methods
1. ASCs were isolated from subcutaneous fat of the SD rats. Observe the morphology of ASCs by inverted microscope. Identify the surface markers of ASCs by flow cytometry and the multi-differentiation potential of ASCs by ASCs differentiation into adipocytes, osteocytes, and chondrocytes.
2. Collagen and chitosan was mixed, freeze-dried and cross-linked to form the scaffolds. The microstructure of the scaffolds was observed by scanning electron microscopy (SEM). The biocompatibility of ASCs with collagen-chitosan scaffolds was proved by detecting cell proliferation situation in collagen-chitosan scaffolds by Methyl thiazolyl blue tetrazolium bromide (MTT) assay and Live/Dead assay.
3. ASCs were cultured in collagen-chitosan scaffolds with all-trans retinoic acid (ATRA) and bone morphogenetic protein 4 (BMP4). At the end of the culture period, matrices were sectioned and labeled with immunomarkers (P63, cytokeratin 19 and cytokeratin 18) to identify cell phenotype.

Results
The isolated ASCs were elongated and spindle-shaped after passage 3, and they could differentiate into adipocytes, osteocytes, and chondrocytes. Flow cytometry revealed that ASCs were positive for CD90, CD105, and CD73, whereas they were negative for CD34, CD45, HLA-DR, and CD11α. Using fluorescence microscope, we observed that ASCs were spindle-shaped and evenly distributed in the scaffolds. MTT assay and Live/Dead assay indicated that the collagen-chitosan scaffolds has good biocompatibility with ASCs, Immunohistochemistry and western blotting assay both showed that ASCs expressed epithelial marker proteins.

Conclusion
In this study, ASCs were successfully transdifferentiation into epidermal cells when cultured in collagen-chitosan scaffolds with ATRA and BMP4.

Keywords
adipose tissue-derived mesenchymal stem cells, epidermal cells, collagen-chitosan scaffolds, all-trans retinoic acid, bone morphogenetic protein 4
SIMPLE CONVERSION OF PRIMED HUMAN IPS CELLS TO NAÏVE-STATE IPS CELLS

Shunsuke Yoshida¹, Brad Hamilton², Robert Annand², Naoki Nishishita³, Mitsuru Inamura¹, Yingju (Miliya) Chen¹

¹Reprocell Co., Yokohama, Japan, ²Stemgent Co., Boston, MA, ³National Inst. of Biomedical Innovation, Osaka, Japan

Human embryonic stem cells (ES cells) and human induced pluripotent stem cells (iPS cells) differ from mouse stem cells in multiple characteristics related to pluripotency and growth potential. Whereas cultured mouse ES cells seem to be derived from the early pre-implantation stage of the embryo, human cells do not. However, recent papers have reported methods to promote human iPSC to acquire nearly equivalent early-stage characteristics. These cell are referred to as naïve human stem cells. We have developed ReproNaive™ cell culture medium for conversion of human iPS (primed-state stem cells) to naïve human iPS cells without genetic modification.

Primed human iPSC grown in ReproNaive™ culture medium on a feeder cell layer (specifically SL10 mouse feeder cells at 4500 cells/cm²) will transition their colony morphology in less than 5 passages. The cell colonies become dome-shape appearing packed and rounded, which is typical of naïve-state cells. The average doubling time of the cells becomes significantly reduced from around 26 hours to approximately 16-20 hours. Nuclear localization of transcription factor TFE3 was shown to be enhanced in these converted cells, consistent with published reports of naïve-state cells. Also, the X chromosome inactive specific transcript gene XIST, which is expressed in primed-state stem cells, is suppressed in the converted cells indicating some X-chromosome reactivation. Finally, these converted cells also retain the expression of the known pluripotent marker genes OCT, NANOG, and TRA1-81.

We have demonstrated that ReproNaive™ -derived naïve state cells can spontaneously differentiate into cells from all three germ-layers, and also can be specifically directed to neuronal cell differentiation with high efficiency. An area of keen interest and intense investigation is whether naïve state human iPSC exhibit less differentiation bias or can give rise to more mature terminally differentiated cell types, as has been shown with rabbit and mouse stem cells. This feature could improve the reliability and efficacy of disease cell models and drug screening.

With ReproNaive™ culture medium you can focus on biology and discovery, and trust ReproCELL to provide quality cell culture tools you need for doing innovative iPSC cell research.
KUF4-MEDIATED SUPPRESSION OF ADIPOGENESIS OF ADIPOSE DERIVED STROMAL CELLS

Jihae Han, Hye Yeon Choi, Ahmed A Dayem, Kyeongseok Kim, Gwangmo Yang, Sohee Lim, Jihye Won, Subbroto K Saha, Sang baek Choi, Yingfu Yin, Ssang-Goo Cho

Department of Animal Biotechnology, Animal Resources Research Center, and Incurable Disease Animal model and Stem cell Institute (IDASI), Konkuk University, 120 Neungdong-ro, Gwangjin-gu, Seoul, 143-701, South Korea

Adipogenesis plays a critical role in human or animal obesity and overweight, which has brought to numerous medical or economic issues for humans and animals in sport or companionship. Here, we tried to induce adipogenic differentiation of 3T3-L1 preadipocytes and adipose-derived stromal cells (ADSCs) as potential therapeutic applications in regenerative medicine or health care for humans and animals. Herein, we focused on natural materials, flavonoids, to determine whether they have the potential to regulate adipogenesis. We screened several decades of flavonoids with different numbers and positions of hydroxyl substitutions, critical factor for its biological activities, and found that the treatment of Konkuk University Flavonoid 4 (KUF4) led to a decrease in the expression of adipogenic markers and lipid deposition. Specific regulation of reactive oxygen species (ROS) generation through differential modulation of the ROS-regulating genes expression was revealed to make important role in the suppression of ADSCs adipogenesis by KUF4 treatment. These results suggest that the flavonoid, KUF4, can be used for regulating adipogenesis of ADSCs for potential safe therapeutic applications in regenerative medicine or health care for humans and many sport or companion animals.
HUMAN IPSC-DERIVED NEURONAL CELL SUB-POPULATIONS THAT ARE SUITABLE FOR DRUG DEVELOPMENT AND THE STUDY OF DIVERGENT NEUROLOGICAL DISORDERS

Brad Hamilton¹, Harumi Kogami², Yuichi Okuda², Kaishu Shiina³, Ryu Yamanaka¹, Paul Cizdziel², Yutaka Shindo³, Yu Ching (Zachary) Lin², Shunsuke Yoshida², Kotaro Oka³, Mitsuru Inamura²

¹Stemgent Co., Boston, MA, ²Reprocell Co., Yokohama, Japan, ³Keio University, Tokyo, Japan

We have developed a comprehensive workflow inclusive of patient-specific primary somatic cell isolation followed by cellular reprogramming to induce pluripotent stem cells, and then directed differentiation to the neural lineage. These derived neurons carry Alzheimer’s disease specific mutations. In addition, by using gene-recombination technology, we have engineered iPS cells from healthy patient tissue to specifically carry the PS1 gene mutation. By regulating the differentiation conditions for these iPS cells, we can control the proportions of various neuronal subtypes, and analyze them functionally and phenotypically by MEA assays, Ca²⁺ imaging, and ELISA.

ReproNeuro™ yields a mixed population of neurons, including dopaminergic, glutamatergic, cholinergic, and GABAergic neurons. These neurons are suitable for patch-clamp, Ca²⁺ imaging, and toxicity assay such as the LDH assay. ReproNeuro DA plus™, on the other hand, is a medium designed to yield a higher proportion of dopaminergic neurons. Because of the enriched neuronal subtype, ReproNeuro DA plus™ –derived cells respond better and more consistently to particular signal molecules. These neurons are also attractive for deriving pathological models of Parkinson’s disease due to the high proportion of dopaminergic neuron. ReproNeuro MQ™ is a cell culture medium designed for multi-electrode-array (MEA) analysis, and neurons cultured in it show a higher-frequency of spontaneous spikes and a better sensitivity to antagonists of glutamate receptors such as AP5 or CNQX.

This comprehensive suite of products and iPSC workflow capabilities enable us to tailor neuronal cell sub-populations in vitro, that are suitable for design of specific disease models to study divergent neurological disorders.
Peripheral blood provides easy access to adult human cell types for reprogramming purposes. Notably, blood-outgrowth endothelial progenitor cells (EPCs) can be clonally isolated from both fresh and frozen human peripheral blood and cord blood. The EPCs' adherent nature and high proliferative capacity, along with a stable cellular phenotype, make them highly desirable for transfection with RNA. Although reprogramming of fibroblasts with messenger RNA (mRNA) was first published in 2010, to date, no group has been able to apply this same technology to reprogram a blood-derived cell type. This limitation has been primarily due to the inability to deliver mRNA to cells originating from blood with good efficiency and without inducing cytotoxicity.

In 2013, published results demonstrated the reprogramming of human neonatal fibroblasts into iPS cells using self-replicative RNA (srRNA), with as few as one transfection. Subsequently, we have extended the application of srRNA for cellular reprogramming to peripheral blood-derived EPCs, cord blood-derived EPCs, as well as adult fibroblasts. The incorporation of reprogramming-associated microRNAs into a two-transfection, no-split protocol on extracellular matrix without the need for conditioned medium has resulted in a simple, reproducible, and robust reprogramming protocol applicable to multiple target cell types.

Integration-free EPC-derived iPS cells exhibit unique genetic stability, making them an exceptional choice for applications requiring clinical grade cells. Lastly, those clinical grade EPC-iPS cells generated using this novel srRNA reprogramming technology present a therapeutic opportunity to treat myeloproliferative disorders in which the disease-causing somatic mutations are restricted to cells in the hematopoietic lineage.

Here we present data demonstrating the unique combined application of microRNA and srRNA for the cellular reprogramming of human EPC lines derived from peripheral blood and cord blood as well as adult fibroblasts into stable, pluripotent, and clinically relevant iPS cells.
MESENCHYMAL STEM CELLS OR ACELULAR DERIVATIVE FROM MESENCHYMAL STEM CELLS PROMOTE LIVER REGENERATION IN INDIVIDUALS WITH STEATOSIS UNDERGOING LIVER RESECTION SURGERY

Javiera P Bahamondes Azcuy, Fernando F Ezquer, Flavia A Bruna, David E Contador, Marcelo E Ezquer

Centro de Medicina Regenerativa, Universidad del Desarrollo, Santiago, Chile

Metabolic syndrome, obesity and diabetes mellitus represent a group of pathologies that have dramatically increased in the last decades. Their hepatic common feature is hepatic steatosis (HS, accumulation of triglycerides in hepatocytes). It is well known that liver has a great regenerative capacity in response to tissue damage, but also there is plenty of clinical evidence showing that HS inhibits the endogenous regenerative processes, greatly increasing morbidity and mortality in patients facing clinical situations such as partial hepatectomy, compared to patients with a normal hepatic condition. Using a murine model of HS, induced by chronic exposition to a high fat diet (HFD), we have proved that the intravenous administration of a single dose of mesenchymal stem cells (MSCs) or conditioned medium (CM) after removal of 70% of liver tissue (Hpx70) induces hepatic regeneration. Male C57BL/6 mice were fed either with HFD or normal rodent diet (RD) for 20 weeks before Hpx70. Immediately after surgery, mice on HFD were treated with either 5x10^5 MSCs (extracted from normal donor mice and ex vivo expanded), MC (obtained from human bone marrow MSC culture) or vehicle. Mice on RD were treated with vehicle and represent animals with normal regeneration capacity (control). Liver cell proliferation (expression of PCNA and incorporation of BrdU), apoptosis (TUNEL), tissue regeneration index (percent of regenerated liver mass), as well as structural (GOT levels) and functional (prothrombin levels) recovery were evaluated two and seven days post-Hpx70. When contrasted with HFD mice treated with vehicle, mice treated with MSCs or CM showed recovery of liver structure and function, increased proliferation and regeneration of hepatic mass, along with decreased apoptosis rate, all in levels similar to control mice, which points towards the secretion of trophic factors as the mechanism by which MSCs induce hepatic regeneration.

Supported by Fondef Ca13I10088
DEVELOPING OF SPATIAL-TEMPORAL-CONTROLLED GENE KNOCKOUT METHOD USING CRISPR SYSTEM IN AXOLOTL TO STUDY SPINAL CORD AND LIMB REGENERATION

Ji-Feng Fei¹, Dunja Knapp¹, Maritta Schuez¹, Prayag Murawala¹, Yan Zou¹, David Drechsel², Elly M Tanaka¹

¹DFG Center for Regenerative Therapies Dresden, Dresden, Germany, ²Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Salamanders can fully regenerate their lost organs, e.g. limb and spinal cord, upon natural injury or amputation, making them a very powerful model for studying tissue regeneration. Understanding the underlying molecular mechanisms of salamander tissue regeneration may also shed light on human regenerative therapies. We previously established an efficient gene knockout method using the CRISPR technique in the salamander Ambystoma mexicanum (axolotl) via injection the mixture of Cas9 mRNA and gRNAs into the fertilized eggs, and showed that knockout of Sox2 resulting in the failure of spinal cord regeneration. Although this constitutive gene knockout approach was successfully used to dissect Sox2 gene function during spinal cord regeneration, the potential application of this method is limited due to the lack of temporal and spatial specificity.

To overcome this roadblock, we demonstrated the establishment of a temporally and spatially controlled gene knockout method by in vivo injecting CRISPR-gRNA complexes into the spinal cord lumen of the axolotl, followed by electroporation. This method leads to comprehensive knockout of Gfp and Sox2 target gene expression. Sox2 knockout in the axolotl spinal cord by CRISPR electroporation gave rise to similar regeneration defects as the phenotype achieved by the zygote-injection protocol. We further showed that simultaneous delivery of CRISPR-gRNA complexes directed against Sox2 and GFP yields efficient knockout of both genes in GFP-reporter animals. Finally we successfully applied this method to other tissues such as skin and limb mesenchyme for GFP knockout. This efficient delivery method opens up the possibility for rapid in vivo genetic screens during axolotl regeneration and can in principle be applied to other vertebrate tissue systems.
Mesenchymal stem cells represent a new source of stem cells for cell therapy nowadays. Our previous work showed that the paracrine factors released by human amniotic fluid-derived mesenchymal stem cells (AF-MSCs) as AF-MSC-conditioned media (AF-MSC-CM) can significantly enhance proliferation of dermal fibroblasts. In this study, we investigated the effect of selenium, which is well-known as ROS scavenger, on the stemness of AF-MSC. We found selenium enhanced the proliferation, survival, stemness and paracrine secretion of AF-MSC. Interestingly, there was a synergy effect between selenium and bFGF (+/+ ) on the proliferation and stemness of AF-MSC compared to selenium and bFGF alone treatment. In addition, +/+ AF-MSC-CM also synergistically increased secretion of cytokines, which increased the proliferation and migration of human dermal fibroblast through TGF-β/SMAD2, PI3K/AKT, and NFkB pathways. Similarly, we also found +/+ AF-MSC-CM improved the wound healing effect in vivo through the SMAD, AKT, NFκB signaling pathway and enhanced the hair growth by activating anagen initiation. Taken together, our findings suggested that selenium can enhance the stemness of AF-MSC and secretion of cytokines to regulate wound healing and hair proliferation.
APPLICATION OF BIO-NANO INTERFACE FOR CONTROLLING STEM CELL FATE

Bayar Hexig

Inner Mongolia University, Research Center for Laboratory Animal Science & College of Life Science, Huhhot, China

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 pharmacological evaluation systems. The reduction or complete removal of serum and animal-derived products is required to satisfy standard for biological ingredients. However, most of the studies reported that proliferation of undifferentiated state and induced differentiation to somatic cells from ES and iPS cells have been based on cell-cell aggregated colony culture system. In colony culturing system, stimulating factors fail to interact with all cells homogeneously and directly in the same time, leading to generate heterogeneous cell population system. Moving towards feeder-free culture systems for hESCs and hiPSCs would represent a significant improvement over conventional culture systems. To address these issues, we developed a novel single cell level culture system applicable for embryonic stem cell maintenance and iPSC cells culture. In the present work, we established a uniform and non-stress single cell level culture system for large scale proliferation of ES and iPS cells. Our novel single cell level culture system was based on an artificial E-cadherin protein bio-nano interface. The results of computer simulation for interaction between culture dish and artificial E-cadherin protein and characterization of protein based bio-nano interface for controlling stem cell fate will be reported.
HIGHLY EFFICIENT AND REPRODUCIBLE DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO PANCREATIC PROGENITORS USING A NOVEL SERUM-FREE MEDIUM.

Michael J Riedel1, Stephanie Lam1, Yvonne Luu1, Simon Hilcove1, Terry E Thomas1, Allen C Eaves1,2, Sharon A Louis1

1STEMCELL Technologies, RND, Vancouver, Canada, 2Terry Fox Laboratory, BC Cancer Agency, Vancouver, Canada

A human pluripotent stem cell (hPSC)-derived regenerative medicine approach to treating diabetes is highly coveted. Current state-of-the-art in vitro protocols generate immature pancreatic precursor cells (PPCs) that can be further matured to endocrine cells in vivo. These hPSC-derived PPCs are a useful tool in studying pancreas development and disease and are being used in clinical trials for treating type 1 diabetes. While several protocols exist to generate PPCs from hPSCs, efficiency and reproducibility across hPSC lines varies. To standardize generation of hPSC-derived PPCs, we developed the STEMdiff™ Pancreatic Precursor Differentiation Kit, a complete and defined medium and supplements that supports efficient generation of PDX1+/NKX6.1+ PPCs from multiple hPSC lines.

hPSCs maintained under defined, feeder-free conditions on Matrigel® in mTeSR™1 were seeded as single cells at 2.1x10^5 cells/cm2. Definitive endoderm (DE) was generated using the STEMdiff™ Definitive Endoderm Kit (Stage 1), which consistently provides >85% CXCR4+SOX17+ DE cells for downstream differentiation. These DE-enriched cells were then differentiated through 3 additional stages (Stages 2 - 4) over 11 days with daily full medium exchanges. At the end of Stage 4, expression of key pancreatic precursor transcription factors was assessed by flow cytometry (FC) and qPCR. By FC, PDX1 expression was: H1, 84.6±2.2% (n=6); H9, 85.5±3.6% (n=5) and WLS-4D1, 83.0±6.8% (n=6; mean±SD). Co-expression of PDX1 and NKX6.1 was: H1, 71.8±4.7%; H9, 73.4±5.5% and WLS-4D1, 67.6±7.0%. Essentially all NKX6.1+ cells were PDX1+. Cell yields of 1.3-1.7x10^7 cells/cm2 were high relative to previously published data, indicating a 6.5 to 8-fold expansion of total cells. This equates to approximately 4.5 to 11.4 PPCs per input hPSC. Furthermore, PPCs generated with this novel medium show upregulation of NKX6.1, NEUROD1, NGN3 and PTF1α mRNA. Insulin and glucagon mRNA was also upregulated at the end of Stage 4.

We have developed a complete, defined and serum-free medium that promotes highly efficient differentiation of hPSCs to PDX1+/NKX6.1+ pancreatic precursor cells. The protocol described here is robust across multiple hPSC lines and can be reliably used in studies aimed at further in vitro β-cell maturation or at understanding pancreatic development.
We have developed ReproRNA™-OSKGM, a synthetic, self-replicating RNA reprogramming vector which expresses reprogramming factors Oct4, Sox2, Klf4, c-Myc, Glis1, and a puromycin selection cassette. To generate human induced pluripotent stem cells (iPSCs), 4 X 10^5 neonatal human foreskin fibroblasts (HFFs) or adult human dermal fibroblasts (HDFs) were transfected once, with 1µg ReproRNA™-OSKGM in duplicates. Transfected cells were plated and exposed to selection in culture medium containing puromycin for 7 days, after which they were harvested, dissociated into single cell suspensions and re-plated on inactivated mouse embryonic fibroblasts (iMEFs) in standard hES medium (DMEM/F12, 20% KOSR, bFGF), or on Matrigel™ in ReproTeSR™ Medium. Embryonic stem cell (ES)-like colonies formed within 2 weeks after transfection of ReproRNA™-OSKGM with cells within all colonies displaying high nucleus to cytoplasm ratio. All colonies were scored after 3 – 4 weeks and then manually selected for subculture in mTeSR™1 maintenance medium. The efficiency of reprogramming was estimated based on percent colony number per cells plated. The results showed that on iMEFs, reprogramming efficiencies for HFFs were 0.07 ± 0.02% (n=2) and 0.12 ± 0.05% (n=6) for HDFs. Reprogramming efficiencies in feeder-free conditions in ReproTeSR™ were similar, at 0.047 ± 0.004% (n=1) and 0.13 ± 0.07% (n=6) for HFFs and HDFs respectively. Individual clones were subcultured in mTeSR™1 and these expressed undifferentiated human pluripotent stem cell markers Oct4, SSEA-3, and Tra-1-60. Moreover, subclones exhibited pluripotent capacity as demonstrated by differentiation to cells of the 3 germ-layers. In summary, our results show efficient reprogramming of fibroblasts to iPSCs with a single transfection of ReproRNA™-OSKGM, a non-integrating, self-replicating RNA vector under feeder-free and defined conditions.
PNEUMACULT™: AN INTEGRATED CULTURE MEDIUM SYSTEM FOR IN VITRO HUMAN AIRWAY MODELING

Juan Hou1, Michael J Riedel1, Terry E Thomas1, Allen C Eaves1,2, Sharon A Louis1

1STEMCELL Technologies, Inc., Research and Development, Vancouver, Canada, 2British Columbia Cancer Agency, Terry Fox Laboratory, Vancouver, Canada

In vitro modeling of the human airway can be used for basic and applied research applications including study of viral infection, drug transport, toxicity testing and disease modelling. Primary human bronchial epithelial cells (HBECs) can be cultured at the air-liquid interface (ALI) using specialized media, resulting in differentiated cultures that exhibit morphological and functional characteristics that mimic the in vivo airway. These HBECs can also be expanded in submerged culture for several passages whilst retaining the ability to differentiate at ALI. Traditional medium formulations for expansion and differentiation of primary human bronchial epithelial cells (HBECs) typically contain undefined components such as Bovine Pituitary Extract (BPE), which contribute to lot-to-lot variability thus leading to inconsistent performance. Recently, we launched PneumaCult™-ALI and PneumaCult™-Ex, both novel defined BPE-free media for differentiation and expansion of primary HBECs, respectively.

Commercially available primary human airway epithelial cells such as Lonza’s HBECs (P1; Cat#: CC-2540s) were thawed and seeded directly into T-25 culture flasks containing PneumaCult™-Ex or control medium (BEGM™; Lonza) at a density of 3.5 x 10³ cells/cm². The culture medium was fully replenished every other day. Cultures were passaged once cells reached approximately 80% confluence. At each passage, cells were enzymatically dissociated and then re-plated at a density of 1 x 10⁴ cells/cm² in PneumaCult™-Ex or control medium for further culture. An aliquot of cells cultured in PneumaCult™-Ex or control medium was also plated in PneumaCult™-ALI according to manufacturer’s instructions to assess differentiation potential. Fold expansion was assessed over 4 passages and the differentiation potential was assessed during the first 3 passages. In four independent donor cell populations, the average fold expansion over 4 passages was similar between cells cultured in PneumaCult™-Ex compared to control (5.55±1.61 vs. 5.12±1.90; mean ± SD). As with control medium, cells cultured in PneumaCult™-Ex exhibited cobblestone morphology and uniformly expressed the basal cell marker p63. Cells cultured in either PneumaCult™-Ex or control medium could be successfully differentiated at each passage during early passages (P1-P3) using PneumaCult™-ALI to generate a pseudostratified mucociliary epithelium as indicated by the qualitative observation of functional cilia and by positive Periodic acid-Schiff (PAS) staining.

Together, PneumaCult™-Ex and PneumaCult™-ALI create a fully integrated, defined and BPE-free tissue culture system for in vitro human airway modeling. This robust and defined system is a valuable tool for basic respiratory research and drug development.
EFFICIENT ESTABLISHMENT AND LONG-TERM MAINTENANCE OF 3-DIMENSIONAL MOUSE INTESTINAL ORGANOIDS USING A NOVEL DEFINED AND SERUM-FREE MEDIUM

Juan Hou¹, Ryan K Conder¹, Michael J Riedel¹, Terry E Thomas¹, Allen C Eaves¹,², Sharon A Louis¹

¹STEMCELL Technologies Inc, Research and Development, Vancouver, Canada, ²British Columbia Cancer Agency, Terry Fox Laboratory, Vancouver, Canada

The intestinal epithelium is rapidly renewed by Lgr5⁺ stem cells located at the crypt base. Recent advances in stem cell culture techniques have given rise to the organoid model that recapitulates the mammalian intestine in vitro. Isolated intestinal crypts are cultured in a 3D, semi-solid environment consisting of Matrigel® and a culture medium that is designed to mimic the intestinal stem cell niche. We have developed a serum-free medium, IntestiCult™ Organoid Growth Medium (OGM) that supports efficient establishment and long-term maintenance of mouse intestinal organoids.

To address the importance of growth medium in this cell culture system, we performed an analysis of key experimental procedures required by users of the organoid system. Crypts were isolated from mouse upper intestines and re-suspended in a 50:50 mix of Matrigel® and IntestiCult OGM. Droplets of the suspension were placed in individual wells of pre-warmed culture plates to create domes containing ~300 crypts each and flooded with IntestiCult OGM. Crypts were cultured at 37°C with 3 medium changes per week. New crypt-like structures typically budded from organoids within 3 days of plating. After 7 days, organoids were dissociated using non-enzymatic medium and mechanical disruption into smaller aggregates. The resultant suspension was split 1:6 to establish secondary and subsequent cultures. Organoids were scored for morphology and characterized by immunocytochemistry (ICC) for specific markers at each passage.

Efficiency of organoid formation from primary crypts was 64 ± 8% (mean ± SD; n=6) by day 5, yielding ~190 organoids per well. Organoids generally consist of an inner lumen surrounded by a complex arrangement of multiple crypt-like buds. Organoid formation efficiency increased to 86 ± 4% (n=4) after the first passage and remained consistently (>85%) over at least 12 passages (90 ± 3%, n=3). Markers for intestinal stem cells (Lgr5), polarized enterocytes (Villin), goblet cells (Muc2), enteroendocrine cells (Chg A), and Paneth cells (Lysozyme) were detected by ICC at each passage.

In summary, IntestiCult™ OGM is a serum-free and defined medium for efficient formation and expansion of primary mouse intestinal organoids that provides a cost effective, valuable tool for studying intestinal function or stem cell biology.
CHD1L MAY PROMOTE NEURONAL DIFFERENTIATION IN HUMAN EMBRYONIC STEM CELLS BY UP-REGULATING PAX6

Dandan Dou¹, Xingwu Wu¹, Zili Li², Yan Zhao², Lingfeng Dong¹, Liang Hu¹,²

¹Central South University, Institute of Reproduction and Stem Cell Engineering, Changsha, China, ²National Engineering Research Center of Human Stem Cells, Changsha, China

In mammals, ATP-dependent chromatin remodeling factors are pivotal in early development since they could regulate many cell process by changing nucleosomes and making chromatin accessible to nuclear factors. For example, Brg-1 is required for primitive ectoderm and trophectoderm. Baf60c is essential for heart development in mice. Mice lacking SNF5 protein stop developing at the preimplantation stage. Distinct assembly of SWI/SNF-like subunits may be involved in neuronal differentiation.

Chromodomain helicase DNA binding protein 1-like gene (CHD1L, also called ALC1), was first isolated as a candidate oncogene in hepatocellular carcinoma. CHD1L also belongs to ATP-dependent chromatin-remodelling factors, containing a conserved SNF2-N domain, a superfamily domain and a macro domain. CHD1L has been proved to be associated with the tumorigenesis, including hepatocellular carcinoma, ovarian carcinoma, bladder cancer, colorectal carcinoma, gastric cancer, etc. Zygote-stage mice embryo, injected with morpholino (MO) targeting Chd1l, did not reach the blastocyst stage and arrested at the compaction stage. These studies suggested that CHD1L was required for mice early development. However, the exact role of CHD1L in development, especially in human, has not been reported.

Here, we found that CHD1L overexpressed in human embryonic stem cells (hESCs) but not differentiated cells. Down-regulation of CHD1L was observed during differentiation of hESC to embryonic bodies (EBs). To investigate the role of CHD1L in hESCs, we conditionally overexpressed CHD1L in hESCs. We found that overexpression of CHD1L in hESCs upregulated ectoderm genes, especially PAX6. Furthermore, Ectopic expression of CHD1L promoted hESC to differentiate into neuroepithelium both in EB differentiation and in directed neuronal differentiation. Knockdown of CHD1L significantly impaired neuroepithelium differentiation of hESCs. Interestingly, CHD1L was highly expressed in cell of ventricular (germinal) zone in fetal mice, and could be colocalized with PAX6 positive population. Our study suggested that CHD1L could promote neuronal differentiation of hESCs and may play an important role in nervous system development.
NETRIN REGULATE GLIOBLASTOMA CELL PROLIFERATION AND STEMNESS

Yizhou Hu\(^1\), Irene Ylivinkka\(^1\), Jorma Keski-Oja\(^1\), Marko Hyytiäinen\(^1\)

\(^1\)Departments of Virology and Pathology, Faculty of Medicine, University of Helsinki, Helsinki, Finland, \(^2\)Helsinki University Hospital, University of Helsinki, Helsinki, Finland

Netrins are secreted laminin-related proteins, which were originally observed to guide neuronal axons during neuronal development. Recently, netrin family members have been found to be expressed in many other tissues and tumor types, and to contribute to the regulation of cell adhesion, migration, proliferation, and apoptosis. Glioblastoma multiforme (GBM) is the most common primary tumor of central nervous system (CNS). Although there is no remedy for this fatal disease, the efficacy of temozolomide (TMZ) -an orally taken alkylating agent- has been demonstrated in the treatment of glioblastoma. Here we have explored the functions and molecular mechanisms of netrins in GBM.

The suppression of netrin-4 expression in GBM cell lines significantly reduced cell proliferation and motility. By using tandem affinity purification and mass spectrometric analysis, we identified the physical interaction between integrin beta-4 and netrin-4. This interaction mediates the activation of AKT/mTOR pathway and concomitant mitogenic effects.

Another member of netrin family, netrin-1, is overexpressed in glioblastoma. In tumor biopsies, it localized to hypoxic structures around necrotic tumor areas. We observed that netrin-1 increased glioblastoma cell invasiveness both in in vitro invasion assays and in in vivo intracranial orthotopic tumor xenografts. Furthermore, we identified that netrin-1 interacts with Notch and activates its downstream signaling which subsequent stimulates glioblastoma cell invasion. As Notch signaling has been linked to cancer cell stemness, our results implicate a role for netrin-1 in the regulation of glioblastoma cell stemness.
DIRECT CONVERSION OF SOMATIC CELLS INTO FUNCTIONAL INTEGRATION-FREE HEPATOCYTES-LIKE CELLS

Seon In Hwang¹, Jonghun Kim¹, Kyung Tae Lim¹, Seung Chan Lee¹, Su Hyun Park², Yong Han Paik², Dong Wook Han¹

¹School of Medicine, Konkuk University, Department of Stem Cell Biology, Seoul, Korea, ²SungKyunKwan University, Samsung Medical Center, Samsung Biomedical Research Institute (SBRI), Samsung Advanced Institute for Health Sciences & Technology (SAIHST, Department of Health Science & Technology), Seoul, Korea

Recent advances in stem cell biology have shown that somatic cells can be directly converted into different types of somatic cells with defined combinations of transcription factors without passing a pluripotent state. Recently, two groups have reported that the defined sets of hepatic transcriptional factors (Hnf4a plus Foxa1, Foxa2 or Foxa3, and Gata4/Hnf1a/Foxa3) directly convert fibroblasts into functional hepatocytes-like cells, namely induced hepatocytes (iHeps) under retro- or lentiviral systems in vitro. However, mutagenesis resulting from viral integration within host genome increases the risk of tumorigenesis. We show that iHeps could be generated from mouse fibroblasts under integration-free vector delivery systems using episomal vectors harboring the previous combination of hepatic transcriptional factors. Integration-free iHeps do not show any evidence of genomic integration of episomal vectors, but also stably maintain the typical hepatic features similar with primary hepatocytes in terms of morphology, marker gene expression, global gene expression pattern and functionality. Taken together, these data suggests that the integration-free iHeps could be an alternative source of hepatocytes for clinical applications including cell therapy.
ENHANCEMENT OF CELL GROWTH AND SELF-RENEWAL OF PLURIPOTENT STEM CELLS AND IMPROVEMENT OF NEUROPROTECTIVE PROPERTIES IN VIVO BY DIHYDROXYFLAVONE TREATMENT

Dawoon Han, Kyeongseok Kim, Sang baek Choi, Sohee Lim, Jihae Han, Hye Yeon Choi, Ahmed A Dayem, Gwangmo Yang, Ji hye Won, Subbroto K Saha, Yingfu Yin, Ssang-Goo Cho

Department of Animal Biotechnology, Animal Resources Research Center, and Incurable Disease Animal model and Stem cell Institute (IDASI), Konkuk University, 120 Neungdong-ro, Gwangjin-gu, Seoul, 143-701, South Korea

Efficient maintenance of the undifferentiated status of embryonic stem (ES) cells may be important for preparation of high quality cell sources that can be successfully used for stem cell research and therapy. Here, we tried to identify a compound that can enhance the quality of pluripotent stem cells. Treatment of ES and induced pluripotent stem (iPS) cells with dihydroxyflavone (DHF) led to increases in cell growth, colony formation, and cell proliferation. Treatment with DHF resulted in high expression of pluripotency markers and significant activation of self-renewal-related kinases. DHF-treated high quality pluripotent stem cells also showed enhanced differentiation potential. In particular, treatment of iPS cells with DHF led to elevated expression of ectodermal differentiation markers and improved differentiation into fully matured neurons. Next, we investigated the in vivo effect of DHF-pretreated iPS (DHF iPS) cells in a peripheral nerve injury model and found that transplantation of DHF iPS cells resulted in efficient axonal regeneration and functional recovery. Upon histopathological and gene expression analyses, we found that transplantation of DHF iPS cells stimulated expression of cytokines in the early phase of injury and successfully reduced convalescence time of the injured peripheral nerve, showing an effective neuroprotective property. Taken together, our data suggest that DHF can be used for more efficient maintenance of pluripotent stem cells as well as for further applications in stem cell research and therapy.
MOLECULAR MECHANISM OF ALTERNATIVE LENGTHENING OF
TELOMERES IN EARLY EMBRYOS AND ITS ROLE IN SOMATIC
CELL REPROGRAMMING

Rongrong Le

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

Telomeres play vital roles in early embryo development, organ homeostasis,
somatic cell reprogramming and tumorigenesis. Telomeres lengthen
significantly in preimplantation embryos via alternative lengthening of
telomeres (ALT) mechanism. However, the molecular mechanism of ALT
in early embryos remains unknown. Importantly, a strong association
between ALT and tumorigenesis has been implicated in recent studies. In
the past a few years, ALT mechanism in abnormal situations has been
intensively studied while how it works in early embryo has been unclear
due to the limit of small amount of samples. However, increasing evidence
indicates that understanding of ALT mechanism in early embryo will
benefit the improvement of induced pluripotent stem cells (iPS) and the
research of ALT in tumors. We used proteomic information of early
embryos to identify candidate genes associated with ALT mechanism. We
found several genes could increase the proportion of Zscan4+ cells and
knockdown of those genes could significantly block the stimulation of
Zscan4 expression by DNA damage reagent. In addition, these genes also
could enhance the induction of iPSCs. Overall, our research will greatly
benefit the understanding of the molecular mechanism of ALT in early
embryos and provide invaluable information for iPS improvement and
cancer therapy.
The existence and identification of leukemia stem cells in adult acute B lymphoblastic leukemia (B-ALL) remain controversial. We examined whether adult B-ALL is hierarchically organized into phenotypically distinct subpopulations of leukemogenic and non-leukemogenic cells or whether most B-ALL cells retain leukemogenic capacity, irrespective of their immunophenotypes. Cells from CD34+CD38-, CD34+CD38+, and CD34-CD38+ subpopulations of primary adult B-ALL xenografts were shown to be capable of reconstituting the original leukemia in NOD-scid-IL2Rg-/- mice. We found that primary leukemia cells from 11 of 25 adult B-ALL patients with different expression profiles of CD34 and CD38 proliferated robustly and became CD34-CD38+ after being co-cultured with OP9 stromal cells. Surprisingly, cultured CD34-CD38+ B-ALL cells were able to reestablish the complete leukemic phenotype in xenografts.

Transcriptomic analysis of CD34+CD38-, CD34+CD38+, and CD34-CD38+ fractions purified directly from xenografts and CD34-CD38+ cells from culture revealed no significantly distinct expression profiles of transcription factors among these populations. Furthermore, 5.7% of single adult primary B-ALL cells engrafted in vivo. These observations suggest that adult B-ALL follows the stochastic stem cell model and that the heterogeneity of CD34 and CD38 expression in B-ALL is reversibly dependent on the microenvironment and is not hierarchically organized.
ENHANCED DIRECT CONVERSION OF FIBROBLASTS INTO HEPATOCYTE-LIKE CELLS VIA ACCELERATED CONVERSION KINETICS

Kyung Tae Lim¹, Seung Chan Lee¹, Eun-Sook Park², Kyun-Hwan Kim², Dong Wook Han¹

¹Department of Stem Cell Biology, School of Medicine, Konkuk University, Seoul, South Korea, ²Department of Pharmacology, School of Medicine, Konkuk University, Seoul, South Korea

Recent studies have shown that defined hepatic factors could lead to the direct conversion of fibroblasts into induced hepatocytes (iHeps). However, the underlying mechanism of the hepatic lineage conversion is largely unknown. Here we report that direct conversion into iHeps is a stepwise transition involving erasure of somatic memory, mesenchymal-to-epithelial transition (MET), and induction of hepatic cell fate in a sequential manner. Through screening for additional factors that could potentially enhance the kinetics of the MET and hepatic programs, we found that the additional transcription factors increased the conversion kinetics, resulting in remarkably improved generation of iHeps (>87 fold). We also demonstrate that combined treatment with small molecules could replace the additional transcription factors and thus lead to the highly efficient conversion of mouse and human fibroblasts into iHeps. The novel reprogramming cocktail defined herein offers a method for obtaining a sufficient number of iHeps for both mechanistic and clinical studies.
IDENTIFICATION AND ESTABLISHMENT OF MOUSE ECTODERM-LIKE STEM CELLS

Chang Liu¹, Ran Wang¹, Jun Chen¹, Guangdun Peng¹, Patrick Tam², Naihe Jing¹

¹Chinese Academy of Sciences, Institute of Biochemistry and Cell Biology, Shanghai, China, ²Children’s Medical Research Institute, Embryology Unit, Sydney, Australia

Ectoderm is the least understood germ layer in mouse embryonic development due to the short time window and lack of molecular markers. To address these issues, we established ectoderm-like stem cell (EctlSC) lines from mouse epiblast stem cells (EpiSCs). EctlSCs expand stably in defined culture condition and display stem cell signatures. The gene expression pattern of EctlSCs is similar to the ectoderm of E7.0 mouse embryo ectoderm region. Furthermore, EctlSCs can differentiate into ectoderm lineages instead of mesendoderm lineages in vitro and in vivo. Taken together, our findings highlight distinct characteristics of EctlSCs and provide a useful tool to study ectoderm specification.
LOW IMMUNOGENICITY OF NEURAL PROGENITOR CELLS DIFFERENTIATED FROM INDUCED PLURIPOTENT STEM CELLS DERIVED FROM LESS IMMUNOGENIC SOMATIC CELLS

Pengfei Liu1,2, Shubin Chen1, Xiang Li1, Guangjin Pan1, Jinglei Cai1, Duanqing Pei1

1Key Laboratory of Regenerative Biology, Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, China, 2Department of Regenerative Medicine, School of Pharmaceutical Science, Jilin University, Changchun, China

The groundbreaking discovery of induced pluripotent stem cells (iPS cells) provides a new source for cell therapy. However, whether the iPS derived functional lineages from different cell origins have different immunogenicity remains unknown. It had been known that the cells isolated from extra-embryonic tissues, such as umbilical cord mesenchymal cells (UMCs), are less immunogenic than other adult lineages such as skin fibroblasts (SFs). In this report, we differentiated iPS cells from human UMCs and SFs into neural progenitor cells (NPCs) and analyzed their immunogenicity. Through co-culture with allogous peripheral blood mononuclear cells (PBMCs), we showed that UMCs were indeed less immunogenic than skin cells to simulate proliferation of PBMCs. Surprisingly, we found that the NPCs differentiated from UMC-iPS cells retained low immunogenicity as the parental UMCs based on the PBMC proliferation assay. In cytotoxic expression assay, reactions in most kinds of immune effector cells showed more perforin and granzyme B expression with SF-NPCs stimulation than that with UMC-NPCs stimulation in PBMC co-culture system, in T cell co-culture system as well. Furthermore, through whole genome expression microarray analysis, we showed that over 70 immune genes, including all members of HLA-I, were expressed at lower levels in NPCs derived from UMC-iPS cells than that from SF-iPS cells. Our results demonstrated a phenomenon that the low immunogenicity of the less immunogenic cells could be retained after cell reprogramming and further differentiation, thus provide a new concept to generate functional lineages with lower immunogenicity for regenerative medicine, especially for application of iPS derived functional cells in allogeneic transplantation.
FROM DNA TO NUCLEOSOMES: PATERNAL SPECIFIC TARGETING OF AN H3.3 CHAPERONE CONTROLS INTEGRITY AND SEGREGATION OF PATERNAL CHROMOSOMES IN MOUSE EARLY EMBRYOS

Zichuan Liu1, Mark E Gill1, Mathieu Tardat1, Antoine Peters1,2

1Friedrich Miescher Institute for Biomedical Research, Epigenetics, Basel, Switzerland, 2University of Basel, Faculty of Sciences, Basel, Switzerland

In mammalian cells centromeres are flanked by stretches of repetitive DNA sequences known as pericentromeric heterochromatin (PCH). As constitutive heterochromatin, PCH is required for faithful segregation of chromosomes during mitosis. The evolutionary conserved Suv39h histone H3 lysine 9 methyltransferases and Hp1 proteins serve crucial functions in this process. On exception to this general mode is PCH at paternally inherited chromosomes in mouse early embryos where a repressive chromatin state is established de novo after fertilization. We have previously shown that members of the canonical PRC1 complex and of PRC2 and associated histone modifications define an alternative repressive state at paternal PCH. Intriguingly, paternal PCH is also enriched for the histone H3.3 variant. Whether such alternative heterochromatic state controls chromosome segregation is unclear. Here, we establish a function for H3.3 in the establishment of paternal PCH. We show that PRC1 is able to target the H3.3 chaperone protein Daxx to PCH in early embryos, facilitating the incorporation of H3.3. Genetic deficiency of either PRC1 components or Daxx abrogates H3.3 incorporation and induces breakage at PCH of exclusively paternal chromosomes, leading to their mis-segregation. We dissect a novel molecular pathway that ensures genome integrity of the paternal genome at the onset of life.
Background:
We had established a novel blue fluorescence as a non-invasive endogenous marker characteristic of ‘primed’ pluripotent stem cells, specifically human pluripotent stem cells. The blue fluorescence shows high correlation with the expression of pluripotency markers (e.g. Oct-4, Sox-2, Nanog) and also appears very early during reprogramming. The fluorescence is localized to perinuclear spherical cytoplasmic lipid bodies or droplets. We determined that the blue fluorescence emanates from retinyl esters sequestered in the lipid droplets. This property was also be used to flow-sort pluripotent stem cells from differentiated cells, with high efficiency (Thangaselvam Muthusamy, Odity Mukherjee, Radhika Menon, P.B. Megha, 2014).

Observations:
Multiple studies indicate that ‘naïve’ pluripotent stem cells and ‘primed’ pluripotent stem cells exist in different metabolic states. Since the lipid bodies and retinol are involved in energy metabolism and affect cellular metabolic states, we hypothesize that lipid bodies in stem cells could reflect the metabolic state of pluripotent stem cells, and/or play a role in the pluripotent state. We have observed that the presence of lipid bodies is influenced by the presence of retinol or retinyl esters and lipids in the culture media. In E8 media, which contains just essential media components, retinol is absent. In this media pluripotent stem cells do not have the lipid bodies. Interestingly, addition of exogenous retinol or retinyl esters causes the lipid bodies to appear. This has allowed us to establish pluripotent stem cell states with and without the lipid bodies. The appearance of these lipid bodies indicates changes in lipid biosynthesis, which is also affected by mitochondrial metabolism. Hence, we have sought to look for differences in various aspects of basal, glycolytic and mitochondrial metabolism in pluripotent stem cells with and without lipid bodies. In addition, we are also examining the reactive oxygen species (ROS) levels and mitochondrial potentials between pluripotent human stem cells with and without lipid bodies.

Conclusion:
Our results should contribute to a better understanding of the novel differences between the ‘primed’ and ‘naïve’ pluripotent stems. These differences indicate the existence of inherently different metabolic pathways which underlie the biology of naïve and primed pluripotent stem cells, and these may contribute to establishing the pluripotent state.

References:
Alopecia is a popular disease and the pivotal key signaling center causing alopecia is the dermal papilla cells (DP cells) which regulate hair growth and regeneration. DP cell engages in crosstalk with the surrounding environment such as extracellular matrix, cytokine, and cell signaling. Previously, we showed that amniotic fluid-derived mesenchymal stem cell (AF-MSC) conditioned media accelerates hair regeneration and growth. In this study, we examined the effects of reprogramming factor on the AF-MSC (RF-AF-MSC). First, Overexpression of reprogramming factor increased proliferation rate, delayed the senescence of AF-MSC, and increased the life span of AF-MSCs compare to empty vector-transduced AF-MSCs. Second, conditioned medium (CM) from RF-AF-MSC was investigated and found that RF-AF-MSC secreted cytokines, related to hair regeneration and growth, such as IGF, PDGF, bFGF, and Wnt7a. In In vivo assay, CM from RF-AF-MSC enhanced the cycle of hair regeneration from telogen to anagen. The expression of follistatin, which is the anagen marker, was increased in CM from RF-AF-MSC compared to CM from AF-MSC. Moreover, CM from RF-AF-MSC increased the ALP expression in the DP area and the hair induction-related genes (LEF, Versican, HEY) were upregulated. Our study demonstrates that overexpression of reprogramming factor promotes AF-MSCs to secret cytokines which enhance the DP’s ability to induce hair regeneration and growth.
SMALL MOLECULE-DIRECTED HIGH EFFICIENT CONVERSION OF PLURIPOTENT HUMAN EMBRYONIC STEM CELLS INTO FUNCTIONAL HUMAN NEURONAL OR CARDIOMYOCYTE CELL THERAPY DERIVATIVES FOR REGENERATIVE MEDICINE

Xuejun H Parsons
XiAn JiaoTong University, College of Nanotechnology and Bioengineering Sciences, Suzhou, China

As neurodegenerative and heart diseases incur exorbitant costs on the healthcare system worldwide, there is a strong focus on providing newer and more efficient solutions for these therapeutic needs. The limited capacity of these two cell systems -- neuron circuitry and cardiomyocytes -- for self-repair makes them suitable for human embryonic stem cell (hESC) derivative-based neuronal and heart therapies. Our recent technology breakthroughs have overcome some major obstacles in moving stem cell research from animals towards humans trials, including resolving minimal essential human requirements for de novo derivation and long-term maintenance of clinically-suitable stable hESC lines and high efficient direct conversion of such pluripotent hESC into a large supply of clinical-grade functional human neuronal or cardiomyocyte cell therapy products by small molecule induction. The openness of pluripotent epigenome differentiates the active pluripotency of normal stable hESC from the repressive pluripotency of abnormal reprogrammed cells and cancer cells. Small Molecules induce a cascade of cardiac or neural lineage-specific differentiation progression towards beating cardiomyocytes or ventral neurons direct from the pluripotent state of hESC by promoting the expression of the earliest cardiac precursor marker Csx/Nkx2.5 or nuclear translocation of the neuronal specific transcription factor Nurr-1. Nuclear translocation of NAD-dependent HDAC SIRT1 and global chromatin silencing lead to small molecule-directed hESC cardiac fate determination or cardiomesoderm specification, while silencing of pluripotence-associated hsa-miR-302 family and drastic up-regulation of neuroectodermal Hox miRNA hsa-miR-10 family lead to small molecule-directed hESC neural fate determination or neuroectoderm specification. Small molecule induction transforms pluripotent hESC into a more cardiomyocyte or neuronal lineage-specific embryonic precursors or progenitors, which yield cardiomyocytes or neurons in high efficiency, than the prototypical neuroepithelial-like cardiac or neural stem cells. Our studies have demonstrated the direct pharmacologic utility and capacity of hESC therapy derivatives for human CNS and myocardium regeneration and, thus, have presented the hESC therapy derivatives as a powerful pharmacologic agent of cellular entity for a wide range of incurable or hitherto untreatable neurodegenerative and heart diseases. Transforming non-functional pluripotent hESC into fate-restricted functional human cell therapy derivatives by small molecule-directed induction dramatically increases the clinical efficacy of graft-dependent repair and safety of hESC-derived cellular products, marking a turning point in cell-based regenerative medicine from current studies in animals towards human trials.
DOES ALLOGENEIC MULTIPOTENT MESENCHYMAL STROMAL CELLS TRANSPLANTATION PROMOTE THE PROGRESSION OF PRECANCEROUS LESIONS?

Anita Plaza-Flores¹, Martha Arango², Flavia Bruna², Iris Espinoza³, Paulette Conget²

¹Universidad Austral de Chile, Doctorado en Ciencias Médicas, Facultad de Medicina, Valdivia, Chile, ²Clinica Alemana Universidad del Desarrollo, Centro de Medicina Regenerativa, Facultad de Medicina, Santiago, Chile, ³DDS, MSc, Specialist in Oral Pathology, Santiago, Chile

Background: Multipotent mesenchymal stromal cells, also referred to as mesenchymal stem cells (MSCs), have been displayed as a tool for the treatment of several diseases due to its potential to: 1) differentiate into various cell types, 2) home into damage tissue (including tumors), 3) secrete trophic and vasculogenic factors and 4) modulate immune response. Although preliminary results are promising, transfer of MSC-based therapy to clinical practice depends, among others, on the generation of empirical data regarding their biosafety. In particular, the role of MSCs in carcinogenesis. MSCs biosafety has been tested in established tumors, however in our knowledge there is no available data concerning to the impact of MSCs transplantation in precancerous lesions.

Aim: The aim of present work was to determine whether local and systemic administration of allogeneic MSCs modify the progression of precancerous lesions, at dysplasia stage, in an animal model of oral squamous cell carcinoma (OSCC).

Methods: OSCC was induced in Syrian hamster by topical application of a tobacco-related carcinogen. Vehicle, 0.1x10⁶, 1x10⁶ or 3x10⁶ allogeneic MSCs were injected nearby dysplastic lesions or intracardially (local and systemic administration respectively). Their evolution (dysplasia→papilloma→carcinoma) was assessed by macroscopy, histology (H&E, immunohistofluorescence) and RT-qPCR.

Results: Although all groups of animals receiving cells showed a tendency to have smaller lesions than those receiving vehicle, there were no significant differences between them. Furthermore, no differences were observed between the administration routes or among the different doses of MSCs. Histologically, in animals receiving MSCs the progression to carcinoma stage was 77.3% or 86.3%, by local or systemic administration respectively; in contrast with animals receiving vehicle that showed 100% of progression up to carcinoma.

Conclusions: Our data suggest that local and systemic administration of allogeneic MSC is harmless to dysplastic lesions in the natural progression of OSCC.
QUANTITATIVE INTEGRATION OF EPIGENETIC VARIATION AND TRANSCRIPTION FACTOR BINDING BY MAMOTIF TOOLKIT
UNVEILED A NOVEL PROMOTER REGULATORY MODULE FOR HUMAN EMBRYONIC STEM CELLS

Zhaohui Gong¹, Jiawei Wang², Yijing Zhang², Zhen Shao¹

¹Shanghai Institute of Biological Sciences, Partner Institute for Computational Biology, Shanghai, China, ²Shanghai Institute of Biological Sciences, Shanghai Institute of Plant Physiology and Ecology, Shanghai, China

We developed a new integrative model called MAmotif, which automatically couples quantitative comparison of ChIP-seq data for the same protein but under different cellular context together with TF-binding motif analysis at its peak regions, to discover TFs whose motif sites have significant association with cell type-biased peaks as candidate cell type-specific co-factors. By applying MAmotif to compare the ChIP-seq data of H3K27ac and H3K9ac generated by ENCODE in more than 15 different cell types, we identified that besides Pou5f1 (Oct4) and Sox2, two zif-finger TF binding motifs are also significantly associated with H1 human embryonic stem cell (hESC)-biased promoter peaks of these two histone marks compared to majority of the other cell types. Then, we comprehensively integrate the motif sites of these four TFs with public ChIP-seq data of TF generated in hESCs, we found these two zif-finger TF binding motifs significantly co-localize with many important TFs including transcription activator EP300, and form a separable regulatory module from POU5F1 and SOX2. Interestingly, opposite from Pou5f1 and Sox2, the numbers of these two zif-finger TFs’ motif sites dramatically decreased in the H3K27ac promoter peaks in mouse ESCs, and they also lose their association with ESC-biased H3K27ac promoter peaks in mouse, indicating severe functional changes exist between human and mouse genomes during evolution.
INHIBITION OF HEPATIC STELLATE CELL ACTIVATION BY BONE MARROW DERIVED STEM CELLS IN A 3D CULTURE MODEL.

Ervinia J Sitanggang¹², Sri W Jusman³, Radiana D Antariantó⁴

¹Biomedical Science Graduate Study Program Faculty of Medicine Universitas Indonesia, Histology, Jakarta, Indonesia, ²Faculty of Medicine Universitas HKBP Nommensen, Histology, Medan, Indonesia, ³Faculty of Medicine Universitas Indonesia, Biochemistry and Molecular Biology, Jakarta, Indonesia, ⁴Faculty of Medicine Universitas Indonesia, Histology, Jakarta, Indonesia

Background:
Transplantation of bone marrow derived stem cells (BMSCs) has been reported to improve liver fibrosis. Several in vitro studies have shown evidence for the mechanism of improvement by co-culturing BMSCs and hepatic stellate cells (HSCs) indirectly or directly in 2D models. In those studies, BMSCs were reported to inhibit HSCs activation as shown by the decrease in α-smooth muscle actin expression, and reduce extracellular matrix deposition as shown by the decrease in collagen type I and III expressions. In this study, we investigated the mechanism by co-culturing BMSCs and HSCs in 3D model as it could better represent the 3D microenvironment with intricate cell-cell and cell-matrix interactions as in the liver tissue.

Methods:
Primary isolated rat HSCs and BMSCs were cultured alone (monoculture) and directly co-cultured at 1:1 ratio with hanging drop method. Characterization of BMSCs was performed by flowcytometry CD90 analysis. The monoculture and co-culture samples were harvested on day 3, 5, and 7 for histology analysis. The samples were analyzed for collagen matrix deposition by Masson’s Trichrome staining, extracellular matrix protein tenascin-C immunocytochemistry, cell proliferation by Ki-67 immunocytochemistry, resting HSC’s state as shown by positive Oil Red O stained cells.

Results:
The percentage of CD90+ cells from the isolated BMSCs was 35.2%. Results of the present study showed that BMSCs have a significant antifibrotic effect as evidenced by the significant decrease in collagen secretion as well as the decrease in tenascin-C expression in the co-culture group (\(p < 0.05\)) compared to the monoculture group on day 7. BMSCs also showed the capacity to inhibit HSCs activation as assessed by the significant increase in Oil Red O-positive cells in the co-culture group (\(p < 0.05\)) compared to the monoculture group on day 7. There was no difference in the proliferation rate between 3D monoculture and co-culture cells.

Conclusions:
These findings demonstrate that BMSCs have a potential therapeutic effect against liver fibrotic process through their effect in minimizing extracellular matrix deposition and their capacity to inhibit HSCs activation.

Keywords:
Hepatic stellate cells; Bone marrow derived stem cells; Tenascin-C; Liver fibrosis; 3D co-culture
Epithelial-mesenchymal interaction occurs during development of various tissues including teeth and bone. Recently, preameloblast-conditioned medium (PA-CM) from mouse apical bud cells (ABCs), a type of dental epithelial cell, was found to induce odontogenic differentiation of dental pulp stem cells and to promote dentin formation. The aims of the present study were to investigate the effects of PA-CM on human bone marrow mesenchymal stem cells (hBMSCs) \textit{in vitro}, and to investigate the bone regenerative capacity \textit{in vivo} through epithelial-mesenchymal interactions of developmental osteogenesis. Co-culturing with ABCs and PA-CM treatment up-regulated osteoblast differentiation markers of hBMSCs compared to cells cultured alone. PA-CM accelerated mineralized nodule formation and also increased BSP promoter activity in hBMSCs. PA-CM facilitated the migration of the hBMSCs but did not significantly influence proliferation. PA-CM promoted bone formation of hBMSCs \textit{in vivo}.

Radiographic and histologic findings showed that PA-CM induced the bony regeneration at calvarial defects in rat. Taken together, these data show that PA-CM enhances the migration and osteogenic differentiation of hBMSCs \textit{in vitro} and induces bone formation \textit{in vivo}. Our results indicate that PA-CM could be applied to implant therapy as a regenerative bioactive material to promote bone regeneration clinically.
LPS-PRECONDITIONED MESENCHYMAL STEM CELLS MODIFY MACROPHAGE POLARIZATION FOR RESOLUTION OF CHRONIC INFLAMMATION VIA EXOSOME-SHUTTLED LET-7B

Dongdong Ti, Haojie Hao, Xiaobing Fu, Weidong Han

Chinese PLA General Hospital, Institute of Basic Medicine Science, College of Life Science, Beijing, China

Introduction Within the last few years, it has become evident that LPS-preconditioned mesenchymal stem cells (LPS pre-MSCs) show enhanced paracrine effects, including increased trophic support and improved regenerative and repair properties. MSCs may release large amounts of exosomes for cell-to-cell communication and maintain a dynamic and homeostatic microenvironment for tissue repair. The present study assesses the therapeutic efficacy and mechanisms of LPS-preconditioned MSC-derived exosomes (LPS pre-exosomes) for chronic inflammation and wound healing.

Methods We extracted exosomes from the supernatant of LPS pre-MSCs using a gradient centrifugation method. In vitro, THP-1 cells were cultured with high glucose (HG, 30 mM) as an inflammatory model and treated with LPS pre-exosomes for 48 hours. The expression of inflammation-related cytokines was detected by real-time RT-PCR, and the distribution of the macrophage subtype was measured by immunofluorescence. Next, the miRNA expression profiles of LPS pre-exosomes were evaluated using miRNA microarray analysis. The molecular signaling pathway responsible for the regenerative potential was identified by western blotting. In vivo, we established a cutaneous wound model in streptozotocin (STZ)-induced diabetic rats, and LPS pre-exosomes were injected dispersively into the wound edge. The curative effects of LPS pre-exosomes on inflammation and wound healing were observed and evaluated.

Results LPS pre-exosomes have a better ability than untreated MSC-derived exosomes (un-exosomes) to modulate the balance of macrophages due to their upregulation of the expression of anti-inflammatory cytokines and promotion of M2 macrophage activation. Microarray analysis of LPS pre-exosomes identified the unique expression of let-7b compared with un-exosomes, and the let-7b/TLR4 pathway served as potential contributor to macrophage polarization and inflammatory ablation. Further investigation of the mechanisms that control let-7b expression demonstrated that a TLR4/NF-κB/STAT3/AKT regulatory signaling pathway plays a critical role in the regulation of macrophage plasticity. Knockdown of AKT in THP-1 cells similarly abolished the immunomodulatory effect of LPS pre-exosomes. In vivo, LPS pre-exosomes greatly alleviated inflammation and enhanced diabetic cutaneous wound healing.

Conclusion LPS pre-exosomes may have improved regulatory abilities for macrophage polarization and resolution of chronic inflammation by shuttling let-7b, and these exosomes carry much immunotherapeutic potential for wound healing.
GMP CULTURE MEDIA DEVELOPMENT FOR THERAPEUTICAL STEM CELLS

Mohan C Vemuri

Thermo Fisher Scientific, Stem Cell Biology, Frederick, MD

Successful cell based therapies are critically dependent on high quality cell culture reagents that are manufactured following Good Manufacturing Practice (GMP) and ISO 13485 guidelines. Stem cell culture reagents are an integral part of the bioprocess technology for production of clinical-grade stem cells to ensure optimal defined quality and safety in cell therapy studies. Large numbers of stem cells with adequate bioprocess control such as safety, sterility, and traceability are required as start-up material to produce fully or partially differentiated cell types for different diseases. The culture of such cells in sufficient numbers required by industry or clinicians is challenging: stem cells are sensitive to culture conditions, and maintaining them in an undifferentiated state is laborious and tedious. In addition, options to use different substrates, culture media, and dissociating enzymes must be carefully considered to maintain the reproducibility, quality, and scalability of the culture process. Cell culture strategies to expand stem cells in particular human MSC, HSC, NSC and T Cells for immunotherapy are presented, with a particular focus on serum free, xeno free and animal origin free GMP systems that are well suited for translational medicine applications.
The promise of human pluripotent stem cells will be realized only when these cells are successfully coaxed into different cell types found in the human body through the process of directed differentiation. This is critical to getting the desired cell types in numbers needed for drug screening, cellular models, and translational cell therapy applications. Many existing methods of differentiation are suboptimal, with laborious mechanical and manual steps, lack of reproducibility, and reduced efficiency to achieve functionally mature lineages. The complex process of differentiation and the challenges associated need to be efficiently deciphered in order to successfully direct the hPSC differentiation to target cell types. In this presentation, challenges associated with hPSC differentiation to neural, cardiac and endodermal lineages are discussed along with ways to overcome some of the hurdles previously mentioned. cGMP media systems designed for efficient differentiation towards ecto-, meso- and endodermal cell lineages are presented.
The Mediator complex orchestrates multiple transcription factors with the Pol II apparatus for precise transcriptional control. However, its interplay with the surrounding chromatin remains poorly understood. Here, we analyze differential histone modifications between WT and MED23-/- (KO) cells and identify H2B mono-ubiquitination at lysine 120 (H2Bub) as a MED23-dependent histone modification. Using tandem affinity purification and mass spectrometry, we find that MED23 associates with the RNF20/40 complex, the enzyme for H2Bub, and show that this association is critical for the recruitment of RNF20/40 to chromatin. In a cell-free system, Mediator directly and substantially increases H2Bub on recombinant chromatin through its cooperation with RNF20/40 and the PAF complex. Integrative genome-wide analyses show that MED23 depletion specifically reduces H2Bub on a subset of MED23-controlled genes. Importantly, MED23-coupled H2Bub levels are inversely regulated during myogenesis and lung carcinogenesis. In sum, these results establish a mechanistic link between the Mediator complex and a critical chromatin modification in coordinating transcription with cell identity control.
MULTIPLEX HIGH-THROUGHPUT ANALYSIS OF IDH-GENE MUTATIONS

Wei Wang1,4, Anna Bertoni1, Andrey Korshunov2, Janna Kirchhof3, Katja Beck3, Peter Lichter1,3, Bernhard Radlwimmer1

1German Cancer Research Center, Molecular Genetics, Heidelberg, Germany, 2German Cancer Research Center, Clinical Cooperation Unit Neuropathology, Heidelberg, Germany, 3Heidelberg Center for Personalized Oncology, Heidelberg, Germany, 4National Center for Tumor Diseases, Translational Oncology, Heidelberg, Germany

Systematic characterization of somatic mutations in cancer genomes has improved our understanding of the disease and provided opportunities for the development of targeted therapies. Validation of key mutations requires sensitive high-throughput techniques. The Isocitrate dehydrogenase 1 and 2 (IDH1/IDH2) genes encode NADP+-dependent isocitrate dehydrogenases playing prominent roles in cellular metabolism including lipid metabolism and glucose sensing. Somatic gain-of-function mutations of IDH have been identified in multiple cancers including diffuse astrocytic glioma, sarcoma and acute myeloid leukemia. Their potential role in disease pathogenesis and prognosis have made IDH1/IDH2 both promising biomarkers and potential therapeutic targets. We established a high-throughput screening method for IDH mutations in early- and late-stage glioma using a multiplex mass spectrometry-based approach. Based on amplicon length and similarity, the 11 most frequent IDH1/IDH2 mutations were grouped in 4 multiplex assays. 139 diffuse astrocytic glioma, including 76 glioblastoma and 63 WHO grade II and III glioma, and 20 cytogenetically normal (CN)–AML samples were analyzed. IDH1/IDH2 combined frequency was approximately 8% (6/76) in GBM, 86% (54/63) the other glioma and 45% (9/20) in AML. Mutations of IDH1-R132 and IDH2-R172 were most frequently mutated in all three tumor entities. IDH1 and IDH2 mutations largely occurred mutually exclusive, and could be detected even when present only in a subpopulation (>5%) of cells. All mutations were independently validated by pyrosequencing with 100% concordance. This study demonstrates that quantitative mutation analysis by MassARRAY is a sensitive and effective method for mutation analysis.
EXOSOMES RELEASED FROM HUMAN IPS-DERIVED MSCS
PROMOTE BONE REPAIR AND REGENERATION BY ACTIVATING
PI3K-AKT SIGNALING PATHWAY

Jieyuan Zhang, Xiaolin Liu, Xin Niu, Zongping Xie, Yang Wang

Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Institute of Microsurgery on Extremities, Shanghai, China

The induced pluripotent stem cells-derived mesenchymal stem cells (iPS-MSCs) have emerged as a promising therapeutic strategy for bone defects. It has been proposed that the protective effect of stem cells on tissue regeneration is not facilitated by their direct differentiation into parenchymal cells which repairs and replaces the damaged tissues, but rather by evoking the regenerative responses from resident cells through paracrine mechanisms. In recent years the therapeutic potential of exosomes, a key component of cell paracrine secretion, has been investigated intensively in various disease models. However, there are few reports regarding the application of exosomes in bone repair and regeneration. Herein, we provided the first demonstration that human iPS-MSCs-derived exosomes (hiPS-MSC-Exos) possessed therapeutic effects in a rat model of critical-sized calvarial bone defects. We found that the implantation of hiPS-MSC-Exos with a classical carrier β-TCP could enhance the percentage of newly formed bones within the defect areas compared with the use of β-TCP alone. Moreover, we found that exosomes treatment profoundly accelerated the proliferation, migration, osteogenic differentiation and mineralization of MSCs in vitro. In addition, we demonstrated that hiPS-MSC-Exos could induce the expression of a class of osteogenesis-related molecules in MSCs. In particular, these exosomes triggered the activation of PI3K/Akt signaling pathway, which was further determined as the critical mediator during the exosomes-induced osteogenic responses from MSCs. Our results indicate that hiPS-MSC-Exos are able to trigger bone regenerative responses from their target tissues and cells, and this process might be attributed to their activation of PI3K/Akt signaling pathway in the recipient cells.

Acknowledgments
This study was financially supported by the National Natural Science Foundation of China (Grant No.:81472152 and 81371938).
ECHINACOSIDE, ACTEOSIDE AND 2-ACETYLACTEOSIDE FROM CISTANCHES HERBA PROMOTES ADULT NSCS PROLIFERATION IN VITRO

Huimin Lin, Jing Han, Weibing Duan, Yan Zhu, Yu Wang

Tianjin University of Traditional Chinese Medicine, Tianjin State Key Laboratory of Modern Chinese Medicine, Tianjin, China

NSCs are functionally defined by their ability to self-renew and differentiate into neurons, astrocytes, and oligodendrocytes, which are broadly used in therapy for neural degeneration and injury disease. Cistanches Herba (CH), clinically used for the prevention and treatment to central nervous system disease in China, consists of phenylethanoid glycosides, iridoids, lignans, amylose and alkaloid. The neuroprotective role of CH is mainly mediated by phenylethanoid glycoside. Acteoside significantly improves scopolamine-induced memory deficits in vivo, while Echinacoside impairs Parkinson’s process in rats by repressing apoptosis. However, their effects on NSCs remain unknown.

We employed the primary NSCs from subventricular zone of adult mice to identify the proliferation-inducing activities of ten monomeric compounds from CH. No matter in presence nor absence of mitogen (EGF and/or FGF), Echinacoside, acteoside and 2-acetylacteoside increased the proliferation of NSCs in 24h, by activating ERK and/or AKT pathway. In this study, we initially elucidate the molecular mechanism of CH on regulating the proliferation of NSCs, which paves the way for CH clinical prevention and treatment to neural degeneration and injury disease.
HEART REGENERATION USING ENGINEERED ECM MIMICKING CARRIERS TO TRANSPLANT CARDIAC PROGENITOR CELLS IN LARGE ANIMAL MODELS

Shuo Tian1, Qihai Liu2, Ienglam Lei1, Xiaoping Li1, Liangxue Lai3, Peter X. Ma2, Zhong Wang1

1University of Michigan Medical School, Department of Cardiac Surgery, Cardiovascular Research Center, Ann Arbor, MI, 2University of Michigan Medical School, Department of Biologic and Materials Sciences, Ann Arbor, MI, 3Chinese Academy of Sciences, Guangzhou Institute of Biomedicine and Health, Guangzhou, China

Cell-based therapy represents a highly promising approach for the treatment of heart diseases but its validation requires extensive preclinical studies. Mouse models have been most prevalent in such studies. However, many therapeutic strategies that showed promising results in mice have not been substantiated in human trials, raising serious concerns over whether mouse models are truly relevant in modeling and treating human diseases. In heart cell-therapy studies, available evidence argues strongly that results gained from mouse studies cannot be translated to human trials. In contrast, due to the similarities between rabbit/pig and human heart, the rabbit and pigs are considered better animal models than the mouse in mimicking the pathogenesis of human heart disease. Therefore, establishing transgenic rabbit and pig models uniquely suited for stem cell-based heart repair will enable us to derive invaluable preclinical insights that would be impossible to achieve using mice. Another major challenge to cell-based therapy is the very low retention and survival rates. Therefore optimal cell carriers and controlled growth factor delivery systems are highly desired to enhance cell retention, survival, and functional engraft.

Our long-term goal is to develop stem cell-based heart therapies to effectively prolong and improve the life of heart patients. We aim first to determine the therapeutic capacity of embryonic cardiac progenitor cells (CPCs) in heart regeneration after myocardial infarction (MI) in rabbit and pig models. ISL1+CPCs, identified from both mice and humans, contribute to multiple heart lineages but their therapeutic potential has not been examined in any detail. To determine the therapeutic potential of ISL1+CPCs, we have generated stable transgenic reporter systems in these large animal models. Meanwhile, we have acquired extensive expertise in CPC isolation and characterization. Furthermore, we have established cutting-edge leadership in developing injectable cell microcarriers for tissue regeneration. In particular, we have developed new nanofibrous hollow microspheres (NF-HMS) that mimic the extracellular matrix architecture at the nanometer scale and shown that the NF-HMS greatly enhance the cell retention and integration in injured heart of large animals. Our progress using these combined approaches in heart regeneration will be presented in the meeting. We expect that our integration of advanced cell source, biomimetic carrier, and large animal models for heart regeneration should provide general principles in developing the most informative model system for regenerative medicine.
The early steps in progression in vivo from a pluripotent hematopoietic stem cell to a lineage restricted precursor are incompletely understood. In particular, normal erythropoiesis proceeds in erythroid islands containing a central macrophage like cell and a number of erythroid precursors. These islands may be derived from a single precursor cell but the trajectory by which the islands develop from the progenitor cell is unknown. The EML cell line is a potential model for this progression. The heterogeneous population of cells present in an EML culture contains a sub-population of CD34+, Sca1 high cells that are capable of rapidly regenerating the full spectrum of cells including differentiation into erythroid precursor cells. Within a couple of days this subpopulation regenerates a heterogeneous EML culture consisting of renewing precursors, together with similar amounts of erythroid committed cells and cells resembling macrophages. We are investigating the transition from precursor to more differentiated cells in this system. The process is strongly influenced by wnt signaling but it is puzzling that healthy precursor cells lack any of the Porcn protein that is required for generation of active extracellular wnt molecules.

To broadly investigate stem cell heterogeneity and early steps in differentiation we have used single cell transcriptome analysis with both normal precursors (HSC and MPP1) and EML cells. Transcriptomes of HSC and MPP1 from 6-8 week old mice fall into several clusters when analyzed by diffusion, t-snet, or standard clustering methods. These include a group of actively cycling cells (predominantly MPP1), a group of quiescent cells, and other cells that are relatively deficient in mRNA but are not evidently apoptotic. Within the HSC and MPP1 there is a relatively random expression of lineage associated transcription factors, with certain notable exceptions. Specifically, Klf1 is expressed rarely and at a low level compared with the other erythroid factors we examined and Cebpa is expressed at a low level compared to most other myeloid factors. We suggest that the small number of factors strongly repressed in the precursor cells include “valve” factors that control the directions of differentiation of the cells expressing them. An additional observation is that the patterns of expression of genes characteristic of different lineage precursors follow different trajectories across the cell cycle.
The aim of the present study was to investigate the effect of the gene 7-Dehydrocholesterol reductase (Dhcr7) and identify signaling pathways involved in regulation of embryonic palatogenesis. The expression of Dhcr7 and its protein product were examined during murine normal embryonic palatogenesis using reverse transcription polymerase chain reaction (RT-PCR) and Western blot (WB); RNAi technology was used to inhibit Dhcr7 expression in palatal shelf culture in vitro. The effects of Dhcr7 on palatogenesis and palatal fusion were examined by scanning electron microscopy (SEM). The expression change of Dhcr7, Sonic Hedgehog (Shh) and Bone morphogenetic protein-2 (Bmp2) were detected by RT-PCR and WB after Dhcr7 gene silencing and addition of exogenous cholesterol. The results showed the palatal shelf failed to complete normal development and fusion when Dhcr7 expression was inhibited. The inhibitory effect of RNAi on the development of the palatal shelf was reversed by adding exogenous cholesterol. Shh and Bmp2 expressions were reduced after Dhcr7 gene silencing; administration of exogenous cholesterol did not affect Dhcr7 expression; however Shh and Bmp2 expressions increased. In conclusion, Dhcr7 plays a role in growth of the palatal shelf and it can affect palatogenesis through alterations in the levels of Shh and Bmp2.

【Key words】 Murine; Palatal shelf; Dhcr7; RNA interference; Organ culture
miR-125b-BCL-2-CASPASE AXIS PARTICIPATES IN ERYTHROBLAST ENUCLEATION

Xiaoyan Xie1,2, Fang Fang1,2, Minyi Qu1,2, Quan Zeng1,2, Junnian Zhou1,2, Jiafei Xi1,2, Wen Yue1,2, Xuetao Pei1,2

1Beijing Institute of Transfusion Medicine, Beijing, China, 2South China Research Center for Stem Cell & Regenerative Medicine, Guangzhou, China

Ex vivo generation of red blood cells (RBCs) from stem cells might meet the urgent requirement in blood transfusion. However, the low erythrocyte enucleation rate remains the bottle neck of this procedure. Here, we propose to take advantage of miR-125b to improve the maturation and enucleation of erythrocytes.

Previous studies demonstrated that miR-125b is a pivotal regulator in hematopoietic stem cell homeostasis as well as a key player for apoptosis and cell cycle regulation in different cells depending on the intracellular context. Interestingly, tracking on miR-125b expression during erythrocyte maturation showed that miR-125b was also enriched in mature RBCs, which hints the involvement of miR-125b in erythrocyte enucleation. Overexpression of miR-125b improved erythroid maturation and enucleation of erythroleukemia cells (K562, TF-1) and of primary proerythroblasts. In contrast, miR-125b inhibitors suppressed erythrocyte enucleation dramatically.

Considering the main models of erythrocyte enucleation are apoptosis and asymmetric cytokinesis, while a key biologic effect of miR-125b is apoptosis regulation, a pro-apoptotic gene Bcl-2 was chosen for miR-125b target study according to bioinformatics analysis. A luciferase reporter assay confirmed the interaction of miR-125b and Bcl-2 3'UTR. During erythrocyte maturation, the down-regulation of Bcl-2 was correlated with the up-regulation of miR-125b. Direct suppressing Bcl-2 in primary erythroblasts and K562 cells presented similar result as miR-125b overexpression. A further exploring of Bcl-2 signal pathway demonstrated that short term activation of Caspase3 followed by ROCK-1 activation was the down-stream effect of Bcl-2 depression, which was a consequence of miR-125b overexpression. And ROCK-1 activation might finally contribute to actin polymization and the terminal maturation/enucleation of erythrocytes.

miR-125b modification for improved erythrocyte enucleation was also confirmed by in vivo studies. Proerythroblasts were transfused into irradiated NOD/SCID mice. The overexpression of miR-125b elevated the engraftment and enucleation of erythroblasts especially for the first 24 hours. Further morphology examination with day 4 transplanted cells demonstrated a typical biconcave disk shape, suggesting the fully maturation of these cells in vivo. Taken together, these results suggest that miR-125b-Bcl-2-Caspase signal axis exerts positive effect on erythrocyte enucleation. miR-125b and small molecules involved in this axis might be further applied in ex vivo RBC generation.
Origin of tissue stem cells (SCs) in organogenesis is considered to be embryonic progenitor cells in a transient state that acquire long-term self-renewal ability and multipotency. Here we want to understand embryonic origin of localized stem cells and the mechanism leading to their specification. Hair follicle (HF) is an ideal system to address this question due to its clear SC identity and stereotypic developmental process. Using a combination of different lineage tracing tools to follow the fate of distinct progenitor cells from the beginning of hair follicle initiation, combined with in vivo cell ablation, unbiased RNA-seq analysis of cells with different fate, and gain/loss of function studies, we unveiled the underlying principle leading to the early specification of dormant and active adult stem cells.
Pulmonary arterial hypertension is characterized by intensive pulmonary vascular remodeling due to proliferation of smooth muscle and endothelial cells. Heterozygous germline mutations of the BMP type II receptor are responsible for the majority of cases of heritable PAH and 15-40% idiopathic PAH. In this study we are aim to clarify the key target of BMPRII in pulmonary vasculature and apply into the drug screening. We identified that the Id (Inhibitor of DNA binding) family of basic-helix-loop helix proteins is a major transcriptional target of BMP signalling in human pulmonary artery vascular cells and is directly affected by mutations or deficiency of BMPR-II by realtime PCR. We are now employing genomic recombineering to generate a human Id1 promoter derived dual reporter in human ES cells to use as a platform to screen small molecules that may identify novel compounds for enhancing Id gene transcription in relevant cell types as potential therapies for PAH. The combination of stem cell technology and cellular molecular mechanism of PAH will enable the development of PAH targeted therapy.
MECHANISM STUDY ON THE EFFECT OF KUS NANOPARTICLES IN INDUCTION OF THE NEURONAL DIFFERENTIATION IN COMPARISON TO RETINOIC-ACID INDUCED NEURONAL DIFFERENTIATION.

Ahmed A Dayem, Yingfu Yin, Gwangmo Yang, Jihae Han, Hye Yeon Choi, Kyeongseok Kim, Sohee Lim, Ji Hye Won, Subroto K Saha, Sangbaek Choi, Ssang-Goo Cho

Department of Animal Biotechnology, Animal Resources Research Center, and Incurable Disease Animal model and Stem cell Institute (IDASI), Konkuk University, 120 Neungdong-ro, Gwangjin-gu, Seoul, 143-701, South Korea

KUS nanoparticles (KUS NPs) possess various medical and industrial applications. In addition, the previous impacted research reports proved the link between these nanoparticles and various biological functions in the cells. In this study, we endeavores to characterize the exact mechanism that reponsable for the potency of KUS NPs in the induction of the neuronal differentiation of the human neuroblasoma cell line in comparison to retinoic acid (RA)-induced neuronal differentiation protocol. Both of KUS NPs and RA treatment led to the phosphorylation of ERK and AKT signalings. However, ERK signaling plays a key role in KUS NPs neuronal differentiation process. In contrast, AKT signaling plays important role in RA-induced neuronal differentiation. Importnatly, reactive oxygen species (ROS) generation in KUS NPs was significantly higher than of induced by RA. Accordingly, treatment with ROS scavenging material, NAC ked to reduction in the phosphorylation of ERK induced by KUS-NPs, but not that induced by RA. Moreover, NAC treatment led to decrease in the neurite length enhanced by KUS-NPs treatmnet. Therefore, ROS is considered as a key signaling molecule in KUS-NPs inuced neuronal differentiation compared to RA. Interestingly, KUS-NPs treatment led to downregulation of the expression level of the phosphatases, but RA treatment led to increase in the expression level of the phosphatases. Based on our research results, KUS-NPs showed different mechanism than of RA. This study can open door for deep study about KUS-NPs effect in the field of the neurodegenerative diseases.
MODELING HUMAN DEVELOPMENT AND DISEASE WITH PLURIPOTENT STEM CELLS

Gordon Keller

University Health Network, McEwen Centre for Regenerative Medicine, Toronto, Canada

The directed differentiation of functional cell types from human pluripotent stem cells (hPSCs) is dependent on our ability to accurately recapitulate the key embryonic stages of development in the culture dish, including the formation of a primitive streak-like (PS) population, the induction of the appropriate germ layer and the specification of this germ layer to the desired lineage. Studies over the past decade have identified the key signaling pathways that control these early developmental steps in the differentiation cultures and have provided strategies for the generation of endoderm-, mesoderm- and ectoderm-derived populations. With these advances, it is now possible to investigate the regulatory pathways that control the development of tissue specific cell subpopulations with the long-term goal of engineering functional tissues. Recent studies in our lab have identified signaling pathways that specifically regulate primitive and definitive hematopoiesis, the generation of sinoatrial node, atrial and ventricular cardiomyocytes, the development of functional hepatocytes and cholangiocytes and the differentiation of articular chondrocytes and cartilage from hPSCs. Findings from the functional analyses of these cells and their application to modeling and treating disease will be presented.
T CELL MEDIATED ACUTE INFLAMMATION IS REQUIRED FOR MUSCLE REGENERATION

Xin Fu, Jun Xiao, Sheng Li, Hongyan Wang, Ping Hu

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

Inflammation is always accompanied with tissue injury. The communication between inflammation and adult tissue regeneration has been a very interesting area to explore. Emerging evidences have suggested that inflammation may play unexpected roles in promoting tissue regeneration. Here we use muscle regeneration as model system to investigate the direct link between inflammation and muscle regeneration. Skeletal muscle regeneration involves a series of physical responses after injury or disease, including activation of quiescent satellite cells (muscle stem cells), proliferation of satellite cells and myoblasts, differentiation of myoblasts, and formation of new myofibers.

We found that T cell mediated acute inflammation is a required positive regulator at early stage of skeletal muscle regeneration. In immunodeficient mice, where the T cell infiltration is diminished while other lymphocytes such as macrophage infiltration remains normal, reparation of muscle injury was dramatically delayed. To further investigate the mechanism of T cell promoting muscle regeneration, we characterized the protein profile of activated T cells. A combination of four factors was identified to be able to promote satellite cell proliferation and long term expansion dramatically in culture. The cultured expanded satellite cells continue to express muscle stem cell marker, and were able to regenerate functional myofibers in vivo. Furthermore, muscular injection of the four factor cocktail could rescue the muscle regeneration defects caused by T cell deficiency. Our results demonstrate that T cell mediated inflammation is required for muscle stem cell proliferation at early stage of post-injury regeneration.
Transcriptional enhancers are critical determinants of cellular identity and influence a variety of cellular processes, but the molecular processes controlling enhancer activation (‘commissioning’) and deactivation (‘decommissioning’) during stem cell development remain largely unexplored. Here we compare the epigenetic state maps, chromatin occupancy of principal lineage transcriptional factors and chromatin modifying enzymes, and gene expression profiles in human primary fetal and adult hematopoietic stem/progenitor cells (HSPCs) and committed erythroid progenitors. Contrasting the similarities and differences between human fetal and adult blood cell development provides critical insights into the enhancer dynamics and gene expression programs directing lineage and developmental stage-specific transcription during hematopoiesis. We find that enhancers are modulated dynamically and serially, and confer higher lineage fidelity. GATA2-to-GATA1 switch is prevalent within transcriptionally primed enhancer elements and drives enhancer commissioning. Further examination of lineage-specific enhancers identified transcriptional factors and combinatorial regulatory patterns with known and unknown roles as putative drivers of enhancer turnover during differentiation. Importantly, by site-directed loss-of-function analysis of lineage-selective enhancers using CRISPR/Cas9-mediated genomic engineering, we start to decipher the causative non-coding regulatory elements underlying blood cell development and disorders. In summary, by comparing the ontogeny of enhancer-mediated gene regulatory networks in human blood stem cell development, we aim to investigate how non-coding genomic elements, lineage-specifying regulators, epigenetic modulators and environmental signals cooperate to control lineage specification, and how dysregulation of enhancer activities contribute to blood disorders.
Microenvironmental regulation of normal and malignant hematopoietic progenitors

Jin-A Kim, Ga-Young Lee, Il-Hoan Oh

Catholic University of Korea, Catholic High-Performance Cell Therapy Center, Seoul, South Korea

Microenvironment of bone marrow plays a key regulatory role to govern the self-renewal and differentiation of normal hematopoietic stem cells (HSCs). Previously we demonstrated that the regulation of HSCs in the stem cell niche involves cross-talk of wnt and notch signals and regenerative activity of HSCs can be modulated by coaxing expression of the cross-talk molecules in the mesenchymal niche. In this presentation, we also show that the mesenchymal niche is involved in the leukemogenic process using the clinical model of human acute myeloid leukemia (AML). We demonstrate that mesenchymal niche in leukemia patients’ bone marrow (BM) is altered under on-going leukemic conditions and that leukemia cells can directly induce transcriptomic reprogramming of mesenchymal cells distinctively from normal hematopoietic cells. The altered leukemic niche provided distinct niche cross-talk (CXCL-12 and Jagged-1) among normal and leukemic cells to selectively suppress the normal primitive HSCs, while supporting leukemogenesis and chemoresistance. Thus, the remodeling of niche by leukemic stem cell is an intrinsic part of leukemogenesis that can lead to clonal dominance of leukemia cells over normal hematopoietic cell. Of note, when screened for the patterns in mesenchymal stroma in AML patients, significant heterogeneity was observed in patients’ bone marrows. Furthermore, each distinct pattern of stromal changes in leukemic bone marrow at initial diagnosis was associated with a heterogeneous clinical course with respect to the maintenance of complete remission for 5–8 years and early or late relapse. Thus, the remodeling of mesenchymal niche by leukemia cells is a self-reinforcing process of leukemogenesis that can be a parameter for the heterogeneity in the clinical course of leukemia and hence serve as a potential prognostic factor.
SSB1/NABP2 AND SSB2/NABP1 HAVE ESSENTIAL AND
OVERLAPPING ROLES IN MAINTAINING HEMATOPOIETIC STEM
AND PROGENITOR CELLS

Wei Shi, Therese Vu, Glen Boyle, Fares Al-Ejeh, Didier Boucher, Steven
Lane, Kum Kum Khanna

QIMR Berghofer Medical Research Institute, Cancer Biology, Brisbane,
Australia

Single-stranded DNA binding (SSB) proteins are essential for a variety of
DNA metabolic processes and the maintenance of genomic stability. We
categorized two SSB proteins, SSB1 and SSB2, in the complexes of
INTS3-SSB1/2-C9orf80, and assessed their physical function in mouse
models whereby they are constitutively or conditionally deleted.
Constitutive double knockout of Ssb1 and Ssb2 (DKO) causes early
embryonic lethality. DKO in adult mice was achieved by using a tamoxifen-
inducible Cre (Ssb1fl/flSsb2fl/flR26-CreERT2), in which Ssb1 and Ssb2 are
conditionally deleted by the administration of tamoxifen. Induced DKO
mice become moribund within seven days post tamoxifen treatment,
showing marked weight loss, severe anaemia, bone marrow deficiency and
intestinal atrophy. DKO bone marrow is markedly hypocellular with
reduction in all lineages of haematopoietic development, leading to the
hypothesis that Ssb1 and Ssb2 are required for the maintenance of
haematopoietic stem and progenitors cells (HSPCs).
Gene expression profile of DKO HSPCs predicates an exacerbated p53/p21
DNA damage response and pronounced interferon response. Stabilization of
p53 and increased apoptotic cell death were observed in DKO bone
marrows and HSPCs. DKO HSPCs have increased expression of IFN
induced surface markers such as Sca1. Functionally, HSPCs in DKO mice
show decreased quiescence at the early stage followed by decreased
proliferation and increased cell loss due to apoptotic cell death at the later
stage, suggesting the imbalanced bone marrow homeostasis upon DKO may
eventually result in exhaustion of the stem cell pool in DKO mice.
Furthermore, bona fide HSPCs intrinsic functional deficiency caused by
DKO was confirmed by competitive bone marrow transplant, where DKO
bone marrows showed abolished differentiation capacity and failed to
repopulate the bone marrows of recipient mice after induction of DKO in
the established engraftments from the Ssb1fl/flSsb2fl/flR26-CreERT2
donors. Mechanistically, DKO HSPCs manifest a profile of stalled
replication forks, unrepair double strand breaks and constitutive presence
of cytosolic single stranded DNAs, correlating with the increased genome
wide double strand breaks, R-loops accumulation and chromosome breaks
found in DKO bone marrow. All together, these results show Ssb1 and Ssb2
have compensatory functions in maintaining genomic stability in HSPCs
and are collectively indispensable for haematopoiesis.
ETV2-MEDIATED SPECIFICATION OF MESODERM CELLS INTO HEMANGIOGENIC FATE

Haiyong Zhao, Kyunghee Choi

Washington University in St. Louis, School of Medicine, Pathology and Immunology, St. Louis, MO

Blood and vessels develop spatially and temporally close to each other in embryogenesis and are therefore hypothesized to share a common mesoderm progenitor - the hemangioblast. However, the existence of the hemangioblast has been questioned due to lack of direct evidence. The transcription factor Etv2 is essential for both blood and vasculature development. It targets a set of critical hematopoietic and endothelial genes, and can induce ectopic expression of these genes in differentiating mouse ES cells and zebrafish embryos. Herein, we investigated if Etv2 directly determines the hemangiogenic fate and can therefore be used as a marker for the hemangioblast and how the Etv2-expressing cells are generated from mesoderm.

We established two ES cell lines, one with fluorescent reporters for Etv2 and its target gene Scl, which is an important transcription factor required for hematopoiesis initiation and is expressed in embryonic endothelium and hematopoietic progenitors, and the other with fluorescent reporters for Etv2 and the mesendoderm marker T/Brachyury. By combining known mesoderm surface markers, PDGFRα and VEGFR2, we monitored the expression dynamics of the reporters during differentiation. We found that only those with Etv2 level above a threshold, not all the Etv2-expressing cells, could activate Scl and be further specified into Scl-high state. Both endothelial and hematopoietic cell lineages were generated from this Scl-high population. We identified cell populations corresponding to multiple differentiation stages from ES cells to Scl-high state, and analyzed their transcriptomes. We also performed CRISPR-screening to identify genes essential for mesoderm commitment or Etv2 expression. Results obtained from these studies will be presented. In conclusion, our work provided a framework for unraveling the hierarchical events leading to the generation of blood and vasculatures.
CONVERSION OF T LYMPHOID-PRIMED PROGENITORS INTO THE MYELOID LINEAGE IN THE ABSENCE OF IRF4 DURING EMBRYOGENESIS

Feng Liu

Institute of Zoology, Chinese Academy of Sciences, State Key Laboratory of Biomembrane and Membrane Biotechnology, Beijing, China

T lymphoid-primed progenitors are hematopoietic progenitors destined to enter the thymus. The in vivo characterization of these embryonic progenitors is challenging, however, due to the intrauterine development of mouse embryos. Thus, how the fate of these cells is determined has not been fully defined in mammals. Here we use zebrafish embryos to show that the homing of T lymphoid-primed progenitors to the thymus is impaired, concomitant with a decrease in ccr9a expression, in the absence of irf4a. Strikingly, fate mapping assays at the single-cell level showed a fate change of irf4a-deficient T lymphoid-primed progenitors to myeloid cells, accompanied by an increase in Pu.1 expression. These data indicate that in addition to regulating ccr9a expression, Irf4a is also essential in T lymphoid-primed progenitors for repressing Pu.1 expression, to prevent an alternate fate. Our findings provide insight into the fate determination mechanism of T lymphoid-primed progenitors.
Pluripotent stem cells have great values for both basic researches and clinical applications. To expand the resources of pluripotent stem cells, we have employed multiple approaches to generate various types of pluripotent stem cell lines. Recently, we have generated haploid embryonic stem cells in mice and rats with both androgenetic and parthenogenetic origins. The androgenetic haploid ES (ahES) and parthenogenetic haploid ES (phES) cell lines can maintain both haploidy and pluripotency. Moreover, the ahES cells and phES cells can functionally replace sperms and oocytes, respectively, and both are capable of producing viable and fertile animals. We also show that the haploid ES cells can serve as a convenient tool for gene functional studies and large-scale genetic screening. From the mechanism aspects, recently we have revealed the regulatory role of the m^6A, an important epigenetic modification on RNA molecules, in promoting cell reprogramming. We have established and banked clinical-grade human pluripotent stem cell lines, and transplanted the ESC-derived functional cells into animal disease models including monkeys, and significant relief of disease symptom was observed without apparent side effects.
CNOT3 PROMOTES DIFFERENTIATION GENE mRNA DEADENYLATION AND DEGRADATION TO MAINTAIN THE PLURIPOTENT STATE IN ESCS AND EPIBLAST.

Xiaofeng Zheng¹, Pengyi Yang¹, Brad Lackford¹, Brian Bennett², David Fargo², Raja Jothi¹, Guang Hu¹

¹National Institute of Environmental Health Sciences, Epigenetics and Stem Cell Biology Laboratory, RTP, NC, ²National Institute of Environmental Health Sciences, Biostatistics & Computational Biology Branch, RTP, NC

The Ccr4-Not complex is the main deadenylase complex in eukaryotic cells and can regulate mRNA decay and stability. We have previously shown that several subunits in this complex, including Cnot1, Cnot2 and Cnot3, are required for the maintenance of both mouse and human embryonic stem cells (ESCs). Here, we show that Cnot3 is also required for the maintenance of epiblast cells during mouse pre-implantation development. Cnot3 is up-regulated at the blastocyst stage and is enriched in the inner cell mass. Its deletion leads to peri-implantation lethality. During diapause, a model commonly used to test the maintenance of epiblast cells, Cnot3 null embryos show a significant decrease in the number of epiblast cells and are quickly lost. Finally, the epiblast cells from the Cnot3 null blastocysts fail to expand and grow in culture. To further elucidate the mechanism through which Cnot3 regulates the pluripotent state in epiblast cells, we derived conditional Cnot3 knockout ESCs. Cnot3 deletion led to ESC differentiation and up-regulation of differentiation genes. Importantly, its deletion led to an increase in the poly(A)-tail length and half-life of differentiation gene mRNAs such as Hand1, Cited1, and Foxa1. Together, we propose the model that Cnot3 maintains the pluripotent state by suppressing differentiation gene mRNA stability via mRNA deadenylation.
OPPOSING ROLES FOR THE IncRNA HAUNT AND ITS GENOMIC LOCUS IN REGULATING HOXA ACTIVATION DURING ESC DIFFERENTIATION

Yafei Yin, Pixi Yan, Jinlong Lu, Xiaohua Shen

Tsinghua University, Tsinghua-Peking Center for Life Sciences, School of Medicine, Beijing, China

Long noncoding RNAs (IncRNAs) have been implicated in controlling various aspects of embryonic stem cell (ESC) biology. However, the functions of specific IncRNAs, and the molecular mechanisms through which they act, remain unclear. Dissection of the precise contribution of RNA transcripts versus genomic DNA sequences is important for functional assessment of IncRNAs.

Here, we show critical roles for a HOXA upstream non-coding transcript (Haunt) in regulating HOXA activation and ESC differentiation. Despite minimal apparent role in ESC self-renewal, Haunt knockout ESCs exhibit a global defect in upregulation of differentiation genes. Interestingly, reducing or enhancing Haunt expression, with minimal disruption of the Haunt locus, led to up- or down-regulation of HOXA genes, respectively. In contrast, increasingly large genomic deletions within the Haunt locus attenuated HOXA activation. We further showed that the Haunt DNA locus contains potential enhancers that interact with HOXA loci on chromatin and are required for HOXA activation. On the other hand, Haunt RNAs directly bind to chromatin and attenuate enhancer-promoter contacts, thereby inhibiting HOXA activation. Thus, Haunt RNA transcripts and its genomic locus exhibit discrete and opposing roles in regulating the HOXA gene cluster during ESC differentiation.

Our findings revealed a multi-faceted model of IncRNA function, highlighting an important role of IncRNA-mediated chromatin and transcriptional regulation in cell-fate transitions. In addition, our work established a rigorous paradigm and illustrate the power of rapid CRISPR/Cas9-based genome editing for assigning IncRNA functions.
GENOME EDITING IN HUMAN PLURIPOTENT STEM CELLS FOR UNDERSTANDING THE MECHANISMS OF PANCREATIC DEVELOPMENT AND DIABETES

Danwei Huangfu
Sloan Kettering Institute, Developmental Biology, New York, NY

Model organisms such as the mouse have greatly facilitated the functional validation of disease-associated genes. However, the ever-increasing number of sequence variants identified by human geneticists challenges us to develop more efficient experimental platforms with the speed and scale that surpasses conventional mouse genetics. An ideal platform should also allow the study of human-specific disease mechanisms.

We combine directed differentiation of human embryonic stem cells (hESCs) with powerful genome-editing technology to study human pancreas development. Through the use of TALENs and CRISPRs, we have generated hESC knockout mutants for all 11 neonatal diabetes-associate transcription factors identified to date. Through directed differentiation to recapitulate pancreatic development, our studies have revealed both conserved and human-specific mechanisms of pancreatic development and neonatal diabetes. We have also uncovered a previously unsuspected connection between pancreatic development and adult-onset type 2 diabetes.

Our findings establish the use of hESCs as a genetic model system for studying human birth defects, and lay the foundation for studying more complex diseases such as type 2 diabetes.
STUDY FAMILIAL HYPERTROPHIC CARDIOMYOPATHY USING PATIENT SPECIFIC INDUCED PLURIPOTENT STEM CELLS

Lei Yang¹, Lu Han¹, Yang Li¹, Kimimasa Tobita¹, Glenna Bett², Randall Rasmusson²

¹University of Pittsburgh, Department of Developmental Biology, Pittsburgh, PA, ²SUNY, Buffalo, Departments of Physiology and Biophysics, Buffalo, NY

Aim: Familial Hypertrophic Cardiomyopathy (HCM) is a primary disorder of cardiac muscle, and is associated with thickened ventricular wall and ventricular septum, increased myocardial fibrosis, disorganized myofibers and always accompanied with arrhythmic heart beatings. It is the most common autosomal dominant cardiovascular disease, with a prevalence of 1:500. The overall annual mortality rate of HCM is 1-5%. It is also the most common cause of sudden cardiac death (SCD) in young people, and accounts for one-third of all SCD in competitive athletes. Mutations in β-myosin heavy chain (β-MHC or MYH7) account for approximately 45% of all identified HCM cases. Studying HCM with patient specific iPS cell derived cardiomyocytes (CMs) would benefit the understanding of HCM mechanism, as well as the development of personalized therapeutic strategies.

Methods and Results: To investigate the molecular mechanism underlying the abnormal cardiomyocyte (CM) functions in HCM, we derived induced pluripotent stem cells (iPSCs) from a HCM patient with a single missense mutation (Arginine442Glycine) in the MYH7 gene. CMs were next enriched from HCM and healthy iPS cells, followed with whole transcriptome sequencing and pathway enrichment analysis. A widespread increase of genes responsible for “Cell Proliferation” was observed in HCM iPSC-CMs when compared with control iPSC-CMs. Additionally, HCM iPSC-CMs exhibited disorganized sarcomeres and electrophysiological irregularities. Furthermore, disease phenotypes of HCM iPSC-CMs were attenuated with pharmaceutical treatments.

Conclusions: Overall this study explored the possible patient-specific and mutation-specific disease mechanism of HCM, and demonstrates the potential of using HCM iPSC-CMs for future development of therapeutic strategies. Additionally, the whole methodology established in this study could be utilized to study mechanisms of other human inherited heart diseases.
A BALANCING ACT BETWEEN STRATIFICATION AND EMT IN HUMAN THYMIC EPITHELIAL CELLS

Matteo Pluchinotta*1,2, Melissa Maggioni*1,2, Tiphaine M Arlabosse*1,2, Paola Bonfanti1, Roxana M Wasnick4, Christèle Gonneau1,2, Ariane Rochat1,2, Yann Barrandon1,2

1Ecole Polytechnique Fédérale Lausanne, Laboratory of Stem Cell Dynamics, Lausanne, Switzerland, 2Centre Hospitalier Universitaire Vaudois, Department of Experimental Surgery, Lausanne, Switzerland, 3University College London, Institute of Immunity and Transplantation, London, United Kingdom, 4University of Giessen, German Center for Lung Research, Giessen, Germany

*: authors contributed equally

The thymus is the primary organ for T cell differentiation and maturation. Its stroma forms a characteristic complex 3D structure mainly composed of thymic epithelial cells (hTECs). Despite of this unconventional epithelial architecture, hTECs express markers associated with epidermal specification and differentiation. We have uncovered that the human thymus contains clonogenic hTECs that can be extensively expanded in a culture system used for skin keratinocyte stem cells. In vitro, these cells express markers of basal cells of stratified epithelia like p63, K5/K14 and CD49f. We demonstrate that EpCAM can be used to split cultured hTECs in two morphologically distinct subpopulations. EpCAM+ hTECs only give rise to stratified colonies that express markers of epidermal differentiation and contain squame-like cells, whereas EpCAM- hTECs mostly give rise to non-stratifying colonies even though they have the capacity to generate EpCAM+ hTECs. EpCAM- hTECs maintain an epithelial identity but display hallmarks of EMT such as the up-regulation of ZEB1, the loss of ECAD and a reduced expression of the miR-200 family members. We were able to show that miR-200c overexpression in EPCAM- hTECs is sufficient to drive a phenotype-switch leading to stratification. This data strongly suggests that the expression of an epidermal stratification program in hTECs is of functional importance rather than linked to promiscuous gene expression. We speculate that the maintenance of a tridimensional epithelial network requires a fine balance between stratification and EMT programs.
TISSUE REGENERATION IN LIVER: PLASTICITY OR STEM CELLS?

Markus Grompe, Bin Li, Willscott Naugler, Branden Tarlow

Oregon Health & Science University, Oregon Stem Cell Center, Portland, OR

Cell replacement in adult organs can be achieved through differentiation of stem cells, replication, or transdifferentiation of existing cells. In the adult liver stem cells have been proposed to replace tissue cells, especially upon injury. Here we will present how specialized cell types are produced in the adult liver during homeostasis and injury. The origin and role of clonogenic biliary cells (Lgr5+ and others) will be discussed. Based on current evidence, we propose that plasticity of differentiated cells rather than stem cells account for tissue repair in the adult mouse liver.
Regeneration of in vivo organ function using pluripotent stem cell (PSC)-derived cells is dependent on the production of functional cells typically prepared in vitro through techniques, such as “directed differentiation.” For example, the directed differentiation of pluripotent stem cells in culture into endodermal lineages typically relies on recapitulation of a sequence of developmental milestones known to occur during embryonic organogenesis of the desired cell or tissue type. However, this approach is limited when the mechanisms regulating in vivo organogenesis are unknown. This presentation will demonstrate the use of pluripotent stem cells to decipher the minimal pathways regulating specification of two endodermal organ primordia, the lung and thyroid, and the validation that these same pathways are evolutionarily conserved in vivo during embryonic organogenesis in Xenopus and mouse foreguts. Importantly, these signaling pathways can be utilized to generate lung and thyroid progenitors from normal mouse and human PSCs as well as from patient-specific iPS cells that carry genetic mutations responsible for clinical hypothyroidism. Finally, the regenerative functional potential of these cells is demonstrated through reconstitution of in vivo thyroid function in hypothyroid mouse recipients following transplantation of purified Nkx2-1+ thyroid progenitors derived from mouse pluripotent stem cells.
THE ROLE OF TET PROTEINS IN HESC PLURIPOTENCY AND DIFFERENTIATION

Nipun Verma1,2, Danwei Huangfu1

1Sloan-Kettering Institute, Developmental Biology, New York, NY, 2Weill Graduate School of Medical Sciences at Cornell University, Tri-Institutional M.D.-Ph.D. Program, New York, NY

Recently a family of Fe(II)/2-oxoglutarate-dependent dioxygenases, the Ten-Eleven Translocation (TET) proteins, were found to oxidize 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC), which could represent intermediates in a DNA demethylation pathway. DNA methylation during mammalian embryonic development is extremely dynamic, with stages marked by waves of demethylation that erase previous methylation patterns and progressive de-novo methylation to establish new configurations.

Previous studies have suggested a role of TET proteins during early development. However, their significance and mechanism of action during the specification of particular lineages has not been investigated. Here we have generated hESC lines with inactivating mutations in TET1, TET2 and TET3 (triple knockout/TKO hESCs). We observe that TKO hESCs exhibit a defect in differentiation into the embryonic germ layers, and most strikingly into the neuroectoderm (NE) lineage. Through phenotype analysis of single and double knockout lines we observe that this NE defect is mainly due to loss of TET1. TKO hESCs show an absence of hydroxymethylation and increased methylation at a regulatory region of PAX6, a critical NE gene. Furthermore upon repair of the mutated TET1 locus, we have rescue of the NE defect, restoration of hydroxymethylation and loss of hypermethylation at the PAX6 regulatory region. Based on these results we will perform rescue experiments involving PAX6 overexpression, 5-Azacytidine inhibition of DNMTs and targeted hydroxymethylation to further investigate the cause of the NE differentiation defect in our TKO hESCs. In addition to being the initial investigation of the role of TET proteins in hESCs, our study provides the first mechanistic evidence for the importance of TET proteins during hESC cell fate specification.
PRIMATE-SPECIFIC ENDOGENOUS RETROVIRUS-DRIVEN TRANSCRIPTION DEFINES NAIVE-LIKE STEM CELLS

Jichang Wang¹, Gangcai Xie¹,², Manvendra Singh¹, Avazeh T Ghanbarian³, Tamás Raskó¹, Attila Szvetnik¹, Huiqiang Cai¹, Daniel Besser¹, Alessandro Prigione¹, Nina V Fuchs¹,⁴, Gerald G Schumann⁴, Wei Chen¹, Matthew C Lorincz⁵, Zoltán Ivics⁴, Laurence D Hurst¹, Zsuzsanna Izsvák¹

¹Max-Delbrück-Center for Molecular Medicine, Robert-Rössle-Strasse 10, 13125 Berlin, Mobile DNA group, Berlin, Germany, ²CAS-MPG Partner Institute for Computational Biology, 320 Yueyang Road, Shanghai, Key Laboratory of Computational Biology, Shanghai, China, ³University of Bath, Department of Biology and Biochemistry, Bath, United Kingdom, ⁴Paul-Ehrlich-Institute, Division of Medical Biotechnology, Langen, Germany, ⁵University of British Columbia, Department of Medical Genetics, Vancouver, Canada

Naive embryonic stem cells hold great promise for research and therapeutics as they have broad and robust developmental potential. While such cells are readily derived from mouse blastocysts it has not been possible to isolate human equivalents easily, although human naive-like cells have been artificially generated (rather than extracted) by coercion of human primed embryonic stem cells by modifying culture conditions or through transgenic modification. Here we show that a sub-population within cultures of human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) manifests key properties of naive state cells. These naive-like cells can be genetically tagged, and are associated with elevated transcription of HERVH, a primate-specific endogenous retrovirus. HERVH elements provide functional binding sites for a combination of naive pluripotency transcription factors, including LBP9, recently recognized as relevant to naivety in mice. LBP9–HERVH drives hESC-specific alternative and chimaeric transcripts, including pluripotency-modulating long non-coding RNAs. Disruption of LBP9, HERVH and HERVH-derived transcripts compromises self-renewal. These observations define HERVH expression as a hallmark of naive-like hESCs, and establish novel primate-specific transcriptional circuitry regulating pluripotency.
OOCYTE FACTORS SUPPRESS MITOCHONDRIAL POLYNUCLEOTIDE PHOSPHORYLASE TO REMODEL THE METABOLOME AND ENHANCE REPROGRAMMING

Shyh-Chang Ng

Genome Institute of Singapore, Stem Cell & Regenerative Biology, Singapore, Singapore

The oocyte contains potent cytoplasmic factors that drive somatic cell nuclear transfer (SCNT) reprogramming, which can also augment the efficiency and quality of induced pluripotent stem cell (iPSC) reprogramming. Here we show that the oocyte-enriched factors, Tcl1 and Tcl1b1, significantly enhance the somatic reprogramming efficiency of mouse fibroblasts into iPSCs. In addition, clonal analysis of pluripotency biomarkers shows that the Tcl1 oocyte factors improve the quality of iPSC reprogramming. Mechanistically, we find that the enhancement effect of Tcl1b1 depends on Akt, one of its putative targets. In contrast, Tcl1 suppresses the mitochondrial polynucleotide phosphorylase (PnPase) to promote reprogramming. Knockdown of PnPase rescues the inhibitory effect from Tcl1 knockdown in iPSC reprogramming, whereas PnPase overexpression abrogates the iPSC enhancement from Tcl1 overexpression.

We further demonstrate that Tcl1 binds and suppresses PnPase’ mitochondrial localization to suppress mitochondrial biogenesis and OxPhos, thus remodeling the metabolome. Hence we have identified the oocyte Tcl1-PnPase pathway as a critical mitochondrial switch during reprogramming into pluripotent stem cells. Since mitochondrial biogenesis and functions are also tightly regulated in pre-fertilization oocytes and early embryonic development, the Tcl1-PnPase switch might not only be relevant for regulating mitochondria in iPSC reprogramming, but also for mitochondrial replacement in aged oocytes and during in-vitro fertilization.
DEVELOPMENT OF AN ENCAPSULATED STEM CELL-BASED THERAPY FOR DIABETES

Olivia G Kelly

ViaCyte, Inc., Research and Development, San Diego, CA

ViaCyte Inc. is a clinical stage company developing a stem cell-based islet replacement therapy for treatment of patients with diabetes. The therapy is a combination product comprised of pancreatic endoderm cells encapsulated within a retrievable delivery device, ENCAPTRA® Drug Delivery System. After implantation, encapsulated progenitor cells differentiate into glucose-responsive, insulin-secreting cells. The renewable starting material for cell product manufacturing is human embryonic stem cells that are directed to differentiate to the pancreatic endoderm cell product using scalable processes. The bio-stable delivery device is designed to fully contain cells and to protect cells from immune attack, with the goal of eliminating the need for immunosuppressant drugs.

Funding in part from California Institute for Regenerative Medicine; SP1-06513, DR1-01423, TR1-01215 and JDRF.
Degeneration of basal forebrain cholinergic neurons (BFCNs) is associated with cognitive impairments of Alzheimer’s disease (AD), implying that BFCNs hold potentials in exploring stem cell-based replacement therapy for AD. However, studies on derivation of BFCNs from embryonic stem cells (ESCs) are limited, and the application of ESC-derived BFCNs remains to be determined. Here, we report differentiation approaches for directing both mouse and human ESCs into mature BFCNs. These ESC-derived BFCNs exhibit features similar to those of their in vivo counterparts, and acquire appropriate functional properties. After transplantation into basal forebrain of AD model mice, ESC-derived BFCN progenitors predominantly differentiate into mature cholinergic neurons that functionally integrate into endogenous basal forebrain cholinergic projection system. The AD mice grafted with mouse or human BFCNs exhibit improvements in learning and memory performances. Our findings suggest the promising perspective of ESC-derived BFCNs in development of stem cell-based therapies for treatment of AD.
Human neural stem cells has been isolated, expanded and stored as banks of cells, HuCNS-SC®. Transplants of these cells offer the prospect to treat a spectrum of neurodegenerative disease or injuries to the brain, spinal cord and eye and represent a potential exciting new medical therapy. Studies transplanting HuCNS-SC into animal models of human diseases or injury have demonstrated protection of host cells and/or improvements in specific functional deficits; neuroprotection and neural replacement that provide the foundational strategies for our clinical studies. Safety of direct transplantation of HuCNS-SC into all three areas of the CNS has been demonstrated in four clinical studies and provides early evidence of biological activity similar to what has been observed in the animal studies.

Studies in spinal cord contused mouse models show that when directly transplanted into the spinal cord above and below the injury site, these cells migrate extensively and differentiate, remyelinate and make synaptic connections with host neurons. HuCNS-SC have also been shown to produce neurotrophic factors implicated in neuroprotection of host cells. The recently completed clinical trial in chronic spinal cord injured patients demonstrated that the surgical transplantation technique and cell dose were safe and well tolerated by all patients and over the course of twelve months showed no abnormal changes in spinal cord function associated with the transplantation technique and no adverse events attributed to the HuCNS-SC cells. Analysis showed multi-segmental gains in sensory function starting around three months after transplantation. Of the twelve patients, three of the seven AIS A patients and four of the five AIS B patients showed signs of positive sensory gains.

Loss of central vision in dry age-related macular degeneration severely impacts the quality of life of the millions of people with this condition and currently there is no treatment. Transplantation of HuCNS-SC into the animal model of retinal degeneration, the RCS rat has demonstrated significant protection of host cone photoreceptors and preservation of vision. Numbers of cone photoreceptors remained constant over an extended period, consistent with the sustained visual acuity and light sensitivity observed in the study.

A Phase I/II dose-escalation trial for patients with the dry form of AMD, also referred to as Geographic Atrophy, investigated the therapeutic potential of HuCNS-SC cells transplanted into the subretinal space of the eye. Result from the AMD studies show that for the majority of the patients in the study, best-corrected visual acuity (BCVA) and contrast sensitivity (CS) improved or remained stable in the treated eye.

The safety and early efficacy results from the Phase I/II studies in spinal cord injury and dry AMD supports the ongoing Phase II controlled proof-of-concept studies in these indications.
NEURAL STEM CELLS, THEIR ROLE IN DEVELOPMENT AND THERAPY DEVELOPMENT

Sally Temple

Neural Stem Cell Institute, Rensselaer, NY

The central nervous system (CNS) is the most complex of tissues, with hundreds of types of neurons and glia patterned into unique regions, connected through intricate, complex circuits. Developmental studies have shown that the initial plate of neuroepithelial cells is regionally patterned by morphogenic signals. This area identity is then interpreted by neural stem cells and more restricted precursor cells, collectively called neural progenitor cells (NPCs), to produce the diverse cells of each CNS region. Progeny arise from NPCs on a defined schedule, and migrate to their final position in the CNS. While most NPCs are extinguished after development, a few are retained in the adult forebrain where they continually make new neurons that contribute to memory formation. Neurodegenerative diseases typically attack specific CNS populations, for example, the hippocampal stem cell system in Alzheimer’s Disease, and midbrain dopaminergic neurons in Parkinson’s disease.

Our studies using long-term timelapse analysis of single cells growing in a controlled environment have demonstrated that NPCs have an inherent timing program that determines their output. This program is acted upon by a variety of environmental factors in the neural stem cell niche to fine-tune the numbers and types of neural cells made. Human NPCs are being harnessed to replace cells lost to disease or damage and are already in clinical trials. In addition, the niche regulators of NPCs offer new therapeutic avenues. The fact that progenitor cells are present in the adult human CNS offers the exciting opportunity for stimulating endogenous reparative processes.

79
Participant List

Dr. Liangxia Ai  
Peking University  
alxmishuchu@163.com

Dr. Trevor K. Archer  
NIEHS/NIH  
archer1@niehs.nih.gov

Ms. Baoling Bai  
Capital Institute of Pediatrics  
604187746@qq.com

Dr. Qing Chang  
Fluidigm instrument tech.  
dana.chang@fluidigm.com

Mr. Shuhan Chen  
Guangzhou Institutes of Biomedicine and Health  
chen_shuhan@gibh.ac.cn

Ms. De Yun Chen  
Chinese PLA General Hospital  
chendeyun301@sina.com

Dr. Yingju (Miliya) Chen  
ReproCELL Japan  
miliya.chen@reprocell.com

Prof. Ting Chen  
National Institute of Biological Sciences  
chenting@nibs.ac.cn

Dr. Lei Cheng  
Cell Research  
lcheng@sibs.ac.cn

Prof. Ssang-Goo Cho  
Konkuk University  
ssangoc@konkuk.ac.kr

Dr. Paul E Cizdziel  
ReproCELL Japan  
paul.cizdziel@reprocell.com

Dr. Wenhao Cui  
Shanghai Tech  
cuiwh@shanghaitech.edu.cn

Dr. Hongkui Deng  
Peking University, College of Life Sciences  
Hongkui_deng@pku.edu.cn

Dr. VALENTINE CHUIJOKE DIKE  
FSH  
infoteachinghospital@gmail.com

Dr. Jiebin Dong  
peking university  
dongjiebin@bjmu.edu.cn

Dr. Andrew Elefanty  
Murdoch Childrens Research Institute  
andrew.elefanty@monash.edu

Dr. Marcelo E Ezquer  
Centro de Medicina Regenerativa, UDD  
mezquer@udd.cl

Dr. Fernando Ezquer  
Facultad de Medicina, Universidad del Desarrollo  
eezquer@udd.cl

Dr. Haiyan Fang  
Shanghai Tongji university  
haiyan_fang@hotmail.com

Dr. Ji-Feng Fei  
DFG-Center for Regenerative Therapies  
Dre  
jlfeng.fei@crt-dresden.de
Dr. Yimeng Gao  
Shanghai Institute for Biological Sciences  
gaoyimeng@sibcb.ac.cn

Ms. Weiwei Gao  
Korea University  
waowei1502@163.com

Dr. Markus Grompe  
Oregon Health and Sciences University  
grompem@ohsu.edu

Ms. Kaili Gu  
Peking University  
cutekelly1992@163.com

Ms. Chunyun Gu  
NISSEI CORPORATION  
guc@nisseicorp.co.jp

Dr. Lin Guo  
Guangzhou Institute of Biomedicine & Health, CAS  
guo_lin09@gibh.ac.cn

Dr. Le Han  
Shimadzu (China) CO., LTD  
sshhl@shimadzu.com.cn

Mr. Dong Wook D Han  
Konkuk University  
dwhan@konkuk.ac.kr

Dr. Hisato Hayashi  
NISSAN CHEMICAL INDUSTRIES, LTD.  
hayashih@nissanchem.co.jp

Prof. Bayar Hexig  
Inner Mongolia University  
bhexig@imu.edu.cn

Dr. Simon Hilcove  
stemcell Tech  
simon.hilcove@stemcell.com

Dr. Juan Hou  
STEMCELL Technologies Inc  
juan.hou@stemcell.com

Dr. Lufeng Hu  
Peking University  
linjasuowei@163.com

Dr. Liang Hu  
CENTRAL SOUTH UNIVERSITY  
lianghu7@gmail.com

Dr. Ping Hu  
Shanghai Institute of Biochemistry and Cell Biology  
hup@sibcb.ac.cn

Dr. Yizhou Hu  
University of Helsinki  
yizhou.hu@helsinki.fi

Dr. Guang Hu  
NIEHS  
hug4@niehs.nih.gov

Dr. Ke Huang  
Guangzhou Institute of Biomedicine and Health  
huang_ke@gibh.ac.cn

Dr. Pengyu Huang  
ShanghaiTech University  
huangpy@shanghaitech.edu.cn

Prof. Yue Huang  
Peking Union Medical College  
huangyue@pumc.edu.cn

Prof. Xuejun Huang Parsons  
XI'AN JIAO TONG UNIVERSITY SUZHOU ACADEMY  
928878079@qq.com
Dr. Ying Lei
Shanghai East Hospital
yinglei78@hotmail.com

Ms. Xirui Li
GIBH
li_xirui@gibh.ac.cn

Ms. Na Li
Institute of zoology, Chinese academy of sciences
linawhere@163.com

Dr. Dangsheng Li
Cell Research
dsli@sibs.ac.cn

Dr. Xueling Li
The Key Laboratory of National Education Ministry
lixueling@hotmail.com

Mr. Dongwei Li
Guangzhou Institutes of Biomedicine and Health
li_dongwei@gibh.ac.cn

Prof. Peng Li
Guangzhou Institutes of Biomedicine and Health
li_peng@gibh.ac.cn

Ms. Songli Li
R&D Systems China
shelly.li@bio-techne.com

Dr. Wenlin Li
Second Military Medical University
liwenlin@smmu.edu.cn

Dr. Qing Li
Shanghai Sixth People’s Hospital
liqing236@gmail.com

Mr. Qing V Li
Memorial Sloan Kettering Cancer Center
liq@sloankettering.edu

Ms. Fei Li
National Institute of Biological Sciences, Beijing
lifei@nibs.ac.cn

Dr. Zhonghan Li
Sichuan University
zhonghan.li@outlook.com

Dr. Dong Liang
Nanjing Maternity and Child Health Care Hospital
liangdong2083@163.com

Dr. Baojian Liao
Guangzhou Institutes of Biomedicine and Health
liao_baojian@gibh.ac.cn

Mr. Kyung Tae Lim
School of Medicine, Konkuk University
lvirus@konkuk.ac.kr

Ms. Huimin Lin
Tianjin State Key Laboratory of Modern Chinese Med
18322597462@163.com

Dr. Chengqi Lin
Southeast University
cqlin@seu.edu.cn

Dr. Guonan Lin
Capital Medical University
linguonan@ccmu.edu.cn

Dr. Chao-Po Lin
University of California, Berkeley
newcplin@gmail.com
Dr. Xiaozhen Liu  
Capital Institute of Pediatrics  
zhen_l@yeah.net  

Dr. Xuqing Liu  
Shanghai Jiao Tong University  
xuqingliu@sjtu.edu.cn  

Dr. Zichuan Liu  
Friedrich Miescher Institute  
zichuan.liu@fmi.ch  

Dr. Chang Liu  
Chinese Academy of Sciences  
liuchang@sibs.ac.cn  

Prof. Lin Liu  
Nankai University  
liulin@nankai.edu.cn  

Mr. Pengfei Liu  
Guangzhou Institute of Biomedicine & Health, CAS  
rockman123456@sina.com  

Prof. Feng Liu  
Institute of Zoology, Chinese Academy of Sciences  
iluf@ioz.ac.cn  

Dr. Guang Liu  
Institute of Basic Medical Sciences, CAMS&PUMC  
iluguang@ibms.pumc.edu.cn  

Ms. Chaojie Liu  
Tongji University  
lcj6190@163.com  

Dr. Yanlin Long Ping  
Hainan Medical University  
1071545806@qq.com  

Dr. Zhiwei Lu  
National Institute of Biological Sciences, Beijing  
luzhiwei@nibs.ac.cn  

Mr. Junhua Lv  
Institute of Zoology, Chinese Academy of Sciences  
huashuo001@126.com  

Ms. Jiao Ma  
Chinese Academy of Sciences  
majiao.2010@foxmail.com  

Dr. SIMEON CHIEMELA MADUGBA  
FSH  
infoteachinghospital@gmail.com  

Mr. Thangaselvam Muthusamy  
National center for biological sciences  
selvam@ncbs.res.in  

Dr. Tao Na  
National Institutes for Food and Drug Control  
natao@vip.163.com  

Dr. CHIKA FORSTER NEBO  
FSH  
infoteachinghospital@gmail.com  

Dr. Shyh-Chang Ng  
Genome Institute of Singapore  
ngsc1@gis.a-star.edu.sg  

Ms. Xin Niu  
Shanghai Sixth Hospital  
niuxin999@163.com  

Dr. Hitoshi Niwa  
RIKEN Center for Developmental Biology  
iwai@cdb.riken.jp
Ms. Lulu  Sun  
Shanghai Institutes for Biological Science  
sunlulu2014@sibcb.ac.cn

Mr. Da  Sun  
Peking University  
sunda_1114@163.com

Dr. Pingxin  Sun  
Second Military Medical University  
13301678592@163.com

Mr. Shicheng  Sun  
University of Edinburgh  
Shicheng.sun@hotmail.com

Dr. Wei  Sun  
Chinese academic of Science  
sunwei@gibh.ac.cn

Dr. Sally  Temple  
Neural Stem Cell Institute  
sallytemple@neuralsci.org

Mr. Fei  Teng  
Chinese Academy of Sciences  
tengfei_ioz@163.com

Dr. Dongdong  Ti  
Chinese PLA General Hospital  
tddee@163.com

Dr. Ann  Tsukamoto  
StemCells, Inc  
ann.tsukamoto@stemcellsinc.com

Dr. NDUKWO KALU  UGURU  
FSH  
infeachinghospital@gmail.com

Dr. Mohan Chari Vemuri  
Thermo Fisher Scientific  
mohan.vemuri@thermofisher.com

Ms. Nipun  Verma  
Sloan Kettering Institute  
niv2005@med.cornell.edu

Prof. Yangming  Wang  
Peking University  
yangming.wang@pku.edu.cn

Dr. Gang  Wang  
Chinese Academy of Sciences  
gwang@sibcb.ac.cn

Mr. Bo  Wang  
Guangzhou Institutes of Biomedicine and Health  
wang_bo2012@gibh.ac.cn

Prof. Yu  Wang  
Tianjin University of Traditional Chinese Medicine  
870303247@qq.com

Dr. Ran  Wang  
Chinese Academy of Sciences  
wangran@sibcb.ac.cn

Ms. Qiming  Wang  
Peking University  
wangqiming970@126.com

Ms. Wenjie  Wang  
National Institute of Biological Sciences  
wangwenjie@nibs.ac.cn

Dr. Ju  Wang  
ShanghaiTech university  
wangju@shanghaitech.edu.cn

Dr. Yang  Wang  
Shanghai Sixth People Hospital  
wangy63cn@126.com
Mr. Jichang Wang
Max Delbrück Center for Molecular Medicine
jichang.wang@mdc-berlin.de

Ms. Xiwen Wang
Peking University
wxw19911010@sina.com

Dr. Wei Wang
German Cancer Research Center
wei.wang@nct-heidelberg.de

Mr. Shaohua Wang
Peking University
wsh20080801@126.com

Dr. Dongye Wang
Guangzhou Institutes of Biomedicine and health
wang_dongye@gibh.ac.cn

Prof. Hongmei Wang
Chinese academy of sciences
wanghm@ioz.ac.cn

Mr. Xiaoshan Wang
Guangzhou Institute of Biomedicine & Health, CAS
wang_xiaoshan@gibh.ac.cn

Dr. Zhong Wang
University of Michigan
mmccotte@umich.edu

Dr. Yonglong Wei
Institute of Zoology, Chinese Academy of Sciences
weiyonglong@126.com

Dr. Lu Wei
East hospital, shanghai
85157732@qq.com

Dr. Sherman M. Weissman
Yale University School of Medicine
sherman.weissman@yale.edu

Dr. Bo Wen
Fudan University
bowen75@fudan.edu.cn

Ms. Jiaying Wu
Shanghai Institutes for Biological Sciences, CAS
wujaying2014@sibcb.ac.cn

Mr. Wenbo Wu
National Institute of Biological Sciences, Beijing
wuwenbo@nibs.ac.cn

Dr. Xunwei Wu
Shandong University
xunwei1971@163.com

Dr. Feng Xiao
Shanghai Institutes for Biological Sciences
smilexiaofeng@sina.com

Ms. Yini Xiao
Shanghai Institutes for Biological Sciences, CAS
xiaoyini2013@sibcb.ac.cn

Prof. Wen-lin Xiao
the Affiliated Hospital of Qingdao University
wenlinxia@sina.com

Dr. Huangfan Xie
Peking University
xiehuangfan@126.com

Dr. Wei Xie
Tsinghua University
xiewei121@tsinghua.edu.cn
Mr. Yuhua Xie  
National Institute of Biological Sciences, Beijing  
xieyuhua@nibs.ac.cn

Dr. Xiaoyan Xie  
Beijing Institute of Transfusion Medicine  
alpha13@126.com

Dr. Jerry Xing  
Lonza  
jerry.xing@lonza.com

Mr. Kai Xu  
Chinese Academy of Sciences  
kaixu104447@sina.com

Dr. Aining Xu  
Ruijin Hospital  
xan36@hotmail.com

Mr. Zijian Xu  
National Institute of Biological Sciences  
xuzijian@nibs.ac.cn

Dr. Jian Xu  
UT Southwestern Medical Center  
jian.xu@utsouthwestern.edu

Dr. Jun Yang  
Chinese academy of sciences  
yanlong@ioz.ac.cn

Dr. Dr. Yuchang Yuan  
Institute of Basic Medical Science, CAMS  
yangjunimb@hotmail.com

Dr. Jiayin Yang  
Shenzhen Cell Inspire Biotechnology Co., Ltd  
yangjiayin@cib.cc

Dr. Aining Xu  
Ruijin Hospital  
xan36@hotmail.com

Prof. Seungkwan You  
Korea University  
bioseung@korea.ac.kr

Ms. Zhou Yu  
National Institute of Biological Sciences, Beijing  
yuzhou@nibs.ac.cn

Dr. Yangyang Yuan  
ShanghaiTech University  
yuanyy@shanghaitech.edu.cn

Dr. Bao-Zhu Yuan  
National Institutes for Food and Drug Control  
fangshi0712@qq.com

Dr. Yixing Yuchi  
Southeast University  
101011981@seu.edu.cn

Dr. Chunmei Yue  
Shanghai Institutes for Biological Sciences, CAS  
cmyue@sibcb.ac.cn
Dr. Xuetao Zhang
Peking University
zhxt51@163.com

Dr. Xin Zhang
University of Oklahoma Health Science Center
xinzhanganan@hotmail.com

Prof. Xiao Zhang
Guangzhou Institute of Biomedicine and Health
zhang_fengxiang@gibh.ac.cn

Mr. Jianquan Zhang
Shenzhen Cell Inspire Biotechnology Co., Ltd
zhangjianquan@cib.cc

Dr. Haiyong Zhao
WUSTL, School of Medicine
hzhao@path.wustl.edu

Dr. Ya Zhao
Chinese Academy of Science
strawberry0928@hotmail.com

Ms. Qian Zhao
Institute of zoology, Chinese academy of sciences
bingxia0923@163.com

Ms. Yi Zhou
Ruijing hospital
778291597@qq.com

Dr. Qi Zhou
Institute of Zoology, CAS
qzhou@ioz.ac.cn

Dr. Ken Zuckerman
Moffitt Cancer Center
ken.zuckerman@moffitt.org
GENERAL INFORMATION

<table>
<thead>
<tr>
<th>Issues</th>
<th>Contacts</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reception</td>
<td></td>
<td>86-512-69568888</td>
</tr>
<tr>
<td>Security</td>
<td>Duty Manager</td>
<td>86-18913579302</td>
</tr>
<tr>
<td>Clinic</td>
<td>Front Office</td>
<td>86-18913579300</td>
</tr>
<tr>
<td>Engineering</td>
<td>Duty Manager</td>
<td></td>
</tr>
<tr>
<td>IT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Front Office</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Dining, Bar Dining
Rooms: Octagon – Floor 1
Suz Garden – Floor 1

Bar:
D Lounge: First Floor
Opening Hours: 09:00 – 00:00

Internet Access
Public Area – Wireless
Room – Wireless / Plug-in

Business Center
Location: First Floor
Opening Hours: 08:00 – 20:00

Gymnasium & Aerobic Room
Location: Mezzanine Floor Opening
Hours: 06:00 – 23:00

Indoor Swimming Pool
Location: Mezzanine Floor Opening
Hours: 06:00 – 23:00

Taxi
Local Taxi:
Hotline: 86-512-67776777
Out of city - eHi Car Rental:
Candy Tang: 86-13764070908
Wing Wu: 86-18939811116

You may contact Cold Spring Harbor Asia Office at 86-512-62729029 for any conference inquiries.