June 13 – 16, 2012

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Final Program

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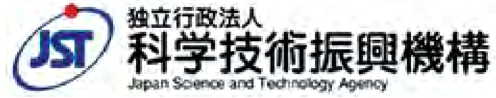

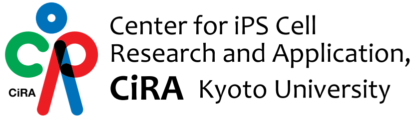

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ISSCR 10th Annual Meeting Co-sponsor

Dear Participants,

I am pleased to welcome you to the 10th Annual Meeting of the International Society for Stem Cell Research. It is a great honor to host the 10th anniversary meeting in Yokohama as the first annual meeting of the ISSCR in Asia, with the cooperation of the Center for iPS Cell Research and Application, Kyoto University.

Stem cell research has been opening a new frontier of science, technology and innovation since 2006, when the iPS cell was successfully generated in Japan. And a desire for further breakthroughs is growing in academia, industry, and general public. Featuring outstanding researchers and significant topics, this meeting will certainly contribute further steps forward to medical innovation. Japan Science and Technology Agency remains committed to foster innovation by funding researchers in Japan and their collaborators around the world.

I heartily hope this meeting to be fruitful for all of you.

Sincerely,

Michiharu Nakamura

Dr. Michiharu Nakamura
President, Japan Science and Technology Agency

Attend the 2012 ASCB Annual Meeting

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For more information go to
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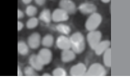

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Dear Colleagues,

Welcome to Yokohama and the 10th Annual Meeting of the International Society for Stem Cell Research!

On behalf of the Center for iPS Cell Research and Application (CiRA) at Kyoto University, it is a pleasure to be your host as an ISSCR co-sponsor during the 10th anniversary of the founding of the ISSCR. We are especially proud to have the ISSCR's first annual meeting in Asia.

With distinctive speakers and supporters from around the world, this annual meeting underscores the latest findings in the rapidly developing field of stem cell biology. We hope you will have a stimulating experience and take advantage of the networking opportunities as you work toward advancing stem cell research.

Enjoy the ISSCR 10th Annual Meeting and the beautiful port city of Yokohama!

Sincerely,

Dr. Shinya Yamanaka
Director,
Center for iPS Cell Research and Application (CiRA), Kyoto University

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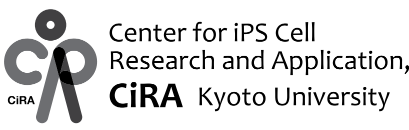

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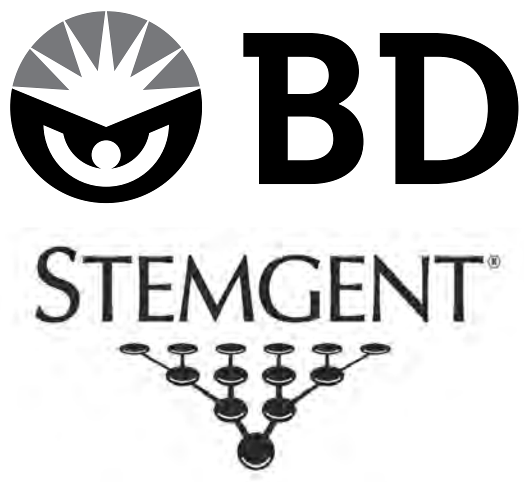

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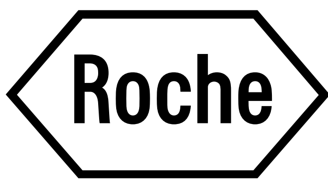

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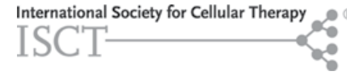

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Letter from the ISSCR

Welcome to the ISSCR 10th Annual Meeting — our first annual meeting in Asia — where we will celebrate our 10th Anniversary. In these next three-and-a-half days, you will hear from more than 150 of the world's best and brightest in the stem cell field — including a record 100 abstract-selected speakers presenting the latest and most exciting research.

Finding Your Way. This program is your guide as you explore cutting-edge research, technologies and techniques offered at ISSCR 2012. It includes a useful map to find where and when the sessions, posters, exhibits and social events are occurring. You can still go online and use the ISSCR Itinerary Builder to plan each day.

Career Development. Early-career investigators are an important part of the ISSCR annual meeting. The ISSCR has planned special events specifically for the junior investigator such as the Career Panel Lunch (Saturday), "Strategies for Success: Forging Seamless Transitions Between Academics and Industry," the Meet the Experts Lunches (Thursday and Friday) and the Junior Investigator Social Event (Thursday evening).

Stay Connected — During and After the Meeting. You, and the thousands of researchers gathered together, are the greatest resource in the field. Share your experience with your peers in real time over the next few days using Twitter hashtag #ISSCR2012. Follow @ISSCR2012 and receive updates to your mobile device regarding meeting resources, opportunities and events. ISSCR will also be launching its new online educational and interactive platform, ***ISSCR Connect.*** Following the meeting, attendees will have the opportunity to view talks you may have missed, view virtual exhibit booths, interact online with your colleagues and more. Additional programming will be available then throughout the year.

Experience Japan. Enjoy all that Yokohama and Japan have to offer. The Yokohama Hospitality Booth and the JTB Housing & Tours Desk are located across from the ISSCR Registration Desk in the National Convention Hall to help you take advantage of the rich culture that surrounds ISSCR 2012.

Learn, interact and enjoy ISSCR 2012 and mark your calendar for ISSCR 2013 in Boston, June 12 –15.

Sincerely,

Larry Goldstein
ISSCR Program Chair

Fred H. Gage
ISSCR President

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Celebrate ISSCR's 10th Anniversary!

ISSCR 10th Anniversary Reception

Wednesday, June 13
7:00 – 9:00 p.m.

ISSCR 2012 Exhibit Hall

Join us and be part of this historic event.

Supported by:

Don't Miss these
Featured Presentations

ISSCR Keynote Speakers

Plenary I:

PRESIDENTIAL SYMPOSIUM — EARLY LIFE DECISION

Wednesday, 1 p.m.

Rudolph Jaenisch is a Founding Member of the Whitehead Institute for Biomedical Research and a Professor of Biology at MIT. In 2005 he established the Human Stem Cell Facility at the Whitehead.

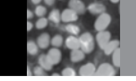
Dr. Jaenisch is a pioneer in making transgenic mice, leading to some important advances in understanding cancer, neurological and connective tissue diseases, and developmental abnormalities. These mice have been used to explore basic questions such as the role of DNA modification, genomic imprinting, X chromosome inactivation, nuclear cloning, and, most recently, the nature of stem cells. The Jaenisch laboratory has used therapeutic cloning and gene therapy to rescue mice having a genetic defect and, using a technique for turning skin cells into stem cells, they have cured mice of sickle cell anemia -- the first direct proof that these easily obtained cells can reverse an inherited disease.

John Gurdon did his undergraduate work in Zoology in the University of Oxford and later a one-year postdoctoral position at CalTech. He returned to Oxford and became a university lecturer in embryology. In 1971 he moved to the MRC molecular biology laboratory in Cambridge, continuing his work on Amphibian developmental biology. In 1983 he moved to the University of Cambridge as John Humphrey Plummer professor of cell biology. He co-founded a research Institute of developmental and cancer biology with Professor Laskey as co-chairman. He remained as Chairman of this Institute until 2002. During his career Dr Gurdon has concentrated on nuclear transplantation in the frog Xenopus. He has also carried out a range of experiments with this material, discovering the value of messenger RNA microinjection, mechanisms of response to morphogen gradients, and, most recently, mechanisms of nuclear reprogramming by Xenopus oocytes and eggs. Dr Gurdon served as Master of Magdalene College Cambridge from 1995-2002. Dr Gurdon has received various recognitions, including, most recently, the Lasker Award for Basic Medical Science.

Austin G. Smith Wellcome Trust Centre for Stem Cell Research, University Of Cambridge, obtained his Ph.D. from the University of Edinburgh in 1986. Following postdoctoral research at the University of Oxford, he joined the Institute for Stem Cell Research at the University of Edinburgh (formerly Centre for Genome Research) in 1990 as a group leader. In 1996, he was appointed Director of the Centre. He was appointed MRC Research Professor in 2003. He took up the post of Director of the Wellcome Trust Centre for Stem Cell Research at the University of Cambridge in the autumn of 2006.

Professor Smith's expertise is in the field of stem cell biology and he has pioneered key advances in the field of Embryonic Stem (ES) Cell research. His research focuses on the molecular and cellular controls of embryonic and somatic stem cells, and on interconversion between pluripotent and tissue-restricted states.

Kazutoshi Takahashi is the first student of Shinya Yamanaka and is a lecturer at Center for iPS Cell Research and Application, Kyoto University where his group works on cellular reprogramming. Takahashi, with his mentor Yamanaka, was the first recipient of the McEwen Award for Innovation, given last year at the ISSCR Annual Meeting in Toronto. In 2010 he was awarded the TOKYO Techno 21 Gold Medal Award and in 2009 the Yukawa-Tomonaga Memorial Prize.



Ernest McCulloch Memorial Lecture
Plenary II:
REGENERATION, ENGRAFTMENT, AND MIGRATION OF STEM CELLS

Wednesday, 4:30 p.m.

Irving L. Weissman, MD, has directed the Institute for Stem Cell Biology and Regenerative Medicine at Sta ford School of Medicine since its founding, providing vision and leadership to build one of the nation's top stem cell programs. In 1988, Dr. Weissman became the first to isolate in pure form any stem cell in any sp cies when he isolated the hematopoietic or blood-forming stem cell in mice. He subsequently isolated the human hematopoietic stem cell, the human neuronal stem cell, and the human leukemia stem cell. His work has opened up an entirely new area of scientific research with enormous potential for life-saving therapies. Dr. Weissman recently made an exciting step toward the goal of transplanting adult stem cells to create a new immune system for people with autoimmune or genetic blood diseases. As published in the November 2007 issue of Science, his lab found a novel way to transplant new blood-forming stem cells into the bone marrow of mice without the tissue-damaging radiation or chemotherapy usually required, thereby effectively replacing their immune systems. Many aspects of this technique will need to be adapted before it can be tested in humans, but when those barriers are surmounted, the benefits could be significant. An immune system transplant, much like a liver or heart transplant, would give a person with an autoimmune disease, such as multiple sclerosis, hope for a healthy future. Dr. Weissman received his medical degree from Stanford in 1965 and, after carrying out research in laboratories provided by the late Henry S. Kaplan, MD, joined the faculty four years later. In addition to being the Virginia and D. K. Ludwig Professor for Clinical Investigation in Cancer Research, he is a professor of pathology and developmental biology, and, by courtesy, professor of neurosurgery and of biological sciences.

Anne McLaren Memorial Lecturer
Plenary VII:
STEM CELLS AND FATE CONTROL

Saturday, 4 p.m.

Fiona Watt obtained her DPhil from Oxford University and was a postdoc at M.I.T. She initially established a laboratory at the Kennedy Institute in London and then moved to the Cancer Research UK (CR-UK) London Research Institute (formerly known as the Imperial Cancer Research Fund). She is currently the Herchel Smith Professor of Molecular Genetics at the University of Cambridge and Deputy Director of the CR-UK Cambridge Research Institute. In 2012 she will be moving to King's College London as Director of the new Centre for Stem Cells and Regenerative Medicine. She is a member of EMBO, a fellow of the Academy of Medical Sciences and a fellow of the Royal Society. Her research focus is on stem cells of adult multi-layered epithelia and the tumours derived from them.

Join Us in Honoring the Recipients of the 2012 ISSCR Awards

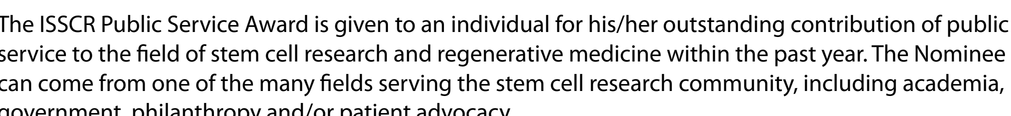
Rob and Cheryl McEwen
McEwen Centre for Regenerative Medicine

ISSCR Public Service Award

Presidential Symposium

ISSCR International Society for Stem Cell Research

Wednesday, 1 p.m.



The ISSCR Public Service Award is given to an individual for his/her outstanding contribution of public service to the field of stem cell research and regenerative medicine within the past year. The Nominee can come from one of the many fields serving the stem cell research community, including academia, government, philanthropy and/or patient advocacy.

Rob and Cheryl McEwen, founders of the McEwen Centre for Regenerative Medicine, Toronto, Canada, are the recipients of the 2012 ISSCR Public Service Award. The McEwens are recognized for their extraordinary support and advocacy for stem cell research and innovation. In 2011, the McEwen's launched the "Stem Cell City" community and other efforts to promote public awareness of the profound impact of stem cell research in healthcare. As founding members of the ISSCR's Global Advisory Council, the McEwens have provided strategic advice and catalyzed the creation of a global network of contributors engaged in accelerating the translation of basic research into treatments.

Join us for this special award presentation which will take place during the Presidential Symposium on the afternoon of Wednesday, June 13, 2012.

Supported by past and present members of the International Society for Stem Cell Research Board of Directors

Cédric Blanpain, MD, PhD
University of Brussels

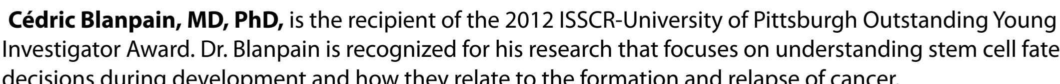
ISSCR-University of Pittsburgh Outstanding Young Investigator Award

Plenary IV:

GENOMICS AND EPIGENOMICS OF STEM CELLS

Friday, 9 a.m.

The ISSCR-University of Pittsburgh Outstanding Young Investigator Award recognizes the exceptional achievements of an investigator in the early part of his or her independent career in stem cell research.

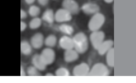


Cédric Blanpain, MD, PhD, is the recipient of the 2012 ISSCR-University of Pittsburgh Outstanding Young Investigator Award. Dr. Blanpain is recognized for his research that focuses on understanding stem cell fate decisions during development and how they relate to the formation and relapse of cancer.

Dr. Blanpain is a tenure researcher of the Belgian FNRS and a WELBIO investigator at University of Brussels (ULB), Belgium where he studies the role of stem cells during development, homeostasis and cancer.

Join us for the 2012 award presentation which will take place during Plenary Session IV on the morning of Friday, June 15.

Supported by the University of Pittsburgh.



Rudolph Jaenisch, MD,
Whitehead Institute for
Biomedical Research, USA

McEwen Award for Innovation

Plenary II:

REGENERATION, ENGRAFTMENT, AND MIGRATION OF STEM CELLS

Wednesday, 4:30 p.m.

The McEwen Award for Innovation, supported by the McEwen Centre for Regenerative Medicine in Toronto, Ontario Canada, recognizes original thinking and ground-breaking research pertaining to stem cells or regenerative medicine that opens new avenues of exploration towards the understanding or treatment of human disease or affliction.

Rudolf Jaenisch, MD, is the recipient of the 2012 McEwen Award for Innovation. He is recognized for his pioneering discoveries in the areas of genetic and epigenetic control of development in mice that directly impact the future potential of embryonic stem cells and induced pluripotent stem cells for therapeutic utility and has made many seminal discoveries in the fields of virology, cancer, epigenetics and regenerative medicine.

Dr. Jaenisch has been a leader in biomedical sciences for more than three decades. Dr. Jaenisch is a Founding Member of the Whitehead Institute for Biomedical Research and Professor of Biology at the Massachusetts Institute of Technology.

Join us for the 2012 award presentation which will take place during the Plenary Session II on the afternoon of Wednesday, June 13.

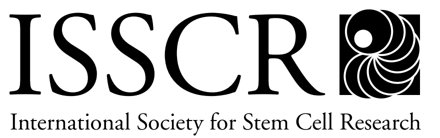
Supported by McEwen Centre for Regenerative Medicine

2012 Conference Series

The ISSCR Conference Series provides an opportunity to interact with local and visiting leaders in stem cell research in a more intimate setting. This year, the ISSCR will collaborate with regional organizations to bring experts from around the world to Brazil and San Francisco to discuss recent research, advances and perspectives.

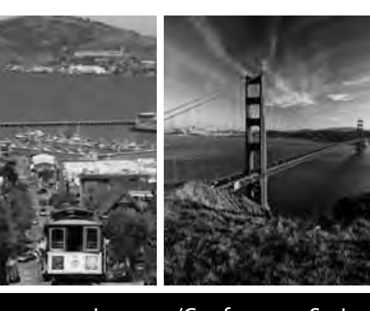
VII Brazilian Congress on Stem Cells and Cell Therapy
Fecomercio Convention Centre, Sao Paulo, Brazil
October 3–6, 2012
Co-Sponsored by ABTCel and ISSCR

www.isscr.org/Conference_Series.htm



Co-Sponsored by the Roddenberry Center for Stem Cell Biology and Medicine at Gladstone and ISSCR

ISSCR - Roddenberry International Symposium 2012 on Cellular Reprogramming
Mission Bay Conference Center at UCSF in San Francisco, California
October 24–25, 2012



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For more information, go to www.isscr.org/Conference_Series.htm

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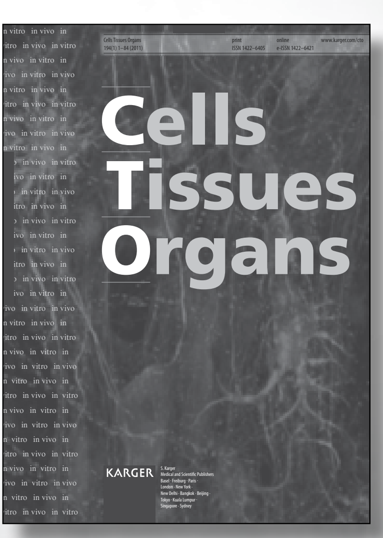
Journal of cell and developmental biology, stem cell research, tissue engineering, and in vitro systems

Cells Tissues Organs



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Cells Tissues Organs aims at bridging the gap between cell biology and developmental biology and the emerging fields of regenerative medicine (stem cell biology, tissue engineering, artificial organs, in vitro systems and transplantation biology). High-quality papers from more traditional fields of experimental biology will also be accepted. CTO offers a rapid and fair peer-review and exquisite reproduction quality. Special topic issues, entire issues of the journal devoted to a single research topic within the range of interests of the journal, are published at irregular intervals.

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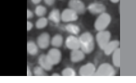

Detailed Schedule at a Glance

Tuesday, June 12

1:00 – 4:00 p.m.	Pre-Meeting Cultural Experience Ikebana (Flower Arranging) & Tea Ceremony	Conf. Center Rm. 413

Wednesday, June 13

8:30 a.m. – 12:30 p.m.	Industry Wednesday Symposia **BD Bioscience** ADVANCED FLOW CYTOMETRIC TECHNOLOGIES FOR ANALYZING AND ISOLATING STEM CELLS DURING STEM CELL DIFFERENTIATION **Thermo Fisher Scientific** IMPROVING THE PRODUCTIVITY OF STEM CELL RESEARCH: INNOVATIVE TOOLS AND PRACTICAL APPLICATIONS **PerkinElmer and Summit Pharmaceuticals International** PRECLINICAL ADVANCES IN STEM CELL RESEARCH- IN VIVO IMAGING ACCELERATING CLINICAL TRANSLATION	Conf. Center Rm. 501 Conf. Center Rm. 502 Conf. Center Rm. 503
8:30 a.m. – 12:30 p.m.	Focus Sessions **ISSCR Ethics and Public Policy Committee** ETHICAL AND POLICY ISSUES IN THE CLINICAL TRANSLATION OF STEM CELLS **Mount Sinai School of Medicine** PROMOTION AND EXPANSION OF PLURIPOTENT STEM CELL RESEARCH	Conf. Center Rm. 301 Conf. Center Rm. 302
12:30 – 1:00 p.m.	Group I Posters Put On Display	Exhibit Halls B & C
1:00 – 3:35 p.m.	**Plenary I** **PRESIDENTIAL SYMPOSIUM – EARLY LIFE DECISION** *Supported by Johnson and Johnson* Chair: **Fred H. Gage,** *Salk Institute for Biological Studies*	National Convention Hall
1:00 – 1:15 p.m.	**Presidential Address**, Fred H. Gage, *ISSCR President*	
1:15 – 1:20 p.m.	JST Welcome	
1:20 – 1:25 p.m.	Mayor of Yokohama, Fumiko Hayashi	
1:25 – 1:35 p.m.	**ISSCR Public Service Award presented to Rob and Cheryl McEwen**	
	Keynote Speakers	
1:35 – 2:05 p.m.	**Rudolf Jaenisch,** *Whitehead Institute for Biomedical Research and Department of Biology, MIT, USA* IPS TECHNOLOGY AND DISEASE RESEARCH	
2:05 – 2:35 p.m.	**Austin G. Smith**, *Wellcome Trust Centre for Stem Cell Research, UK* THE CORE OF EMBRYONIC STEM CELLS	
2:35 – 3:05 p.m.	**John Gurdon**, *The Gurdon Institute, UK* DIRECT REPROGRAMMING OF SOMATIC CELL NUCLEI BY OOCYTES	
3:05 – 3:35 p.m.	**Kazutoshi Takahashi**, *CiRA, Kyoto University, Japan* WHAT IS THE BUG IN THE PROGRAM OF PLURIPOTENCY	
3:00 – 8:00 p.m.	Exhibits Open	Exhibit Halls B & C
3:00 – 8:00 p.m.	Posters Open for Viewing	Exhibit Halls B & C
3:30 – 4:30 p.m.	Refreshment Break *Supported by Beckman Coulter*	Exhibit Halls B & C
4:30 – 7:00 p.m.	**Plenary II** **REGENERATION, ENGRAFTMENT, AND MIGRATION OF STEM CELLS** *Supported by The New York Stem Cell Foundation (NYSCF)* Chair: **David T. Scadden**, *Harvard Stem Cell Institute*	National Convention Hall
4:30 – 4:40 p.m.	**McEwen Award for Innovation**	
4:41 – 5:06 p.m.	**Jane E. Visvader**, *Walter & Eliza Hall Institute of Medical Research, Australia* DELINEATING THE MAMMARY STEM CELL HIERARCHY AND ITS MOLECULAR REGULATORS	
5:07 – 5:32 p.m.	**Yoshiki Sasai**, *Center for Developmental Biology RIKEN, Japan* SELF-ORGANIZATION OF THREE-DIMENSIONAL TISSUE STRUCTURES IN ES CELL CULTURE	
5:33 – 5:58 p.m.	**Elaine Fuchs,** *Howard Hughes Medical Institute, The Rockefeller University, USA* SKIN STEM CELLS IN HOMEOSTASIS, WOUND REPAIR AND CANCER	
5:59 – 6:09 p.m.	**Poster Teasers**	
6:10 – 6:45 p.m.	**Ernest McCulloch Memorial Lecture** **Irving L. Weissman**, *Stanford University School of Medicine, USA* NORMAL AND NEOPLASTIC STEM CELLS	
6:45 – 7:00 p.m.	**Charles Sabine**, *Patient Advocate, UK* WHY WHAT YOU DO MATTERS	
7:00 – 9:00 p.m.	ISSCR 10th Anniversary Reception	Exhibit Halls B & C

SAVE THE DATE

June 12 – 15, 2013

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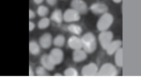

Detailed Schedule at a Glance

Thursday, June 14

Time	Event	Location
8:00 – 9:00 a.m.	Morning Coffee	Marine Lobby
9:00 – 11:25 a.m.	**Plenary III** **LOST IN TRANSLATION: THE DIFFICULT PATH FOR STEM CELLS TO THE CLINIC –** Joint session with Japan Society for Regenerative Medicine (JSRM) *Supported by iPS Academia Japan, Inc.* Chair: **Deepak Srivastava**, *J. David Gladstone Institutes*	National Convention Hall
9:02 – 9:27 a.m.	**Ann Tsukamoto**, *StemCells Inc, USA* CLINICAL TRANSLATION OF HUMAN NEURAL STEM CELLS, HUCNS-SC: WHERE ARE WE TODAY?	
9:28 – 9:53 a.m.	**Katarina Le Blanc**, *Karolinska Institutet, Sweden* MESENCHYMAL STEM CELLS FOR TREATMENT OF GRAFT-VERSUS-HOST DISEASE	
9:54 – 10:19 a.m.	**Masayo Takahashi**, *RIKEN CDB, Japan* TRANSPLANTATION OF IPS CELL-DERIVED RETINAL PIGMENT EPITHELIAL CELLS	
10:20 – 10:45 a.m.	**Jan Helge Solbakk**, *University of Oslo, Norway* THE TRAGEDY OF TRANSLATION: EPISTEMOLOGICAL AND ETHICAL CHALLENGES PERTAINING TO BRINGING STEM CELLS TO THE CLINIC	
10:46 – 11:11 a.m.	**Hideyuki Okano**, *Keio University School of Medicine, Japan* TRANSLATION OF REPROGRAMMING TECHNOLOGIES FOR CNS DISORDERS	
11:13 – 11:25 a.m.	**Poster Teasers**	
11:00 a.m. – 8:00 p.m.	Exhibit Hall Open, Posters Open for Viewing	
11:25 a.m. – 1:30 p.m.	Lunch on your Own	
11:45 a.m. – 1:15 p.m.	Meet the Experts Lunch pre-registration required	Conf. Center Rm. 315
11:45 a.m. – 12:15 p.m.	Innovation Showcases	
	BD Bioscience Christian Carson CELL SURFACE MARKER DISCOVERY USING ANTIBODY LIBRARIES AND HIGH-THROUGHPUT FLOW CYTOMETRY	Conf. Center Rms 501 – 502
	Qiagen Michiyo Koyanagi-Aoi LARGE SCALE ANALYSES OF MOLECULAR SIGNATURES AND BEHAVIORS OF HUMAN PLURIPOTENT STEM CELLS	Conf. Center Rms 301 – 304
	Molecular Devices LLC Oksana Sirenko and Evan F Cromwell PREDICTIVE ASSAYS FOR HIGH THROUGHPUT ASSESSMENT OF DRUG TOXICITY USING IPSC DERIVED CELL MODELS	Conf. Center Rms 411 – 412
	Union Biometrica Rock Pulak LARGE PARTICLE FLOW CYTOMETRY FOR CELL CLUSTERS (EBS, SPHEROIDS) AND MICROCARRIES OF 3D CELL CULTURES	Conf. Center Rm. 503
12:30 – 1:00 p.m.	Innovation Showcases	
	BD Biosciences Nil Emre ISOLATION AND ANALYSIS OF HESCS, HIPSCS AND THEIR ECTODERM AND ENDODERM DERIVATIVES BY FLOW CYTOMETRY	Conf. Center l Rms 501 – 502
	BioLamina Karl Tryggvason LAMININS- EXTRACELLULAR MODULATORS OF STEM CELLS AND CELL LINEAGES	Conf. Center Rms 301 – 304
	Cellular Dynamics International Chris Parker ADVANCEMENT IN IN VITRO MODELING USING IPSC-DERIVED CELLS	Conf. Center. Rms 411 – 412
	Merck Millipore Vi Chu ADVANCES IN REPROGRAMMING AND NEURAL INDUCTION OF HUMAN IPS CELLS	Conf. Center Rm. 503
1:30 – 3:15 p.m.	**Concurrent Session I Track A** **Pluripotent Stem Cells** Co-Chair: **George Q. Daley**, *Children's Hospital Boston, USA* Co-Chair: **Mitinori Saitou**, Kyoto University, Japan	National Convention Hall
1:30 – 1:35 p.m.	**George Q. Daley**, *Children's Hospital Boston, USA* Introduction and overview	
1:35 – 2:00 p.m.	**Mitinori Saitou**, *Kyoto University, Japan* TOWARDS IN VITRO RECONSTITUTION OF MAMMALIAN GERM CELL DEVELOPMENT	
2:00 – 2:15 p.m.	**Takanori Takebe**, *Yokohama City University, Yokohama, Japan* CREATION OF VASCULARIZED HUMAN ORGAN FROM INDUCED PLURIPOTENT STEM CELLS	

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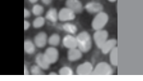

Detailed Schedule at a Glance

Thursday, June 14 (cont.)

Time	Session	Location
2:15 – 2:30 p.m.	**Nadav Sharon**, *Hebrew University, Israel* DUXO, A NOVEL DOUBLE HOMEOBOX TRANSCRIPTION FACTOR, IS REQUIRED FOR GASTRULA ORGANIZER FORMATION IN HUMAN EMBRYONIC STEM CELLS	
2:30 – 2:45 p.m.	**Amar M. Singh**, *University of Georgia, USA* CROSS-TALK OF SIGNALING NETWORKS REGULATE HUMAN STEM CELL PLURIPOTENCY	
2:45 – 3:00 P.M.	**Dieter Egli**, *New York Stem Cell Foundation, USA* REPROGRAMMING HUMAN SOMATIC CELLS TO A PLURIPOTENT STATE USING OOCYTES	
3:00 – 3:15 p.m.	**Bernadett Papp**, *University of California, Los Angeles, USA* OPPOSING WNT SIGNALLING TRANSCRIPTIONAL EFFECTORS ARE CRITICAL FOR REPROGRAMMING TO PLURIPOTENCY	
1:30 – 3:15 p.m.	**Concurrent Session I Track B** **New Technologies for Controlling and Observing Stem Cell Behaviour** Co-Chair: **Derek van der Kooy,** *University of Toronto, Canada* Co-Chair: **Atsushi Miyawaki,** *RIKEN Brain Science Institute, Japan*	Conf. Center Main Hall
1:30 – 1:35 p.m.	**Derek van der Kooy**, *University of Toronto, Canada* Introduction and overview	
1:35 – 2:00 p.m.	**Atsushi Miyawaki**, *RIKEN Brain Science Institute, Japan* A CHEMICAL APPROACH FOR HIGH-RESOLUTION FLUORESCENCE IMAGING AND 3D RECONSTRUCTION OF TRANSPARENT MOUSE BRAIN	
2:00 – 2:15 p.m.	**Eli R. Zunder**, *Stanford University, USA* DYNAMIC PROGRESSION ANALYSIS OF IPS CELL REPROGAMMING AND DIFFERENTIATION BY HIGH-DIMENSIONAL MASS CYTOMETRY	
2:15 – 2:30 p.m.	**Lieven Haenebalcke**, *DMBR, VIB-UGent, Zwijnaarde (Gent), Belgium* GENERATION AND USE OF A ROSA26-BASED CONDITIONAL AND INDUCIBLE (COIN) ES/IPS CELL SYSTEM TO STUDY CELLULAR REPROGRAMMING AND GENE FUNCTION DURING LINEAGE-DIRECTED DIFFERENTIATION	
2:30 – 2:45 p.m.	**Alice N. Yamada**, *Agilent Technologies, USA* CULTIVATING A SINGLE CELL VISUALIZATION AND MANIPULATION WORKFLOW: A COMBINED FLUORESCENCE AND ATOMIC FORCE MICROSCOPY APPROACH	
2:45 – 3:00 p.m.	**Roger S. Lasken**, *J. Craig Venter Institute, USA* GLOBAL GENE EXPRESSION ANALYSIS BY SEQUENCING OF cDNA DERIVED FROM SINGLE NUCLEI	
3:00 – 3:15 p.m.	**Jeroen Krijgsveld**, *EMBL, Germany* A QUANTITATIVE PROTEOMIC PANORAMA OF CELLULAR REPROGRAMMING	
1:30 – 3:15 p.m.	**Concurrent Session I Track C** **Stem Cells and Tissue Engineering** Co-Chair: **Christine Mummery**, *Leiden University, Netherlands* Co-Chair: **Stephen Badylak**, *McGowan Institute for Regenerative Medicine, USA*	Conf. Center Rms 301 - 304
1:30 – 1:35 p.m.	**Christine Mummery**, *Leiden University, Netherlands* Introduction and overview	
1:35 – 2:00 p.m.	**Stephen Badylak**, *McGowan Institute for Regenerative Medicine, USA* FUNCTIONAL TISSUE RECONSTRUCTION: SEED VS. SOIL	
2:00 – 2:15 p.m.	**Ophelia K. Veraitch**, *Keio University School of Medicine, Japan* HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED KERATINOCYTE PRECURSORS CROSSTALK WITH HAIR INDUCTIVE MESENCHYME AND RECONSTITUTE HAIR FOLLICLES IN VIVO	
2:15 – 2:30 p.m.	**Akira Myoui**, *Osaka University Hospital, Japan* A PROSPECTIVE CLINICAL STUDY TO EVALUATE THE SAFETY AND EFFICACY OF AUTOLOGOUS MARROW-DERIVED MESENCHYMAL CELLS INTEGRATED WITH POROUS CERAMICS SCAFFOLD FOR BONE DEFECT AFTER BONE TUMOR REMOVAL	
2:30 – 2:45 p.m.	**Saik Kia Goh**, *University of Pittsburgh, USA* PERFUSION-DECELLULARIZATION OF PANCREAS AS A SCAFFOLD FOR THE DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS INTO INSULIN-PRODUCING CELLS	
2:45 – 3:00 p.m.	**Gershon Finesilver**, *The Hebrew University, Israel* KIDNEY DERIVED MICRO ORGAN MATRICES PROVIDE AN OPTIMIZED ENVIRONMENT TO SUPPORT THE DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS TOWARDS A RENAL LINEAGE	
3:00 – 3:15 p.m.	**Hiroki Saito**, *University of Tokyo, Japan* PRODUCTION OF FUNCTIONAL ISLETS WITH A THREE-DIMENSIONAL STRUCTURE FROM IPS CELLS IN VITRO	
1:30 – 3:15 p.m.	**Concurrent Session I Track D** **Stem Cell Signalling and Niches** Co-Chair: **Elaine Fuchs**, *Rockefeller University, USA* Co-Chair: **Inke Nathke**, *University of Dundee, UK*	Conf. Center Rms 501 - 502

Welcome Letter from Yokohama Mayor

June 13-16, 2012

A Welcome Message from the City Mayor

It is with great pleasure that I welcome you to the City of Yokohama upon this opening of the 10th Annual Meeting of the International Society for Stem Cell Research (ISSCR2012). On behalf of our 3.7 million citizens, I am delighted to have this opportunity to offer a warm greeting to the stem cell researchers who have gathered from over 50 countries around the world.
I am greatly honored that the City of Yokohama was selected to host ISSCR2012, an event which engages the attention and expectations of the world as a platform for discussions on the latest in stem cell research, and I truly hope that researchers will benefit from fruitful discussions on this occasion.

For those who are fortunate to have free time during their stay, the City of Yokohama boasts numerous spots for sightseeing, including the Sankeien Garden, which features valued traditional architecture designated as cultural properties and prides enchanting natural scenery for each season; the world's largest China Town; and the Minato Mirai 21 District where the venue for this meeting is located. I hope that all of you will have enough time to fully enjoy the attractiveness of our city during your stay.

In closing, I would like to express my deepest gratitude for all of the strenuous efforts of the people who made ISSCR2012 possible, and my wishes for the success of this annual meeting and further developments in the field of stem cell research.

横浜市長 林 文子

Fumiko Hayashi
Mayor, City of Yokohama

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1-1 Minato-cho, Naka-ku,
Yokohama 231-0017 Japan

Detailed Schedule at a Glance

Thursday, June 14 (cont.)

1:30 – 1:35 p.m.	**Elaine Fuchs**, *Rockefeller University, USA* Introduction and overview	
1:35 – 2:00 p.m.	**Inke Nathke**, *University of Dundee, UK* GUT EPITHELIAL TISSUE MAINTENANCE IN HEALTH AND DISEASE	
2:00 – 2:15 p.m.	**Salvador Aznar Benitah**, *Center for Genomic Research (CRG), Spain* COMPLEX TIMING OF HUMAN EPIDERMAL STEM CELL FUNCTION BY THE CIRCADIAN MOLECULAR CLOCK	
2:15 – 2:30 p.m.	**Eric Deneault**, *University of Montreal, Canada* A NOVEL OSTEOCLASTIC NETWORK DETERMINES IN VITRO NICHE FOR MOUSE AND HUMAN HEMATOPOIETIC STEM CELLS	
2:30 – 2:45 p.m.	**Ryohichi Sugimura**, *Stowers Institute, USA* INACTIVATION OF FRIZZLED5, MEDIATING NON-CANONICAL WNT SIGNALING, AFFECTS HSC MAINTENANCE IN THE PERIVASCULAR BUT NOT IN THE ENDOSTEAL NICHES	
2:45 – 3:00 p.m.	**Deana Janzen**, *University of California, Los Angeles, USA* ESTROGEN AND PROGESTERONE EXPAND AND REJUVENATE MOUSE ADULT UTERINE EPITHELIAL STEM CELLS	
3:00 – 3:15 p.m.	**Naisana S. Ashli**, *Victor Chang Cardiac Research Institute, Australia* SIGNALING NETWORKS REGULATING ADULT CARDIAC STEM CELLS	
1:30 – 3:15 p.m.	**Concurrent Session I Track E** **Stem Cells, Injury and Regeneration** Co-Chair: **Janet Rossant**, *Hospital for Sick Children, Canada* Co-Chair: **Kiyokazu Agata**, *Kyoto University, Japan*	Conf. Center Rm 503
1:30 – 1:35 p.m.	**Janet Rossant**, *Hospital for Sick Children, Canada* Introduction and overview	
1:35 – 2:00 p.m.	**Kiyokazu Agata**, *Kyoto University, Japan* INSIGHTS INTO THE FUNCTION OF ERK SIGNALING IN PLURIPOTENT STEM CELL SYSTEMS FROM AN EVOLUTIONARY VIEWPOINT	
2:00 – 2:15 p.m.	**Meritxell Huch**, *Hubrecht Institute, Netherlands* UNLIMITED IN VITRO EXPANSION OF ADULT LIVER AND PANCREAS PROGENITORS THROUGH A WNT/LGR5 REGENERATIVE RESPONSE	
2:15 – 2:30 p.m.	**Tohru Itoh**, *University of Tokyo, Japan* CRITICAL ROLE OF FGF7 IN REGULATING MOUSE ADULT LIVER STEM/PROGENITOR CELLS AND REGENERATION IN DAMAGED LIVERS	
2:30 – 2:45 p.m.	**Catherine P. Lu**, *Rockefeller University, USA* DEFINING THE ORIGINS, PROPERTIES AND MOLECULAR CHARACTERISTICS OF SWEAT GLANDS AND THEIR STEM CELLS	
2:45 – 3:00 p.m.	**Christian Goritz**, *Karolinska Institute, Sweden* PERICYTE-DERIVED SCAR FORMATION FOLLOWING LESIONS TO THE CENTRAL NERVOUS SYSTEM	
3:00 – 3:15 p.m.	**Caghan Kizil**, *Center for Regenerative Therapies Dresden (CRTD), Germany* INJURY-INDUCED EXPRESSION OF THE TRANSCRIPTION FACTOR GATA3 IS A PRO-REGENERATIVE CUE IN ADULT VERTEBRATE BRAIN	
3:15 – 4:15 p.m.	Refreshment Break	Exhibit Halls B & C
4:15 – 6:00 p.m.	**Concurrent Session II Track A** **Neural Stem Cells** *Supported by F. Hoffmann - La Roche Ltd.* Co-Chair: **Hideyuki Okano**, *Kieo University School of Medicine, Japan* Co-Chair: **Arnold R. Kriegstein**, *University of California, San Francisco, USA*	National Convention Hall
4:15 – 4:20 p.m.	**Hideyuki Okano**, *Kieo University School of Medicine, Japan* Introduction and overview	
4:20 – 4:45 p.m.	**Arnold R. Kriegstein**, *University of California, San Francisco, USA* NEURAL STEM AND PROGENITOR CELLS IN HUMAN CORTICAL DEVELOPMENT AND EVOLUTION	
4:45 – 5:00 p.m.	**Jovica Ninkovic**, *Helmholtz Zentrum München, Germany* IDENTIFICATION OF THE MINIMAL MOLECULAR NETWORK NECESSARY FOR VOLUNTARY AND FORCED NEUROGENESIS	
5:00 – 5:15 p.m.	**Bilal E. Kerman**, *Salk Institute for Biological Studies, USA* DEVELOPMENT OF AN EMBRYONIC STEM CELL-BASED MYELINATION ASSAY	
5:15 – 5:30 p.m.	**David J. Ryan**, *Wellcome Trust Sanger Institute, United Kingdom* NAIVE SANGER HUMAN INDUCED PLURIPOTENT STEM CELLS (SH-IPS) HAVE NEURAL INDUCTION REQUIREMENTS SIMILAR TO MOUSE EMBRYONIC STEM CELLS	

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Liang Xiaoting
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Lior Zangi
Jiwang Zhang
Sheng Zhong
Yang Zhou
Jianhong Zh

Detailed Schedule at a Glance

Thursday, June 14 (cont.)

5:30 – 5:45 p.m.	**Kunimasa Ohta**, *Kumamoto University, Japan* TSUKUSHI MAINTAINS THE GROWTH AND UNDIFFERENTIATED PROPERTIES OF NEURONAL STEM/PROGENITOR CELLS AS A NICHE MOLECULE	
5:45 – 6:00 p.m.	**Yusuke Kishi**, *University of Tokyo, Japan* GLOBAL REGULATION OF THE CHROMATIN STATE IN MOUSE NEURAL STEM CELLS DURING THE NEOCORTICAL DEVELOPMENT	
4:15 – 6:00 p.m.	**Concurrent Session II Track B** **Cell Fate Conversion** Co-Chair: **Hiromitsu Nakauchi,** *University of Tokyo, Japan* Co-Chair: **Gerald R. Crabtree,** *Stanford University, USA*	Conf. Center Main Hall
4:15 – 4:20 p.m.	**Hiromitsu Nakauchi**, *University of Tokyo, Japan* Introduction and overview	
4:20 – 4:45 p.m.	**Gerald R. Crabtree**, *Stanford University, USA* CONVERTING FIBROBLASTS TO NEURONS WITH A MICRO-RNA CHROMATIN SWITCH	
4:45 – 5:00 p.m.	**Esther Y. Sun**, *Harvard University, USA* GENERATION OF PATIENT-SPECIFIC MOTOR NEURONS FROM FIBROBLASTS BY DEFINED FACTORS	
5:00 – 5:15 p.m.	**Ernesto Lujan**, *Stanford University, USA* DIRECT CONVERSION OF MOUSE FIBROBLASTS TO SELF-RENEWING, TRIPOTENT NEURAL PRECURSOR CELLS	
5:15 – 5:30 p.m.	**Pengyu Huang**, *Chinese Academies for Sciences, China* INDUCTION OF FUNCTIONAL HEPATOCYTES FROM FIBROBLASTS BY DEFINED FACTORS	
5:30 – 5:45 p.m.	**Masato Fujioka**, *Keio University School of Medicine, Japan* IN VIVO TRANSDIFFERENTIATION OF SOX2 EXPRESSING COCHLEAR SUPPORTING CELLS TO AUDITORY HAIR CELLS BY GAMMA SECRETASE INHIBITOR CONTRIBUTES TO HEARING RECOVERY IN NOISE INDUCED DAMAGED MICE	
5:45 – 6:00 P.M.	**Andrew C. Perkins**, *University of Queensland, Australia* A LONG NON-CODING RNA ANTISENSE TO EVX1 IS NECESSARY AND SUFFICIENT FOR MESODERM GENERATION	
4:15 – 6:00 p.m.	**Concurrent Session II Track C** **Modeling Human Disease** *Supported by F. Hoffmann - La Roche Ltd.* Co-Chair: **Alan Colman**, *Singapore Stem Cell Consortium, Singapore* Co-Chair: **Zhong Zhong**, *GlaxoSmithKline, China*	Conf. Center Rms 301 - 304
4:15 – 4:20 p.m.	**Alan Colman**, *Singapore Stem Cell Consortium, Singapore* Introduction and overview	
4:20 – 4:45 p.m.	**Zhong Zhong**, *GlaxoSmithKline R&D, China* MODELING HUMAN NEURODEGENERATION IN HES/IPS DERIVED NEURONS	
4:45 – 5:00 p.m.	**Steve S.W. Han**, *Massachusetts General Hospital, USA* MISLOCALIZATION OF FUS/TLS IN SPINAL MOTOR NEURONS DERIVED FROM ALS PATIENT-SPECIFIC INDUCED-PLURIPOTENT STEM CELLS	
5:00 – 5:15 p.m.	**Muotri Alysson**, *University of California, San Diego, USA* GLUTAMATE INTAKE UNBALANCE IN RETT SYNDROME NEURAL CELLS	
5:15 – 5:30 p.m.	**Luis F.Z. Batista**, *Stanford University, USA* CONSEQUENCES OF DYSFUNCTIONAL TELOMERE HOMEOSTASIS IN HUMAN PLURIPOTENT CELLS	
5:30 – 5:45 p.m.	**Younkyoung Lee**, *Harvard University, USA* ANALYSIS OF PRIMARY ADIPOCYTE DISORDER: USING HUMAN PLURIPOTENT STEM CELL-DERIVED ADIPOCYTES TO STUDY LIPODYSTROPHIES	
5:45 – 6:00 p.m.	**Asif M. Maroof**, *Sloan-Kettering Institute for Cancer Research* SELECTIVE INDUCTION OF DISTINCT NKX2.1+ NEURONAL POPULATIONS FROM HUMAN PLURIPOTENT STEM CELLS WHOSE PATTERNING DEPENDS ON TIMING OF SHH EXPOSURE	
4:15 – 6:00 p.m.	**Concurrent Session II Track D** **Pluripotent Stem Cells II** *Supported by F. Hoffmann - La Roche Ltd.* Co-Chair: **Kathrin Plath**, *University of California, Los Angeles, USA* Co-Chair: **Kevin Eggan**, *Harvard University, USA*	Conf. Center Rms 501 - 502
4:15 – 4:20 p.m.	**Kathrin Plath**, *University of California, Los Angeles, USA* Introduction and overview	
4:20 – 4:45 p.m.	**Kevin C. Eggan,** *Harvard University, USA* EROSION OF DOSAGE COMPENSATION IMPACTS HUMAN IPSC DISEASE MODELING	
4:45 – 5:00 p.m.	**Yohei Hayashi**, *J. David Gladstone Institutes, USA* BMP-SMAD-ID AXIS PROMOTES REPROGRAMMING TO PLURIPOTENCY	

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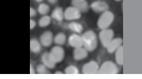

Detailed Schedule at a Glance

Thursday, June 14 (cont.)

5:00 – 5:15 p.m.	**Melissa L. Wilbert**, *University of California, San Diego, USA* LIN28 INTERACTS WITH DISCRETE BINDING MOTIFS IN MESSENGER RNA AND REGULATES ALTERNATIVE SPLICING THROUGH MODULATION OF SPLICING FACTORS	
5:15 – 5:30 p.m.	**Ricardo A. Rossello**, *Duke University, USA* ELUSIVE NON-MAMMALIAN TRANSGENICS: IN-VIVO PLURIPOTENCY IN NON-MAMMALIAN IPS-LIKE CELLS, INDUCED BY MAMMALIAN GENES	
5:30 – 5:45 p.m.	**Kento Onishi**, *University of Toronto, Canada* ENGINEERING LOCAL MICROENVIRONMENTS RE-ACTIVATES DOWNREGULATED SIGNALING PATHWAYS, LEADING TO THE IDENTIFICATION AND ISOLATION OF NAIVE MOUSE AND HUMAN PLURIPOTENT STEM CELLS	
5:45 – 6:00 p.m.	**Robert L. Judson**, *University of California, San Francisco, USA* MICRORNA-BASED DETECTION OF MRNA NETWORKS THAT REGULATE DE-DIFFERENTIATION TO INDUCED PLURIPOTENT STEM CELLS	
4:15 – 6:00 p.m.	**Concurrent Session II Track E** **Stem Cell Aging and Metabolism** Co-Chair: **Sean Morrison**, *Children's Research Institute at UT Southwestern, USA* Co-Chair: **Thomas A. Rando**, *Stanford University, USA*	Conf. Center Rm 503
4:15 – 4:20 p.m.	**Sean Morrison**, *Children's Research Institute at UT Southwestern, USA* Introduction and overview	
4:20 – 4:45 p.m.	**Thomas A. Rando**, *Stanford University, USA* EPIGENETIC MECHANISMS OF STEM CELL AGING AND REJUVENATION	
4:45 – 5:00 p.m.	**Gen Shinoda**, *Children's Hospital Boston, USA* REGULATION OF ORGANISMAL GROWTH AND METABOLISM BY THE STEM CELL FACTOR LIN28	
5:00 – 5:15 p.m.	**Daisuke Nakada**, *Baylor College of Medicine, USA* LKB1-DEPENDENT METABOLIC REPROGRAMMING OF MOUSE HEMATOPOIETIC STEM CELLS DURING DEVELOPMENT	
5:15 – 5:30 p.m.	**Christos Gekas**, *Centre for Genomic Regulation (CRG), Spain* INTEGRIN AIIB (CD41) IDENTIFIES A MYELOID-BIASED MOUSE HEMATOPOIETIC STEM CELL SUBSET THAT ACCUMULATES WITH AGE	
5:30 – 5:45 p.m.	**Keiyo Takubo**, *Keio University School of Medicine* PDK PLAYS A CRITICAL ROLE IN MAINTAINING QUIESCENCE OF HEMATOPOIETIC STEM CELLS THROUGH GLYCOLYTIC METABOLIC PROGRAM	
5:45 – 6:00 p.m.	**Maria Carolina Florian**, *University of Ulm, Germany* CDC42 ACTIVITY REGULATES HEMATOPOIETIC STEM CELL AGING AND REJUVENATION	
6:00 – 8:00 p.m.	Group 1 Poster Presentation and Exhibit Reception	Exhibit Halls B & C
8:00 p.m.	Group 1 Posters dismantle, Group II Poster Put on Display	
9:00 – Midnight	Junior Investigator Social Event *Supported by Stemgent*	

Friday, June 15

8:00 – 9:00 a.m.	Morning coffee	Marine Lobby
9:00 – 11:30 a.m.	**Plenary IV** **GENOMICS AND EPIGENOMICS OF STEM CELLS** *Supported by Fluidigm Corporation* Chair: **Sir Ian Wilmut**, *University of Edinburgh, United Kingdom*	National Convention Hall
9:00 – 9:25 a.m.	**ISSCR Business Meeting**	
9:25 – 9:50 a.m.	**Bing Ren**, *University of California, San Diego, USA* CHARTING THE MAMMALIAN EPIGENOME	
9:50 – 10:15 a.m.	**Anjana Rao,** *La Jolla Institute for Allergy & Immunology, USA* TET PROTEINS AND 5-METHYLCYTOSINE OXIDATION	
10:15 – 10:40 a.m.	**Huck-Hui Ng**, *National University of Singapore, Singapore* SYSTEMS BIOLOGY OF STEM CELLS	
10:40 – 10:55 a.m.	**Poster Teasers**	
10:55 – 11:30 a.m.	**Outstanding Young Investigator Award Winner** **Cedric Blanpain**, *IRIBHM, Université Libre de Bruxelles, Belgium* STEM CELLS DURING EPITHELIAL HOMEOSTASIS AND CANCER INITIATION	
11:00 a.m. – 8:00 p.m.	Exhibit Hall Open	
11:30 a.m.	Group 2 Posters put on display	
11:30 a.m. – 1:30 p.m.	Lunch on your Own	

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Detailed Schedule at a Glance

Friday, June 15 (cont.)

Time	Event	Location
11:45 a.m. – 1:15 p.m.	Meet the Experts Lunch, pre-registration required	Conf. Center Rm. 315
11:45 a.m. – 12:15 p.m.	Innovation Showcases	
	BD Biosciences Marshall Kosovsky A NOVEL ECM MIMETIC SURFACE FOR STEM CELL EXPANSION: ANALYSIS OF HUMAN MESENCHYMAL STEM CELLS UNDER XENO-FREE CONDITIONS	Conf. Center Rms. 501 – 502
	STEMCELL Technologies Michael Riedel EFFICIENT DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO DEFINITIVE ENDODERM USING THE FULLY DEFINED, XENO-FREE STEMDIFF™ DEFINITIVE ENDODERM KIT	Conf. Center Rms. 301 – 304
	Life Technologies Mark Tomoshima LEAVING NOTHING TO CHANCE: INCREASING PRECISION IN MAKING AND MODIFYING IPSCS	Conf. Center Rms. 411 – 412
	Miltenyi Biotec GmbH Sebastian Knöbel STREAMLINING THE IPS CELL WORKFLOW FROM REPROGRAMMING TO DIFFERENTIATION	Conf. Center Rm. 503
12:30 – 1:00 p.m.	Innovation Showcases	
	BD Biosciences Bob E. Newman ISOLATION AND EXPANSION OF HUMAN BONE MARROW-DERIVED MESENCHYMAL STEM CELLS AND HUMAN ADIPOSE-DERIVED STEM CELLS IN A SERUM-FREE MEDIUM	Conf. Center Rms. 501 – 502
	Cellectis Bioresearch Inga Gerard TALENS®: THE NEXT GENERATION GENE CUSTOMIZATION TOOLS FOR AFFORDABLE AND PRECISE REPROGRAMMING OF PLURIPOTENT STEM CELLS	Conf. Center Rms. 301 – 304
	Life Technologies Nirupama (Rupa) Shevde EVOLUTION OF IPSC CULTURE MEDIUM	Conf. Center Rms. 411 – 412
	Miltenyi Biotec GmbH Stefan Miltenyi CELLULAR THERAPY: MANY TALK ABOUT IT - WE ENABLE IT	Conf. Center Rm. 503
1:30 – 3:15 p.m.	**Concurrent Session III Track A** **Hematopoietic Stem Cells** Co-Chair: **Leonard I. Zon**, *Children's Hospital Boston, USA* Co-Chair: **Toshio Suda**, *Keio University, Japan*	National Convention Hall
1:30 – 1:35 p.m.	**Leonard I. Zon**, *Children's Hospital Boston, USA* Introduction and overview	
1:35 – 2:00 p.m.	**Toshio Suda,** *Keio University School of Medicine, Japan* GLYCOLYTIC METABOLISM IN HEMATOPOIETIC STEM CELLS	
2:00 – 2:15 p.m.	**Huafeng Xie**, *Dana-Farber Cancer Institute, USA* POLYCOMB REPRESSIVE COMPLEX 2 IS ESSENTIAL FOR THE MAINTENANCE AND DIFFERENTIATION OF MOUSE HEMATOPOIETIC STEM CELLS	
2:15 – 2:30 p.m.	**Louise N. Winteringham**, *University of Western Australia, Australia* MYELOID LEUKEMIA FACTOR 1 AFFECTS HEMOPOIETIC STEM CELL DEVELOPMENT	
2:30 – 2:45 p.m.	**Katie L. Kathrein**, *Children's Hospital Boston, USA* A LARGE-SCALE REVERSE GENETIC SCREEN IN ZEBRAFISH IDENTIFIES THE CHROMATIN FACTORS REQUIRED FOR HEMATOPOIETIC STEM CELL SPECIFICATION	
2:45 – 3:00 p.m.	**Sho-ichi Hirose**, *Terumo Corporation, Japan* POTENTIAL APPLICATION OF AN IMMORTALIZED ERYTHROCYTE-PRODUCING CELL LINE DERIVED FROM HUMAN PLURIPOTENT STEM CELLS	
3:00 – 3:15 p.m.	**Polynikis Kaimakis**, *Erasmus MC Erasmus Stem Cell Institute, Netherlands* PROGRAMMING THE ENDOTHELIAL-TO-HEMATOPOIETIC STEM CELL TRANSITION IN LY6A AORTIC CELLS DURING DEVELOPMENT OF THE MOUSE HEMATOPOIETIC SYSTEM	
1:30 – 3:15 p.m.	**Concurrent Session III Track B** **Epigenetics of Stem Cells** Co-Chair: **Fiona M. Watt**, *Cancer Research UK, Cambridge Research Institute, UK* Co-Chair: **Toshie Kai**, *Temasek SG, Singapore*	Conf. Center Main Hall

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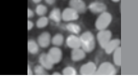

Detailed Schedule at a Glance

Friday, June 15 (cont.)

1:30 – 1:35 p.m.	**Fiona M. Watt**, *Cancer Research UK, Cambridge Research Institute, UK* Introduction and overview	
1:35 – 2:00 p.m.	**Toshie Kai,** *Temasek Life Sciences Laboratory and Dept of Biological Sciences, National University of Singapore, Singapore* TUDOR DOMAIN PROTEINS AND PIWIS FUNCTION IN NUAGE FOR PIRNA AMPLIFICATION IN STEM CELLS AND GERMLINE CELLS	
2:00 – 2:15 p.m.	**Tahsin Stefan Barakat**, *Erasmus MC, University Medical Center Rotterdam, Netherlands* RNF12 ACTIVATES XIST AND IS ESSENTIAL FOR X INACTIVATION IN FEMALE MOUSE EMBRYONIC STEM CELLS	
2:15 – 2:30 p.m.	**Sheng Zhong**, *University of Illinois at Champaign-Urbana, USA* COMPARATIVE EPIGENOMICS	
2:30 – 2:45 p.m.	**Alexander Meissner**, *SCRB, Harvard University, USA* DNA METHYLATION DYNAMICS IN STEM CELLS AND DEVELOPMENT	
2:45 – 3:00 p.m.	**Montserrat Anguera**, *Massachusetts General Hospital, USA* MOLECULAR SIGNATURES OF HUMAN INDUCED PLURIPOTENT STEM CELLS HIGHLIGHT SEX DIFFERENCES AND CANCER GENES	
3:00 – 3:15 p.m.	**Naoki Hattori**, *National Cancer Center Research Institute, Japan* DEVELOPMENT OF A NOVEL TECHNIQUE TO VISUALIZE A COMBINATION OF HISTONE MODIFICATIONS	
1:30 – 3:15 p.m.	**Concurrent Session III Track C** **Stem Cell Therapies (including mesenchymal)** *Supported by International Society for Cellular Therapy (ISCT)* Co-Chair: **Kurt C. Gunter**, *ISCT President* Co-Chair: **Pete Coffey**, *University College London, UK*	Conf. Center Rms. 301 - 304
1:30 – 1:35 p.m.	**Kurt C. Gunter**, *ISCT President* Introduction and over\view	
1:35 – 2:00 p.m.	**Pete Coffey**, *University College London, UK and NRI, UCSB, USA* STEM CELL THERAPIES FOR AGE-RELATED MACULAR DEGENERATION: THE CHALLENGES AHEAD	
2:00 – 2:15 p.m.	**Francesco Saverio Tedesco**, *University College London, United Kingdom* RE-ESTABLISHMENT AND GENETIC CORRECTION OF STEM/PROGENITOR CELLS FROM MUSCULAR DYSTROPHIES VIA REPROGRAMMING OF AUTOLOGOUS CELLS	
2:15 – 2:30 p.m.	**Tetsuya Nakamura**, *Tokyo Medical and Dental University, Japan* FUNCTIONAL ENGRAFTMENT OF COLON EPITHELIUM EXPANDED IN VITRO FROM A SINGLE ADULT LGR5+ STEM CELL	
2:30 – 2:45 p.m.	**Astrid G. Limb**, *University College London, UK* HUMAN MÜLLER STEM CELLS CAN BE INDUCED TO DIFFERENTIATE INTO RETINAL NEURAL PHENOTYPES IN VITRO AND EXERT FUNCTIONALITY IN VIVO UPON TRANSPLANTATION	
2:45 – 3:00 p.m.	**Christopher L. Smith**, *Johns Hopkins University, USA* IN VITRO MODELS OF ENHANCED HUMAN MESENCHYMAL STEM CELL HOMING TO BRAIN TUMORS	
3:00 – 3:15 p.m.	**Barbara von Tigerstrom**, *University of Saskatchewan, Canada* STEM CELL-BASED THERAPIES - PRODUCT OR PRACTICE?	
1:30 – 3:15 p.m.	**Concurrent Session III Track D** **Growth Factors Controlling Stem Cell Behaviour** Co-Chair: **Hans Clevers**, *Hubrecht Institute, Netherlands* Co-Chair: **Alexandra L. Joyner**, *Memorial Sloan-Kettering Cancer Center and Weill Cornell Graduate School of Medicine, USA*	Conf. Center Rms. 501 - 502
1:30 – 1:35 p.m.	**Hans Clevers**, *Hubrecht Institute, Netherlands* Introduction and overview	
1:35 – 2:00 p.m.	**Alexandra L. Joyner**, *Memorial Sloan-Kettering Cancer Center and Weill Cornell Graduate School of Medicine, USA* MULTIPLE ROLES FOR HEDGEHOG-GLI SIGNALING IN DIVERSE ADULT STEM CELL POPULATIONS	
2:00 – 2:15 p.m.	**Lily T. Cho**, *University of Cambridge, UK* CONVERSION FROM MOUSE EMBRYONIC TO EXTRAEMBRYONIC STEM CELLS REVEALS ROLES FOR FGF SIGNALLING AND PRIMITIVE ENDODERM TRANSCRIPTION FACTORS	
2:15 – 2:30 p.m.	**Alan C. Mullen**, *Whitehead Institute for Biomedical Research, USA* COORDINATED CONTROL OF CODING AND LONG NON-CODING RNA DURING ENDODERMAL DIFFERENTIATION	
2:30 – 2:45 p.m.	**Brendan A.S. McIntyre**, *McMaster Stem Cell and Cancer Research Institute, Canada* GLI3 MEDIATED HEDGEHOG INHIBITION AUGMENTS AND CONTROLS ADULT VERSUS EMBRYONIC HEMATOPOIETIC PROGRAMMING OF HUMAN STEM CELLS	

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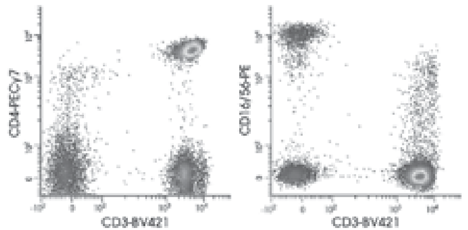
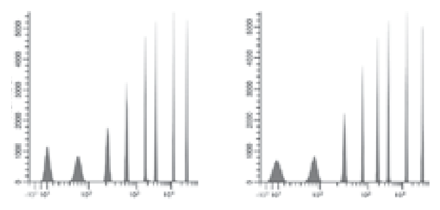


Resolution of immunophenotyping subsets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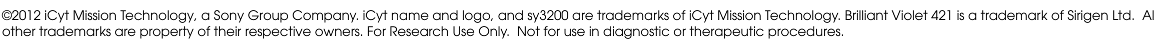

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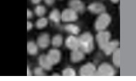

Detailed Schedule at a Glance

Friday, June 15 (cont.)

Time	Session	Location
2:45 – 3:00 p.m.	**Xuetao Pei**, *Beijing Institute of Transfusion Medicine, China* MESENCHYMAL STEM CELLS FROM PRIMARY BREAST CANCER TISSUE PROMOTE CANCER PROLIFERATION AND ENHANCE MAMMOSPHERE FORMATION	
3:00 – 3:15 p.m.	**Bruna Corradetti**, *Università Politecnica delle Marche, Italy* MOUSE EMBRYONIC STEM CELL RENEWAL BY AFFINITY TARGETED PARACRINE STIMULATION	
1:30 – 3:15 p.m.	**Concurrent Session III Track E** **Genomic Integrity** Co-Chair: **Ihor R. Lemischka**, *Black Family Stem Cell Institute, USA* Co-Chair: **Kun Zhang**, *University of California, San Diego, USA*	Conf. Center Rm. 503
1:30 – 1:35 p.m.	**Ihor R. Lemischka**, *Black Family Stem Cell Institute, USA* Introduction and overview	
1:35 – 2:00 p.m.	**Kun Zhang**, *University of California, San Diego, USA* GENETIC AND EPIGENETIC INTEGRITY OF REPROGRAMMED HUMAN PLURIPOTENT STEM CELLS	
2:00 – 2:15 p.m.	**Linzhao Cheng**, *Johns Hopkins University, USA* WHOLE GENOME VIEW OF DNA SEQUENCE VARIATIONS IN HUMAN IPS CELL LINES GENERATED WITH NON-INTEGRATING PLASMID VECTOR EXPRESSION	
2:15 – 2:30 p.m.	**Uri Ben-David**, *The Hebrew University, Israel* HIGH PREVALENCE OF EVOLUTIONARILY-CONSERVED AND SPECIES-SPECIFIC GENOMIC ABERRATIONS IN MOUSE, RHESUS AND HUMAN PLURIPOTENT STEM CELLS	
2:30 – 2:45 p.m.	**Jill M. Dowen**, *Whitehead Institute for Biomedical Research, USA* CONTROL OF MOUSE EMBRYONIC STEM CELL STATE BY CHROMOSOME MAINTENANCE PROTEINS	
2:45 – 3:00 p.m.	**XinQuan Ge**, *Yale University School of Medicine, USA* THE ROLE OF DORMANT REPLICATION ORIGINS IN MAINTAINING MOUSE EMBRYONIC STEM CELL GENOME STABILITY DURING SELF-RENEWAL AND DIFFERENTIATION	
3:00 – 3:15 p.m.	**Panagiota A Sotiropoulou**, *Université Libre de Bruxelles, Belgium* CRITICAL ROLE OF BRCA1 DURING THE SPECIFICATION OF PROSPECTIVE MOUSE ADULT HAIR FOLLICLE STEM CELLS	
3:15 – 4:00 p.m.	Refreshment Break	Exhibit Halls B & C
4:00 – 6:00 p.m.	**Plenary V** **TISSUE ENGINEERING** Chair: **Daniel R. Marshak**, *PerkinElmer, Inc.*	National Convention Hall
4:01 – 4:26 p.m.	**Sangeeta N. Bhatia,** *Massachusetts Institute of Technology, USA* BUILDING HEPATIC TISSUES FOR DISEASE MODELING AND THERAPY	
4:27 – 4:52 p.m.	**Hiromitsu Nakauchi,** *University of Tokyo, Japan* GENERATION OF FUNCTIONAL ORGANS FROM PLURIPOTENT STEM CELLS: TOWARD THE NEXT GENERATION OF REGENERATIVE MEDICINE	
4:53 – 5:18 p.m.	**Kristi S. Anseth**, *University of Colorado at Boulder, USA* SYNTHETIC HYDROGELS AS DYNAMICALLY TUNABLE STEM CELL CULTURE NICHES	
5:19 – 5:44 p.m.	**Teruo Okano**, *Tokyo Women's Medical University, Japan* CELL SHEET ENGINEERING: CURRENT CLINICAL APPLICATION AND PREPARATION OF 3D TISSUES	
5:45 – 6:00 p.m.	**Poster Teasers**	
6:00 – 8:00 p.m.	Group 2 Poster Presentation and Exhibit Reception	Exhibit Halls B & C

Saturday, June 16

Time	Session	Location
8:00 – 9:00 a.m.	Morning Coffee	Marine Lobby
9:00 – 11:15 a.m.	**Plenary VI** **RNA CONTROL OF STEM CELL BEHAVIOR** Chair: **Haifan Lin**, *Yale University School of Medicine*	National Convention Hall
9:02 – 9:27 a.m.	**John Rinn,** *Broad Institute of MIT and Harvard University, USA* LINKING RNA TO HUMAN HEALTH AND DISEASE	
9:29 – 9:54 a.m.	**Narry Kim**, *Seoul National University, Korea* LIN28 SHAPES THE TRANSLATIONAL LANDSCAPE IN EMBRYONIC STEM CELLS	
9:56 – 10:21 a.m.	**Yumiko Saga**, *National Institute of Genetics, Japan* NANOS2-MEDIATED RNA REGULATION IN GERM CELL DIFFERENTIATION	
10:23 – 10:48 a.m.	**Kevin Struhl, BCMP,** *Harvard Medical School, USA* TRANSCRIPTIONAL REGULATORY CIRCUITS INVOLVED IN THE DYNAMIC EQUILIBRIUM BETWEEN CANCER STEM CELLS AND THEIR NON-STEM CANCER CELL COUNTERPARTS	
10:50 – 11:15 a.m.	**Robert Martienssen**, *Cold Spring Harbor Laboratory, USA* INHERITANCE AND REPROGRAMMING OF HETEROCHROMATIN WITH SMALL RNA	
11:00 a.m. – 4:00 p.m.	Exhibit Hall Open, Posters Open for Viewing	

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The ISSCR is a global trans-disciplinary science-based organization dedicated to stem cell research. The ISSCR Annual Meeting has become the premier international forum for the presentation of stem cell science. Founded in 2002, the society brings together investigators researching stem cells in many different organ systems and models. The ISSCR seeks to:

- Promote and foster the exchange and dissemination of information and ideas relating to stem cells
- Encourage the general field of research involving stem cells
- Promote professional and public education in the areas of stem cell research and applications

ISSCR membership is open to stem cell and regenerative medicine investigators and clinicians in all environments including universities, medical schools, government agencies, for-profit and not-for-profit research laboratories and industries worldwide. ISSCR membership has grown to more than 3,800 in just nine years and includes the world's leading stem cell scientists.

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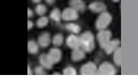

Detailed Schedule at a Glance

Saturday, June 16 (cont.)

Time	Event	Location
11:30 a.m. – 1:30 p.m.	Lunch on your Own	
11:45 a.m. – 1:15 p.m.	JI Career Panel Lunch *Supported by Stemgent* STRATEGIES FOR SUCCESS: FORGING SEAMLESS TRANSITIONS BETWEEN ACADEMICS AND INDUSTRY	Conf. Center Rm. 315
11:45 a.m. – 12:15 p.m.	Innovation Showcases	
	Fluidigm Brittnee Jones and Reef Hardy NOVEL DISCOVERIES IN STEM CELL BIOLOGY THROUGH SINGLE-CELL GENOMIC APPLICATIONS: PART I	Conf. Center Rms. 411 – 412
	Nikon Corporation Lee Rubin LONG-TERM CELL CULTURE OBSERVATION SYSTEM AND ADVANCED IMAGE ANALYSIS TECHNOLOGY	Conf. Center Rms. 301 – 304
	Biological Industries Israel Beit Haemek Ltd. David Fiorentini ADVANCED SERUM-FREE, XENO-FREE CULTURE SYSTEM FOR HUMAN MESENCHYMAL STEM CELLS	Conf. Center Rms. 501 – 502
	STEMCELL Technologies Jennifer Antonchuk STEMdiff™ CARDIOMYOCYTE KIT FOR DIFFERENTIATION OF HUMAN IPSCS TO THE CARDIOMYOCYTE LINEAGE	Conf. Center Rm. 503
12:30 – 1:00 p.m.	Innovation Showcases	
	Fluidigm Corporation Brittnee Jones and Reef Hardy NOVEL DISCOVERIES IN STEM CELL BIOLOGY THROUGH SINGLE-CELL GENOMIC APPLICATIONS: PART II	Conf. Center Rooms 411-412
	R&D Systems Joy Aho DEFINING STEM CELL POPULATIONS FOR EXPERIMENTAL SUCCESS: CHARACTERIZATION AND SELECTION *IN VITRO*	Conf. Center Rms. 501 – 502
	Stemgent Brad Hamilton mRNA FOR INTEGRATION-FREE CELL FATE MANIPULATION	Conf. Center Rm. 503
	Corning, Inc. Julien Maruotti CORNING® SYNTHEMAX® SURFACE: A NOVEL SYNTHETIC, XENO-FREE REAGENT FOR STEM CELL CULTURE IN FEEDER-FREE, SERUM-FREE ENVIRONMENT	Conf. Center Rooms 301-304
1:30 – 3:15 p.m.	**Concurrent Session IV Track A** **Stem Cells in Organ Development** Co-Chair: **Sally Temple**, *Neural Stem Cell Institute, USA* Co-Chair: **Kenneth D. Poss**, *Duke University, USA*	National Convention Hall
1:30 – 1:35 p.m.	**Sally Temple**, *Neural Stem Cell Institute, USA* Introduction and overview	
1:35 – 2:00 p.m.	**Kenneth D. Poss**, *Duke University, USA* TISSUE SPECIFIC TRANSLATIONAL PROFILING DURING ZEBRAFISH HEART REGENERATION	
2:00 – 2:15 p.m.	**Yunhua Zhu**, *ICMB, Singapore* APOPTOSIS-DEPENDENT LINEAGE TRACING FROM CRYPT BASE COLUMNAR CELLS IN THE MOUSE INTESTINE	
2:15 – 2:30 p.m.	**Masaya Ueno**, *University of California, Los Angeles, USA* HGF/C-MET SIGNALING PATHWAY GOVERNS SYNCYTIOTROPHOBLAST STEM CELL MAINTENANCE AND CELL POLARITY DURING PLACENTAL DEVELOPMENT	
2:30 – 2:45 p.m.	**Kenshiro Hara**, *National Institute of Basic Biology, Japan* DIRECT OBSERVATION OF THE BEHAVIORS OF UNDIFFERENTIATED SPERMATOGONIA IN MOUSE TESTIS	
2:45 – 3:00 p.m.	**Pamela G. Robey**, *NIDCR, NIH, DHHS, USA* SECRETED FRIZZLED RELATED PROTEIN 2 MEDIATES FORMATION OF BONE AND THE HEMATOPOIETIC MICROENVIRONMENT BY MULTIPOTENT BONE MARROW STROMAL CELLS (SKELETAL STEM CELLS)	
3:00 – 3:15 p.m.	**Samantha S. Hodgson**, *University of Queensland, Australia* A MOUSE MODEL FOR IN VIVO QUANTITATIVE MONITORING OF WNT/B-CATENIN SIGNALLING IN SKIN REVEALS HAIR CYCLE DYNAMICS IN SOX18 MUTANT MICE	

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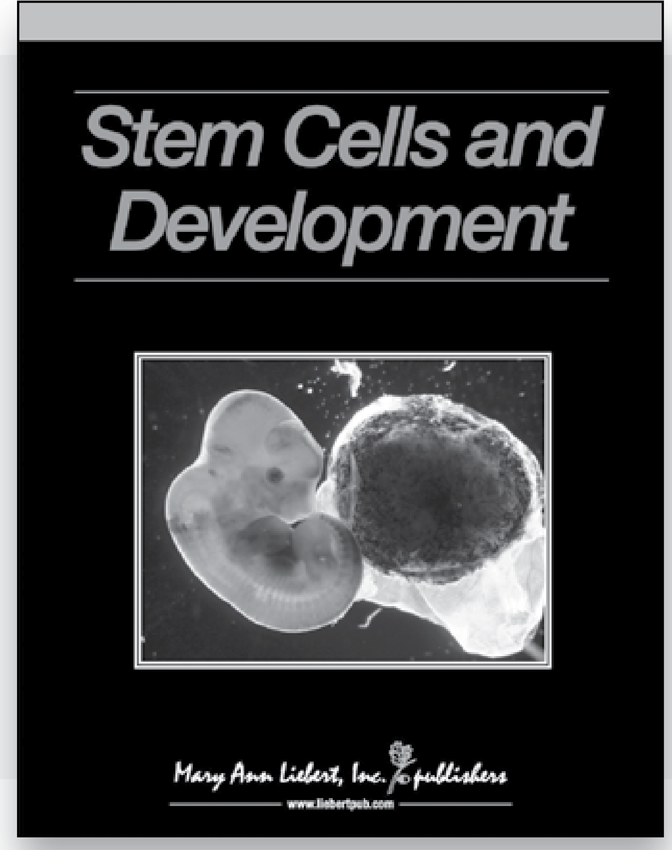

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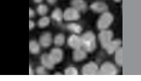

Detailed Schedule at a Glance

Saturday, June 16 (cont.)

Time	Session	Location
1:30 – 3:15 p.m.	**Concurrent Session IV Track B** **Self-Renewal Mechanisms** Co-Chair: **Thomas Graf,** *Center for Genomic Regulation, Spain* Co-Chair: **Ian Chambers,** *University of Edinburgh***,** *UK*	Conf. Center Main Hall
1:30 – 1:35 p.m.	**Thomas Graf**, *Center for Genomic Regulation, Spain* Introduction and overview	
1:35 – 2:00 p.m.	**Ian Chambers**, *University of Edinburgh, UK* TRANSCRIPTION FACTOR CONTROL OF TRANSITIONS IN PLURIPOTENT CELL STATES	
2:00 – 2:15 p.m.	**Ting Chen**, *Rockefeller University, USA* AN RNAI SCREEN UNVEILS A NEW PLAYER IN MOUSE SKIN STEM CELL SELF-RENEWAL AND LONG-TERM REGENERATION IN VIVO	
2:15 – 2:30 p.m.	**Michael H. Sieweke**, *Centre d'Immunologie de Marseille Luminy (CIML), France* ACTIVATION OF UNLIMITED SELF RENEWAL ON A CELL TYPE SPECIFIC ENHANCER PLATFORM	
2:30 – 2:45 p.m.	**Chih-Hong Lou**, *University of California, San Diego, USA* THE RNA DECAY PATHWAY, NMD, PROMOTES THE STEM-LIKE STATE	
2:45 – 3:00 p.m.	**Christopher P. Arnold**, *Stanford University, USA* MIR-181A MEDIATES THE CONTROL OF MURINE EMBRYONIC STEM CELL SELF RENEWAL	
3:00 – 3:15 p.m.	**Jean Lu**, *Academia Sinica, Taiwan* NME6 AND NME7 IS ESSENTIAL FOR MOUSE EMBRYONIC STEM CELL RENEWAL	
1:30 – 3:15 p.m.	**Concurrent Session IV Track C** **Immunology and Stem Cells** Chair: **Hongkui Deng**, *Peking University, China* Co-Chair: **Robert Hariri**, Celgene Cellular Therapeutics, USA	Conf. Center Rms. 301 - 304
1:30 – 1:35 p.m.	**Hongkui Deng,** *Peking University, China* Introduction and overview	
1:35 – 2:00 p.m.	**Robert Hariri**, *Celgene Cellular Therapeutics, USA* PLACENTAL DERIVED STEM CELLS: A THERAPEUTIC PLATFORM FOR THE TREATMENT OF AUTOIMMUNE DISEASE	
2:00 – 2:15 p.m.	**Toshinobu Nishimura**, *University of Tokyo, Japan* ANTIGEN-SPECIFIC T CELL INDUCTION FROM HUMAN INDUCED PLURIPOTENT STEM CELLS	
2:15 – 2:30 p.m.	**Dan S. Kaufman**, *University of Minnesota, USA* DEVELOPMENT OF NATURAL KILLER CELLS FROM HUMAN PLURIPOTENT STEM CELLS USING DEFINED CONDITIONS SUITABLE FOR CLINICAL TRANSLATION	
2:30 – 2:45 p.m.	**Masakazu Kamata**, *University of California, Los Angeles, USA* ANTIBODY-BASED IMMUNOTHERAPY USING REPROGRAMMED ANTIGEN-SPECIFIC EFFECTOR B-CELLS	
2:45 – 3:00 p.m.	**Suzanne N. King**, *University of Wisconsin – Madison, USA* COMPARATIVE ANALYSIS OF THE IMMUNOREGULATORY AND REGENERATIVE PROPERTIES OF HUMAN MESENCHYMAL STROMAL CELLS -HYALURONIC ACID HYDROGEL CONSTRUCTS	
3:00 – 3:15 p.m.	**Yumi Matsuzaki**, *Keio University, Japan* MINOR ANTIGEN-MISMATCHED MSC REACT WITH RESIDUAL HOST T CELLS TO TRIGGER THE PROGRESSION OF CHRONIC GVHD	
1:30 – 3:15 p.m.	**Concurrent Session IV Track D** **Chemical Control of Stem Cell Behaviour** Co-Chair: **Nissim Benvenisty**, *Hebrew University, Israel* Co-Chair: **Lee Rubin**, *Harvard University, USA*	Conf. Center Rms. 501 - 502
1:30 – 1:35 p.m.	**Nissim Benvenisty**, *Hebrew University, Israel* Introduction and overview	
1:35 – 2:00 p.m.	**Lee Rubin**, *Harvard University, USA* FINDING THERAPEUTICS FOR MOTOR NEURON DISEASES	
2:00 – 2:15 p.m.	**Stuart M. Chambers**, *Sloan Kettering Institute, USA* COMBINED SMALL MOLECULE INHIBITION ACCELERATES DEVELOPMENTAL TIMING AND CONVERTS HUMAN PLURIPOTENT STEM CELLS INTO NOCICEPTORS	
2:15 – 2:30 p.m.	**Christopher E. Henderson**, *Columbia Stem Cell Initiative, USA* HIGH-THROUGHPUT SCREENS USING MOUSE ES CELL-DERIVED NEURONS IDENTIFY DRUGS THAT ENHANCE AXONAL REGENERATION	
2:30 – 2:45 p.m.	**Roberto Iacone**, *Hoffmann-La Roche, Switzerland* 2D VASCULAR CONVERSION OF HUMAN PLURIPOTENT STEM CELLS BY A NOVEL GSK3β INHIBITOR - A CELLULAR MODEL TO EVALUATE ENDOTHELIAL DYSFUNCTION IN TYPE 2 DIABETES VASCULAR COMPLICATIONS	

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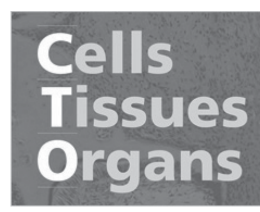

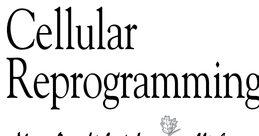

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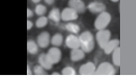

Detailed Schedule at a Glance

Saturday, June 16 (cont.)

2:45 – 3:00 p.m.	**Lauren Drowley**, *AstraZeneca, UK* A REGENERATIVE MEDICINE APPROACH TO DIABETES AND OBESITY - DIFFERENTIATING ADIPOSE AND SKELETAL MUSCLE PROGENITOR CELLS INTO FUNCTIONAL BROWN ADIPOSE TISSUE	
3:00 – 3:15 p.m.	**Roger E. Rönn**, *Lund University, Sweden* IMPROVED GENERATION OF HUMAN HEMATOPOIETIC STEM/PROGENITOR CELLS FROM ES AND PATIENT DERIVED IPS CELLS USING SMALL MOLECULES AND FACTORS	
1:30 – 3:15 p.m.	**Concurrent Session IV Track E** **Stem Cells and Cancer** Co-Chair: **Connie Eaves**, *Terry Fox Lab, BC Cancer Agency, Canada* Co-Chair: **Ramkumar Mandalam**, *Cellerant Therapeutics, USA*	Conf. Center Rm. 503
1:30 – 1:35 p.m.	**Connie Eaves**, *Terry Fox Lab, BC Cancer Agency, Canada* Introduction and overview	
1:35 – 2:00 p.m.	**Ramkumar Mandalam**, *Cellerant Therapeutics, USA* ANTIBODY-BASED TARGETING OF CANCER STEM CELLS IN HEMATOLOGICAL MALIGNANCIES	
2:00 – 2:15 p.m.	**Allison N. Lau**, *Children's Hospital Boston, USA* CD24 MARKS METASTATIC LUNG TUMOR-PROPAGATING CELLS WITH ACTIVE HIPPO SIGNALING	
2:15 – 2:30 p.m.	**Andrew S. Goldstein**, *University of California, Los Angeles, USA* DISTINCT SELF-RENEWING STEM-LIKE CELL POPULATIONS INITIATE AND MAINTAIN AGGRESSIVE HUMAN PROSTATE CANCER	
2:30 – 2:45 p.m.	**Haruko Shima**, *National Cancer Center Research Institute, Japan* ROLES OF RING1A/B IN STEM CELL POTENTIAL OF MOZ AND OTHER ACUTE MYELOID LEUKEMIAS	
2:45 – 3:00 p.m.	**Robert Vanner**, *University of Toronto, Canada* IDENTIFICATION OF RELATIVELY QUIESCENT SOX2-EXPRESSING MOUSE BRAIN TUMOUR STEM CELLS IN MEDULLOBLASTOMA IN VIVO	
3:00 – 3:15 p.m.	**Athanasia D. Panopoulos**, *The Salk Institute, USA* PARALLEL FUNCTIONS OF THE ENDOPLASMIC RETICULUM CHAPERONE PROTEIN GRP78 IN TUMORIGENESIS AND THE INDUCTION OF PLURIPOTENCY	
3:15 – 4:00 p.m.	Refreshment Break	Exhibit Halls B & C
3:45 – 4:00 p.m.	Group 2 Posters Dismantle	
4:00 – 6:30 p.m.	**Plenary VII** **STEM CELLS AND FATE CONTROL** *Supported by Salk Institute for Biological Studies* Chair: **Arturo Alvarez-Buylla**, *University of California, San Francisco*	National Convention Hall
4:00 – 4:15 p.m.	**President-Elect Address, Shinya Yamanaka**, *CiRA, Kyoto University, Japan*	
4:15 – 4:30 p.m.	**Leonard I. Zon**, *Children's Hospital, Boston, USA* Ten Years of ISSCR	
4:30 – 4:55 p.m.	**Hans C. Clevers**, *Hubrecht Institute, Netherlands* LGR5 STEM CELLS IN SELF-RENEWAL AND CANCER	
4:55 – 5:20 p.m.	**Hongjun Song**, *Johns Hopkins, USA* ANALYSIS OF NEURAL STEM CELLS IN THE ADULT BRAIN, ONE AT A TIME	
5:20 – 5:45 p.m.	**Oliver Hobert**, *Columbia University Medical Center, USA* REPROGRAMMING GERM CELLS INTO NEURONS	
5:45 – 6:20 p.m.	**Anne McLaren Memorial Lecturer** **Fiona M. Watt**, *Cancer Research UK Cambridge Research Institute, UK* **INTEGRATING INTRINSIC AND EXTRINSIC SIGNALS TO REGULATE EPIDERMAL STEM CELL FATE**	
6:20 p.m.	**Closing Remarks**, *Fred H. Gage, ISSCR President*	
6:30 – 7:30 p.m.	Closing Reception *Supported by Children's Hospital of Boston/Stem Cell Program, Harvard Stem Cell Institute and Massachusetts General Hospital for Regenerative Medicine.*	Marine Lobby

Sunday, June 17

Post Meeting Tours

9:30 a.m. – 5:30 p.m.	"Experience Tokyo"	
9:30 a.m. – 5:30 p.m.	"Captivating Kamakura"	
4:00 – 6:00 p.m.	ISSCR Public Symposium ON THE CUTTING EDGE: IPS CELLS AND THE FUTURE OF REGENERATIVE MEDICINE	Maraikan National Museum of Emerging Science and Innovation, Tokyo

Exhibits and Exhibitor Floor Plan

125 226 224 123 222 121 220 218
227 225 324 223 322 221 318
325 426 424 323 420 319 418
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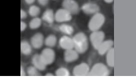

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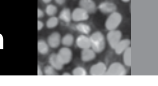

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Led by Dr. Shinya Yamanaka, the pioneer of iPS cell technology, the Center for iPS Cell Research and Application (CiRA), located in Kyoto, Japan, focuses on seamlessly conducting fundamental studies as well as preclinical and clinical research.

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HSCI is a scientific collaborative consisting of the schools of Harvard University and its eleven affiliated teaching hospitals and research institutions. Founded in 2004, HSCI has pushed the field of stem cell biology forward and made substantial progress toward the ultimate goal of understanding disease processes and the development of treatments

Innovative Cell Techonogies, Inc. – Booth 615

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International Society for Cellular Therapy

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iwamoto_y@khi.co.jp
www.khi.co.jp/english/

Kawasaki's wide range of technologies contributes to automated manufacturing of cells required for drug discovery, regenerative medicine, and also cell therapy. This system enables mass production of cells in safety, steadily and stably, without CPC.

Keyence Corporation – Booth 211

Takashi Fujiwara,Marketing Manager
1-3-14, Higashinakajima, Higashiyodogawa-ku
Osaka, 533-8555
Japan
Phone: +81-(0)6-6379-1141
Fax:+81-(0)6-6379-1140
fujiwara-ta@keyence.co.jp
http://www.keyence.com/products/microscope/microscope/bz9000/bz9000.php

Introduction of fully automated all-in-one fluorescence microscope (Biorevo) from Keyence.

Built-in sample enclosure eliminates the need for a darkroom. It makes it possible to install the system anywhere. Full electronics control of motorized components simplifies setup and operation.

Life Technologies – Booth 518

Dorene Hertlein, Assoc. Project Management Specialist
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USA
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Fax: +1-240-379-4750
Dorene.hertlein@lifetech.com
www.lifetechnologies.com

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As a leadeing supplier of tools and reagents for the science community, Life Technologies is the leadeing provider of novel tools for stem cell research, providing more than 1,500 products used by scientists in basic research, screening, and drug discovery.

LMS Co. Ltd. – Booth 323

Mr. Yoichi Kadooka, Division Manager
Tanaka Bldg. 3-6-7
Bunkyo-Ku Hongo Tokyo 113-0033
Japan
Phone: +81 3 5842 4301
Fax: +81 3 3811 0033
y-kadooka@lms.co.jp
www.lms.co.jp

EVOS fl Digital microscope, Luna Automated Cell counter

Lonza – Booth 501

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Phone: +1-443-223-9543
scott.dandrea@lonza.com
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Massachusetts General Hospital Center for Regenerative Medicine

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185 Cambridge St., CPZN 4264
Boston, MA 02114
USA
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Fax: +1-617-724-2662
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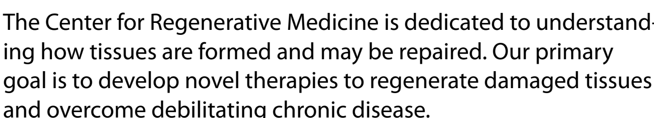
www.massgeneral.org/regenmed



The Center for Regenerative Medicine is dedicated to understanding how tissues are formed and may be repaired. Our primary goal is to develop novel therapies to regenerate damaged tissues and overcome debilitating chronic disease.

Matrigen Life Technologies – Booth 708

Sonia Patel, Director of Operations
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Phone: +1-508-755-5070
soina@matrigen.com
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Merck Millipore – Booth 303

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Miltenyi Biotec GmbH – Booth 308

Dr. Thorsten Decker, Product Manager Stem Cells
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Fax: +49-2204-85197
info@mac-stemcells.com
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The New York Stem Cell Foundation (NYSCF) – Booth 712

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USA
Phone: +1-212-365-7444
Fax: +1-212-787-5844
schang@nyscf.org
www.nyscf.org

NYSCF is a nonprofit organization dedicated to accelerating stem cell research through its independant research laboratory, collaborations, grants, scientific conferences and public education.

Nikon Corporation – Booth 524

Nikon Instruments Company
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Nippon Vision Engineering Co., Ltd. – Booth 521

Yoshinori Hosogi, General Manager
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Phone: +81 45 935 1117
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Stephanie Peacock, Director of Marketing
8100 Southpark Way, A-8
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PeproTech, Inc – Booth 419

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PerkinElmer Japan Co., Ltd. – Booth 625

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Primorigen Biosciences, Inc. – Booth 403

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brad.garcia@primorigen.com
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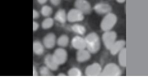

Exhibitor/Supporter Directory

Promega Corporation – Booth 109

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PromoCell GmbH – Booth 613

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Qatar Biomedical Research Institute (QBRI) is engaged in applied biomedical research and the translation of new basic science discoveries into personalized solutions for diseases of high prevalence in Qatar and in the world by exploiting genomics, metabolomics, and stem-cell science applications.

QIAGEN K.K. – Booth 523

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ReproCELL – Booth 611

Yoshinori Tanizawa, Specialist, Technical Support Department
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Japan
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Fax: +81-45-474-1006
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Scottish Development International (SDI) – Booth 301

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Glasgow, G2 8LU
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Scotland is a world leader in stem cell and regenerative medicine R&D, both academic and commercial. Scottish Development International's aim is to assist in the growth of the Scottish economy, by encouraging inward investment and helping Scottish-based companies develop international trade.

SCRUM Inc. – Booth 103

Takemitsu Furuta, Executive Director
A&Y Bldg. 1-8-9 Midori Sumida-ku
Tokyo, 130-0021
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SERVA Electrophoresis GmbH – Booth 222

Mr. Jens Boettcher, Product Manager Life Science
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Sony Corporation/iCyt – Booth 113

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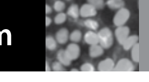

Exhibitor/Supporter Directory

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Stem Cell Network – Booth 602

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www.stemcellnetwork.ca

The Stem Cell Network is a not-for-profit organization, supporting leading-edge research and training at more than 30 institutions across Canada, with a goal to catalyze the translation of stem cell research into clinical applications, commercial products and public policy.

STEMCELL Technologies Inc – Booth 312

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Jeffrey Stern Ph.D., M.D, President
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jstern@nynsci.org
www.stemcultureinc.com

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Stemgent – Booth 513

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Terumo BCT – Booth 322

Mark Flower, Senior Marketing Manager
10810 W Collins Ave
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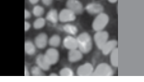

Exhibitor/Supporter Directory

Unitech Co., Ltd. – Booth 427

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University of Pittsburgh, McGowan Institute for Regnerative Medicine

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Alan Russell, PhD, Director
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VERITAS Corporation – Booth 121

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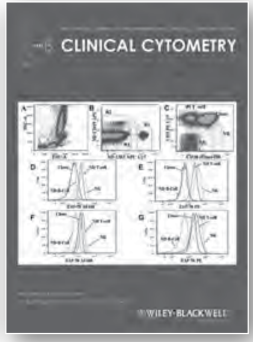

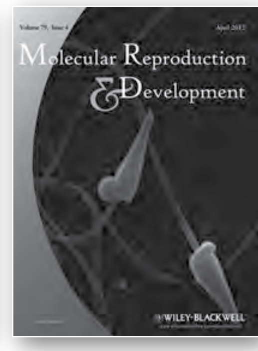

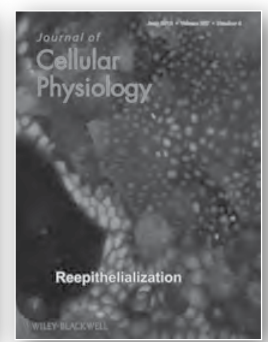

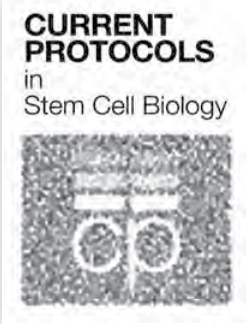

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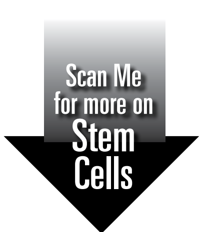
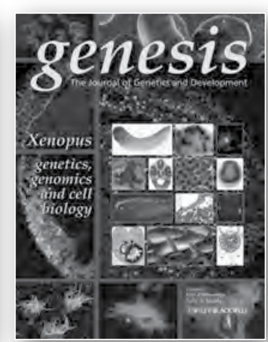
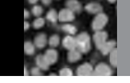






Detailed Program and Abstracts — Wednesday, June 13

Presidential Symposium: Early Life Decision

Supported by Johnson & Johnson

Wednesday, June 13, 2012, 1:00 pm – 3:30 pm

IPS TECHNOLOGY AND DISEASE RESEARCH

Jaenisch, Rudolf
Whitehead Institute for Biomedical Research and Department of Biology, MIT, Cambridge, MA, USA

Direct reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) can be achieved by over-expression of Oct4, Sox2, Klf4 and c-Myc transcription factors. Reprogramming is a continuous stochastic process where almost all donor cells eventually give rise to iPSCs though the process is highly inefficient. To get insight into the mechanism of reprogramming we have used single cell gene expression analysis of cells at different stages of reprogramming. Our results reveal considerable variation in gene expression at early times followed by a more hierarchical phase at later stages. In addition, we have identified a subset of pluripotency genes the activation of which can predict successful iPS cell generation. The iPS approach will allow the generation of patient specific iPS cells that can be used to study complex human diseases in the Petri dish. Progress in using iPS cells for therapy and for the study of complex human diseases will be summarized. The presence of reprogramming vectors in the iPS cells and the inefficiency of gene targeting represent two important impediments for realizing the potential of ES and iPS cells to study human diseases. We have designed strategies that efficiently allow the generation of vector-free iPS cells. In addition, we have used Zn finger and TALEN mediated genome editing to establish efficient protocols to target expressed as well as silent genes in human ES and iPS cells.

THE CORE OF EMBRYONIC STEM CELLS

Smith, Austin G.
Centre for Stem Cell Research, Stem Cell Institute, University of Cambridge, Cambridge, United Kingdom

Pluripotency is the capacity of an individual cell to produce all lineages of the mature organism including the germline in response to extrinsic cues. In mice and rats this naïve state at the foundation of mammalian development can be captured in culture in the form of self-renewing embryonic stem (ES) cells. ES cells cultured in serum are highly mosaic and appear metastable. More homogenous propagation is achieved using two selective kinase inhibitors that target the mitogen-activated protein kinase (Erk) cascade and glycogen synthase kinase-3 (Gsk3) respectively. We have suggested that ES cells maintained using the two inhibitors (2i) along with the cytokine leukaemia inhibitory factor (LIF) are anchored in a self-renewing ground state. Our current research is centred on: (i) defining the transcription factor hub that sustains naïve pluripotency; (ii) elucidating the exit route that allows entry into multi-lineage commitment and differentiation. Our research is supported by The Wellcome Trust, the Medical Research Council, the Biotechnology and Biological Sciences Research Council, and the European Commission Framework 7 Program.

WHAT IS THE BUG IN THE PROGRAM OF PLURIPOTENCY

Takahashi, Kazutoshi
Center for IPS Cell Research & Application, Kyoto, Japan

Bugs in computer software programs often lead vulnerability and instability. In most situations, such errors are fixed by updating the algorisms used. iPS cell technologies have also been improved by engineering innovations such as integration-free vectors and xeno-free culture conditions. However, the latest program in any field always faces new issues.

Recent advances in the pluripotent stem cell field have ignited debates about reprogramming errors, such as genetic alterations and epigenetic memories. To argue these points, it is necessary to understand the origins and causes of such errors. In addition, it is also necessary to distinguish reprogramming-mediated errors from inheritable characters. We have performed a series of comparison analyses of the global gene expression, DNA methylation and exon sequences among more than 50 human pluripotent stem cell lines. In my talk today, I would like to discuss the current issues and future directions of human pluripotent stem cell research for clinical use.

DIRECT REPROGRAMMING OF SOMATIC CELL NUCLEI BY OOCYTES

Gurdon, John, Jullien, Jerome, Pasque, Vincent, Miyamoto, Kei, Halley-Stott, Richard
Wellcome Trust/CR UK Gurdon Institute, University of Cambridge, Cambridge, United Kingdom

In normal development, cells become increasingly committed to the lineage on which they have embarked. Their differentiated state becomes progressively more stable. The more differentiated a cell becomes, the harder it is to reverse its differentiation back to an embryonic state by nuclear transfer or by iPS technology. Our aim is to understand the mechanisms by which somatic cell nuclei are made to reverse differentiation and acquire the characteristics of embryo cells. Eggs and oocytes are able to reprogram somatic cell nuclei by their natural components, as normally happens after fertilization. The complete reprogramming of a differentiated cell of one type to another entirely unrelated type requires two major steps. The first is to reverse the differentiated state of the cell back to that of an uncommitted embryo cell. After that, the derived embryo cell needs to be directed to a new cell fate. We study the first of these two major steps in reprogramming. The amphibian oocyte, a first meiotic prophase progenitor of a fertilizable egg, is especially well suited to an analysis of transcriptional reprogramming. Our first aim is to identify the components of oocytes that re-set transcription to an embryonic type. We transplant mammalian somatic or cultured cell nuclei to the amphibian oocyte, and then analyse changes in transcription over the next few days. The transplanted nuclei do not divide or replicate their DNA, but undergo direct changes in transcription. An initial major change in the transplanted nuclei is the decondensation of their chromatin to remove differentiation marks and to open up genes that have become quiescent in the course of differentiation, including the known pluripotency factors. Particularly important for this is the oocyte-specific linker histone B4 (HF1oo in mammals). After chromatin decondensation intense transcriptional activity is promoted by special factors in oocytes that include polymerized actin and Wave-1. In this way, we can follow transcriptional events at the biochemical level by ChIP analysis as well as in individual transplanted nuclei by confocal microscopy. The second and complementary direction of our

Detailed Program and Abstracts — Thursday, June 14

work is to understand the molecular basis of the resistance shown by somatic nuclei to the reprogramming components of oocytes. We find enormous differences between different cell-types in the extent to which a gene such as Oct4, or Sox2 is reprogrammed by oocytes. For example, Sox2 is about 10 times more easily reprogrammed in mouse embryonic fibroblast than in C2C12 nuclei and Oct4 is about 5 times more strongly transcribed in the nuclei of C2C12 than in mouse embryonic fibroblast nuclei. We are able to progressively remove proteins and RNA from somatic nuclei to identify those components which account for the resistance to reprogramming by oocytes. Non-coding RNAs seem to have no role in the resistance to reprogramming, and most chromosomal proteins can be removed without changing resistance. DNA methylation does not explain most of the resistance observed. We consider the resistance components to be those which have an important role in stabilizing different lineage pathways in normal development.

Plenary II - Regeneration, Engraftment and Migration of Stem Cells

Supported by The New York Stem Cell Foundation (NYSCF)

Wednesday, June 13, 2012, 4:30 pm - 7:00 pm

DELINEATING THE MAMMARY STEM CELL HIERARCHY AND ITS MOLECULAR REGULATORS

Visvader, Jane E.[1], Pal, B[2], Bouras, T[2], Sheridan, J[2], Vaillant, F[2], Shi, W[2], Smyth, G[2], Lindeman, G[3]

[1]Walter & Eliza Hall Institute of Medical Research and The University of Melbourne, Parkville, Australia, [2]Walter & Eliza Hall Institute of Medical Research, Parkville, Australia, [3]Walter & Eliza Hall Institute of Medical Research and Royal Melbourne Hospital, Parkville, Australia

To understand relationships between breast tissue and 'cells of origin' of cancer in breast tumors, it is necessary to dissect the normal mammary epithelial hierarchy. We have isolated discrete populations of mouse and human mammary epithelial cells on the basis of cell surface markers and identified subsets that are highly enriched for mammary stem (MaSC), luminal progenitor and mature luminal cells. Recent studies have revealed that MaSCs are highly responsive to steroid hormones despite lacking expression of the estrogen and progesterone receptors. Transcriptome analyses of mouse and human mammary epithelial populations have led to the identification of multiple conserved genes/pathways and potential effectors of hormone action. In addition, the roles of a number of transcriptional regulators have been pinpointed along the mammary hierarchy using targeted mice. High throughput functional shRNA screens are currently being performed to identify novel genes governing stem cell proliferation and differentiation. To extend these studies, we have determined the epigenomes of primary mammary epithelial subtypes. Comparison of the whole-genome histone modification maps with the transcriptomes of these subpopulations will provide insight into molecular mechanisms that control cell-fate and differentiation programs in stem and progenitor cells.

SELF-ORGANIZATION OF THREE-DIMENSIONAL TISSUE STRUCTURES IN ES CELL CULTURE

Sasai, Yoshiki

RIKEN Center for Developmental Biology, Kobe Hyogo, Japan

Over the last several years, much progress has been made for in vitro culture of mouse and human ES cells. Our laboratory focuses on the molecular and cellular mechanisms of neural differentiation from pluripotent cells. Pluripotent cells first become committed to the ectodermal fate and subsequently differentiate into uncommitted neuroectodermal cells. Both previous mammalian and amphibian studies on pluripotent cells have indicated that the neural fate is a sort of the basal direction of the differentiation of these cells while mesoendodermal differentiation requires extrinsic inductive signals. ES cells differentiate into neuroectodermal cells with a rostral-most character (telencephalon and hypothalamus) when they are cultured in the absence of strong patterning signals. In this talk, I first discuss this issue by referring to our recent data on the mechanism of spontaneous neural differentiation in serum-free culture of mouse ES cells. Then, I focus on self-organization phenomena observed in 3D culture of ES cells, which lead to tissue-autonomous formation of regional structures such as layered cortical tissues. We also discuss our new attempt to monitor these in vitro morphogenetic processes by live imaging, in particular, self-organizing morphogenesis of optic cup and pituitary gland in three-dimensional cultures.

SKIN CELLS IN HOMEOSTASIS, WOUND REPAIR AND CANCER

Fuchs, Elaine, Chen, Ting, Lu, Catherine P., Oshimori, Naoki, Schober, Marcus

Howard Hughes Medical Institute, The Rockefeller University, New York, NY, USA

Embryonic epidermis begins as a single layer of unspecified progenitors. During development, it receives external cues to undergo a series of morphogenetic events which culminate in the production of a stratified epidermis replete with hair follicles and sweat glands. Postnatally, these tissues undergo self-renewal which requires stem cells. We've demonstrated that resident stem cells exist in the epidermis, hair follicle and sweat glands. How these cells develop and how they balance self-renewal and differentiation is of fundamental importance to our understanding of normal tissue maintenance and wound repair, and to compare how this happens to states, e.g. cancer, where the balance goes awry. Using skin as our paradigm, we've been dissecting how extrinsic signaling to stem sets off a cascade of changes in transcription that governs the activation of stem cells during tissue development, homeostasis and hair cycling. Our findings have provided us with new insights into our understanding of the process of stem cell activation, and in so doing have revealed mechanisms which are also deregulated in a variety of different human cancers. In this talk, I will review some of our studies that implicate a complicated cross-talk between stem cells and their niche microenvironment, and how these communication circuitries change in normal homeostasis and wound repair and in tumor progression.

Ernest Mcculloch Memorial Lecture

NORMAL AND NEOPLASTIC STEM CELLS

Weissman, Irving L.

Dept of Pathology, Stanford University, Stanford, CA, USA

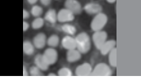
ABSTRACT NOT AVAILABLE AT TIME OF PRINTING

WHY WHAT YOU DO MATTERS

Sabine, Charles

Patient Advocate, Gloucestershire, United Kingdom

Emmy-award winning TV journalist, Charles Sabine, worked for US network NBC news for 26 years. That career took him; via twelve wars, six revolutions, and four earthquakes; to most of the news events of Europe, the Middle East, Africa and Asia since the early



Detailed Program and Abstracts — Thursday, June 14

1980s. There, he learnt first-hand the extraordinary limits that the human spirit is capable of reaching, in the face of tragedy inflicted by both nature and mankind. In 2008, he decided to put the lessons of those experiences to a different use, when he became a pioneering spokesman for freedom of scientific research, and sufferers of degenerative brain illnesses - in particular, Huntington's disease, which has ravaged his family. That role has led to Sabine speaking at prestigious venues across the world, among them; the European and British parliaments; the Royal Institution in London and the World Congress on Freedom of Scientific Research. No two presentations of Charles Sabine's are the same, as he recognises that no two audiences are the same; but all draw on the lessons of his travels with NBC and the stark reality of battling with one of the most devastating illnesses known to man. For the ISSCR 2012 audience, the central theme of his talk will be the importance to patients and their families of work that goes on within laboratories on their behalf, and the need for researchers to realise just how even unsuccessful research is of value; because; in a world of total darkness, the very faintest glimmer of light emboldens the human spirit to go on. He will also talk of his belief that the future of healthcare and research must involve greater collaboration between all key protagonists: researchers, governments, pharmaceutical companies, and patients and their families. Lastly, he will explain why every aspect of research, however far it may seem to be removed from the patient, is part of a greater good of aspiring to care for the infirm; what Darwin called 'the noblest part of our nature'.

Plenary III — Lost in Translation: The Difficult Path for Stem Cells to the Clinic

Joint session with Japan Society for Regenerative Medicine (JSRM)
Supported by iPS Academia Japan, Inc.

Thursday, June 14, 2012, 9:00 am – 11:25 am

CLINICAL TRANSLATION OF HUMAN NEURAL STEM CELLS, HUCNS-SC: WHERE ARE WE TODAY?

Tsukamoto, Ann[1], Capela, Alexandra[1], Huhn, Stephen[1], Weissman, Irving L.[2], Gage, Fred H.[3], Uchida, Nobuko[1]
[1]StemCells Inc, Palo Alto, CA, USA, [2]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA, USA, [3]Laboratory of Genetics, Salk Institute, La Jolla, CA, USA

Currently, there are few effective therapies for the treatment of neurodegenerative diseases or injuries to the brain, spinal cord and eye. Human neural stem cell transplants offer the prospect to treat such conditions and represent a potential exciting new medical therapy. A highly purified composition of human neural stem cells has been isolated, expanded and stored as banks of cells, HuCNS-SC®. When transplanted into the brain of immunodeficient rodents, human neural stem cells reside and proliferate in host neurogenic sites, such as the subventricular zone and dentate gyrus of the hippocampus. Their progeny migrate globally throughout the brain and differentiate in a site appropriate manner into neurons, astrocytes and oligodendrocytes. When transplanted into the spinal cord above and below the injury site, these cells also migrate extensively and differentiate, remyelinate and make synaptic connections with host neurons. HuCNS-SC have also been shown to produce soluble proteins such as housekeeping lysosomal enzymes, neurotrophic factors, and chemokines which may protect damaged host cells and also become mature oligodendrocytes which myelinate dys- or demyelinated host axons. Moreover, these human cells survive long-term in the host brain with no signs of tumor formation or adverse effects. Therefore, a single transplant of human neural stem cells offers the prospect of a durable clinical benefit. Transplantation of HuCNS-SC into animal models of human diseases or injury have been performed to assess the cells' biological properties including their impact on these specific targets. These preclinical efficacy studies have demonstrated protection of host cells and/or improvements in specific functional deficits and provided the foundation for the neuroprotection and neural replacement strategies to support initiation of our clinical studies. Three clinical studies have been initiated to date and a fourth is authorized for initiation. The first clinical study in Batten disease, a fatal lysosomal storage disease, has been completed and the surviving patients are now ~4-5 years post-transplant with no safety concerns. A trial in Pelizaeus-Merzbacher disease (PMD), a fatal myelination disorder, is completing and clinical data will be presented. A trial in chronic thoracic spinal cord injury has completed dosing of the most severely injured patients (AIS-A), and will now enroll those with incomplete injuries, AIS-B and C. The Company has recently been authorized to begin a trial in dry AMD. Preclinical studies in a rat model of retinal degeneration, the RCS rat, have shown cone photoreceptor protection and visual preservation following subretinal transplants of HuCNS-SC. The clinical data derived from these studies should facilitate future clinical testing of HuCNS-SC cells in a broad range of other neurological disorders including Alzheimer's disease, stroke, and cerebral palsy.

MESENCHYMAL STEM CELLS FOR TREATMENT OF GRAFT-VERSUS-HOST DISEASE

Le Blanc, Katarina
Centre for Allogeneic Stem Cell Transplantation, Karolinska Institutet, Stockholm, Sweden

Mesenchymal Stromal Cells (MSCs) are non-hematopoietic progenitor cells that have immune-modulatory properites and promote peripheral tolerance. MSCs suppress alloreactive donor anti-host T-cell responses. Based on the immunomodulatory properties of MSCs along with the cells' ability to promote repair of injured tissue, it was hypothesized that MSCs may be beneficial in reversing inflammation. To date, MSCs have been infused intravenously to several hundred patients. No acute infusional toxicity has been reported. Many questions remain to be answered to optimize MSC treatment. As MSCs are poor stimulators of alloresponses, the majority of patients have received MSCs derived from third-party mismatched donors. However, if and to what degree HLA-matching influences response in humans remains unclear. Furthermore, it is well established that MSC are rare cells in vivo and that culture ex vivo is necessary to obtain a sufficient number of cells for a therapeutic effect. The influence of culture conditions and media supplements on the efficacy of the cells needs to be established in clinical trials. This is particularly true since no efficacy marker has been established that predicts the clinical outcome of patients treated with MSCs. For example, measurements of MSC-induced lymphocyte suppression in mixed lymphocyte culture does not correlate with clinical response. Trials have used MSCs expanded in the presence of either fetal calf serum or platelet lysate. In vitro properties of MSC expanded in the two media are comparable, but undetected differences may still influence patient responses. Response rates of MSC-treated patients with graft-versus-host disease and various automimmune disorders indicate that MSCs are a promising treatment

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tool. However, optimal cell expansion and donor selection will need to be evaluated in clinical trials.

TRANSPLANTATION OF IPS CELL-DERIVED RETINAL PIGMENT EPITHELIAL CELLS

Takahashi, Masayo
Laboratory for Retinal Regeneration, RIKEN Center for Developmental Biology, Kobe, Japan

Transplantation of embryonic stem (ES) cell-derived cells into the spinal cord and retina has begun. This pioneering work in ES cell therapy paves the way for cell transplantation therapy using induced pluripotent stem (iPS) cells. Previously we have established a culture method that induces directed differentiation of human ES/iPS cells into mature retinal cells such as photoreceptor cells and retinal pigment epithelial (RPE) cells. We are now planning to transplant autologous iPS cell-derived RPE cells that have an advantage of showing no rejection following transplantation. We have already purified terminally differentiated RPE cells from human iPS cells. RPE cells derived from iPS cells showed the characteristic morphologies, secretion of growth factors and the gene expression pattern similar to the authentic human RPE cells. We are now preparing the Standard Operating Procedure (SOP) for a clinical trial of cell therapy of wet type age-related macular degeneration. We also prepared cell processing center suitable for clinical use. After confirming safety, we will conduct clinical studies. Experience gained through transplantation of iPS cell-derived RPE cells can be also applied to photoreceptor transplantation.

THE TRAGEDY OF TRANSLATION: EPISTEMOLOGICAL AND ETHICAL CHALLENGES PERTAINING TO BRINGING STEM CELLS TO THE CLINIC

Solbakk, Jan Helge
Centre for Medical Ethics, Faculty of Medicine, University of Oslo, Oslo, Norway

There is a profound moral question at the heart of all translational research: Who should go first in human trials when the risks are not possible to estimate, the trial highly observed, and the effects of failure far-reaching? This paper aims at addressing this question by unveiling the epistemological problem at its core. Second, the paper will draw attention to some of the unresolvable ethical challenges underlying any type of translational research involving human beings.

TRANSLATION OF REPROGRAMMING TECHNOLOGIES FOR CNS DISORDERS

Okano, Hideyuki
Dept. of Physiology, Keio University School of Medicine, Tokyo, Japan

There has been increasing enthusiasm in the reprogramming technologies for induced pluripotent stem cells (iPS cells) and direct induction in terms of cell therapies for regenerative medicine and stem cell modeling of human disease. On the other hand, recent reports have emphasized the pitfalls of iPSC technology, including the potential for genetic and epigenetic abnormalities, tumorigenicity, and immunogenicity of transplanted cells. These constitute serious safety-related concerns for iPSC-based cell therapy (Okano et al. Circulation Res, 2012). In fact, we have characterized iPS cells as a source of neural stem/progenitor cells (NS/PCs), which can be obtained from adult somatic tissues, to develop cell therapy for damaged CNS. We found that mouse (Miura et al., Nat. Biotech, 2009) and human iPS cells can be induced for NS/PCs by the similar method for neural differentiation of mouse (Okada et al., Stem Cells, 2008; Naka et al., Nature Neurosci, 2008) and human ES cells, respectively. We have examined the therapeutic potential of mouse and human iPSCs-derived neural progenitor cells in a mouse spinal cord injury (SCI) model. As a result, we found that mouse (Miura et al., Nat. Biotech, 2009) and human iPSCs-derived NS/PCs showed varied tumor forming propensities depending their parental iPSC lines. On the other hand, non-tumorigenic iPSCs-derived NS/PCs can be selected through the analysis of gene expression, flowcytometric analysis, and transplantation to the brains of NOD/SCI mice. When these "safe" mouse (Tsuji et al., PNAS, 2010) and human (Nori et al. PNAS, 2011) iPS-derived NS/PCs were transplanted into mouse and non-human primates SCI models 9 days after contusive injury, they differentiated into neurons and glia without forming tumors. They also induced the re-myelination, axonal regrowth of host 5HT(+) serotonergic fibers and participated in synaptogenesis with host neurons, and various trophic actions, which collectively promoted the locomotor function recovery. Thus, safety and effectiveness of human and mouse iPSCs-derived NS/PCs for SCI has been demonstrated for SCI using mouse and non-human primates SCI models upon selection of appropriate well-reprogrammed iPSC lines. Previous studies including ours have suggested that the key factors for successful transplantation for SCI models will include transplantation of NS/PCs that are non-tumorigenic and can produce both neurons and glia within several weeks after the injury. From this aspect, there is emerging interest in the directly induced NS/PCs from the fibroblasts which can be produce both neurons and glia within a few weeks after the gene transfer of 4 reprogramming factors. Their basic characterization and potential application for transplantation therapy and development of diseases-model cells will be referred in the talk.

Concurrent Session IA: Pluripotent Stem Cells I

Thursday, June 14, 2012, 1:30 pm - 3:15 pm

TOWARDS IN VITRO RECONSTITUTION OF MAMMALIAN GERM CELL DEVELOPMENT

Saitou, Mitinori
Dept of Anatomy and Cell Biology, Graduate School of Medicine, Institute for Integrated Cell-Material Sciences, and Center for iPS Cell Research and Application, Kyoto University and JST, ERATO, Kyoto, Japan

The germ cell lineage ensures the creation of new individuals, thereby perpetuating and diversifying the genetic and epigenetic information across the generations. We have been investigating signaling, global transcription and epigenetic dynamics associated with germ cell specification, and have proposed a concept that germ cell specification involves an integration of three key events: repression of the somatic program, re-acquisition of potential pluripotency, and an ensuing genome-wide epigenetic reprogramming. Recently, using pluripotent stem cells (embryonic stem cells and induced pluripotent stem cells), we have succeeded in precisely reconstituting the mouse germ-cell specification pathway in culture. This work will serve as a foundation for the better elucidation of early germ-cell biology, including the mechanism of genome-wide epigenetic reprogramming, as well as for the reconstitution of the entire germ-cell development process in vitro both in males and females, not only in mice but also in other mammals, including humans.

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CREATION OF VASCULARIZED HUMAN ORGAN FROM INDUCED PLURIPOTENT STEM CELLS

Takebe, Takanori, Sekine, Keisuke, Enomura, Masahiro, Suzuki, Yuka, Koike, Hiroyuki, Zhang, Ran-Ran, Koike, Naoto, Ueno, Yasuharu, Zheng, Yun-Wen, Taniguchi, Hideki

Yokohama City University, Yokohama, Japan

Since the discovery of embryonic stem cells in1981, years of laboratory studies have failed to generate vital organs from pluripotent stem cells, which is an ultimate goal of regenerative medicine. One rationale approach to create a complex and vascularized organ is to recapitulate the cellular interactions during organogenesis. Excellent animal studies revealed that the initial step of liver organogenesis, liver bud formation, requires the dynamic orchestrations between epithelial cells, mesenchymal cells and endothelial cells prior to vascular function. Here, we successfully created human liver bud-like tissues in vitro from human induced pluripotent stem cells (hiPSC) by recapitulating organogenesis. Without the aid of scaffolds, hepatic specified hiPSC were three-dimensionally organized into liver bud (hiPSC-LB) under the presence of endothelial and mesenchymal lineages. hiPSC-LB shared many similarities with murine E10.5 liver bud determined by histological and gene expression analyses. Furthermore, hiPSC-LB was successfully stimulated and organized into mature liver tissue via in vivo transplantation without any recipient liver damage. As a transplant model, we used a transparency cranial window model because of the high vascularity and easy optical access to monitor the structural organization. Surprisingly, gross observation showed quick perfusion of hiPSC-LB through the host blood vessels within just a few days post transplantation. Intra-vital confocal microscopy confirmed that fluorescence-labeled human endothelial networks are fully functional inside the liver tissue. Quantitative analyses of generated vasculatures resembled that of adult liver tissues. Inside the created liver tissues, human CD31 positive endothelial cells formed microvascular networks confirmed by whole mount immunostaining. Enzyme-linked immunosorbent assay (ELISA) showed the human albumin production from the collected serum samples in vivo, indicating the emergence of hepatic functions. They also showed human-specific drug metabolizing capabilities 30 days post transplantation. Immunohistological analyses revealed the albumin, cytokeratin 8.18, and ZO-1 positive hepatic cord formation. To the best of our knowledge, this is the first report demonstrating the creation of human functional organ with vascular networks from pluripotent stem cells. Cell replacement therapy is currently the major way of regenerative medicine. In fact, tremendous number of studies reported the success in functional cell production via stepwise differentiation or direct reprograming approach. However, the disappointing clinical outcomes of cell transplantation methods highlighted the novel strategies aiming at three-dimensional human organ generation. Our techniques discovered here pave the new way for generating a complex and vascularized organ as an alternative to organ transplantation.

DUXO, A NOVEL DOUBLE HOMEOBOX TRANSCRIPTION FACTOR, IS REQUIRED FOR GASTRULA ORGANIZER FORMATION IN HUMAN EMBRYONIC STEM CELLS

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The mammalian body plan is established by the organizer during gastrulation, concomitant with the differentiation of the epiblast into the three germ layers. Previously, we differentiated human embryonic stem cells (hESCs) into GOOSECOID (GSC) expressing cells, and showed that they harbor the function of the gastrula organizer, as they induce secondary axes when injected into frog embryos. Here we report that these human organizer cells express DUXO (DUX of the Organizer), a novel member of the Double-homeobox (DUX) family of transcription factors, an enigmatic group of genes unique to placental mammals. Both of DUXO's homeodomains share high similarity with those of siamois and twin, the initial organizer inducers in the amphibian embryo. DUXO over expression in hESCs induces organizer related genes, whereas its knock down hampers formation of the organizer and its derivatives. Furthermore, we show that DUXO regulates GSC, the canonical organizer marker, in a direct manner. Finally, injection of DUXO mRNA into Xenopus embryos together with low amounts of WNT induced secondary axes, proving that DUXO can indeed initiate the organizer molecular network. Thus, we conclude that DUXO is a major regulator of the human organizer.

CROSS-TALK OF SIGNALING NETWORKS REGULATE HUMAN STEM CELL PLURIPOTENCY

Singh, Amar M.[1], Reynolds, David[1], Cliff, Tim[1], Ohtsuka, Satoshi[1], Mattheyses, Alexa L.[2], Sun, Yuhua[1], Menendez, Laura[1], Kulik, Michael[1], Dalton, Stephen[1]

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The mechanisms by which signaling networks integrate to regulate the balance between self-renewal and differentiation remain largely unclear. By maintaining human pluripotent stem cells in a fully defined, serum-free media, we have dissected the interacting signaling cascades critical to self-renewal. Here we will describe how an elaborate cross-talk between the PI3K/Akt, Activin A/Smad2,3, Raf/Erk, and Wnt/β-catenin signaling pathways cooperate to regulate self-renewal mechanisms. Specifically, we find that PI3K/Akt plays a dual role to inhibit Erk and regulate Activin A/Smad2,3 thresholds to promote self-renewal. Upon differentiation by loss of PI3K/Akt, Wnt and Erk cooperate to inactivate Gsk3β, enabling β-catenin to synergize with Smad2,3 to activate target genes. Our findings provide important insight into regulation of self-renewal and differentiation of human pluripotent stem cells, while resolving the numerous conflicting reports in the literature (see Singh et al., Cell Stem Cell, In Press).

REPROGRAMMING HUMAN SOMATIC CELLS TO A PLURIPOTENT STATE USING OOCYTES

Egli, Dieter

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With continued analysis of induced pluripotent stem cells (iPSc), questions have arisen regarding the equivalency of these cells to human embryonic stem cells (hESc) based on gene expression profiles, DNA methylation profiles, novel mutations, copy number variations and differentiation capacity. Stem cells derived through mouse somatic cell nuclear transfer (SCNT) have been shown to be more faithfully reprogrammed than iPSCs; such analyses following human SCNT has not previously been possible. We have recently derived pluripotent stem cell lines by nuclear transfer into oocytes. These stem cell lines contain a diploid genome of the somatic cell as well as a haploid genome of the oocyte. Genome-wide quantification of expressed SNPs revealed allelic

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ratios consistent with equal expression from each genome, suggesting that somatic cell memory was erased. Thus, the ability of the human oocyte to reprogram somatic cells to a pluripotent state was demonstrated. These cell lines now allow us to compare reprogramming using SCNT to reprogramming using defined factors. Sequencing of several SCNT cell lines, as well as of iPS cell lines made from the same somatic cell has been undertaken and we will present our progress in comparing iPS cells and SCNT ES cells as well as our continued progress in reprogramming human cells using oocytes.

OPPOSING WNT SIGNALLING TRANSCRIPTIONAL EFFECTORS ARE CRITICAL FOR REPROGRAMMING TO PLURIPOTENCY

Papp, Bernadett[1], Ho, Ritchie[1], Hoffman, Jackson A.[2], Merrill, Bradley J.[3], Plath, Kathrin[1]

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WNT signalling stimulates self-renewal and regulates differentiation of mouse embryonic stem cells (ESCs) and can enhance transcription factor-mediated reprogramming to pluripotency. Our work focuses on the critical gap in understanding how Wnt signalling and its transcriptional effectors (the Tcf famliy members: Tcf3, Tcf4, Tcf1, Lef1) function in reprogramming. We find that the Tcf3 expression level rises during the early phase of reprogramming and that overexpression of Tcf3 enhances early reprogramming steps, depending on its DNA-binding or co-repressor interaction domain. Conversely, deletion of Tcf3 enhances reprogramming at late steps and can even induce the conversion of late reprogramming intermediates to pluripotency. Molecularly, upregulation of pluripotency genes is critical in the final steps of reprogramming. Genomewide Tcf3 ChIP-SEQ analysis in the starting cell type and late intermediate cells identified that majority of Tcf3 targets change during reprogramming. Tcf3 leaves its somatic targets and is recruited to specific differentiation genes as well as key pluripotency targets in late reprogramming intermediates. In agreement with these data, Tcf3 knockdown causes downregulation of many of these differentiation genes, as well as allows upregulation of Tcf3 targeted pluripotency genes. Overexpression completely blocks the induction of pluripotency genes. Together, our data indicate that Tcf3 promotes the early reprogramming steps but impairs late events. Strikingly, brief overexpression of Tcf3 early in reprogramming combined with knockdown of Tcf3 in the late phase allows very efficient reprogramming even in the absence of Sox2 (e.g. Oct4-cMyc-Klf4 (“OCK”)). Normally OCK reprogramming completely fails to reprogram. These data confirm our conclusion that elevated Tcf3 drives early reprogramming productively but inhibits late ones, and define two phases of reprogramming with distinct requirements for the transcriptional repressor Tcf3. Expanding our analysis to other Tcf family members, we found that Tcf4 expression and function relatively similar to that of Tcf3; its knockdown induces pluripotency in late reprogramming intermediates. In contrast, Tcf1 is upregulated during reprogramming and knockdown of Tcf1 impairs the establishment of pluripotency triggered by Tcf3 or Tcf4 knockdown in late intermediates. Lef1 expression is highest in the starting fibroblasts and reduction of Lef1 levels enhances early events. Overexpression of Tcf3, but not of Tcf4, Lef1 or Tcf1 enhances early colony formation, suggesting a specific function of Tcf3 early in reprogramming.
In summary, we propose that reprogramming is bi-phasic with respect to the activitites of all WNT effector transcription factors. We suggest that the competion of different Tcf family members matters for successful reprogramming. Tcf3/Tcf4 and Lef1/Tcf1 have opposing roles in reprogramming, and their requirement is different in early and late phases of reprogramming. Currently we are analyzing how the activity of the Wnt signalling pathway modulates the activiy of the Tcf factors in reprogramming.

Concurrent Session IB — New Technologies for Controlling Stem Cell Behavior

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A CHEMICAL APPROACH FOR HIGH-RESOLUTION FLUORESCENCE IMAGING AND 3D RECONSTRUCTION OF TRANSPARENT MOUSE BRAIN

Miyawaki, Atsushi, Hama, Hiroshi, Kurokawa, Hiroshi, Kawano, Hiroyuki, Sakaue-Sawano, Asako

RIKEN Brain Science Institute, Wako, Saitama, Japan

Optical methods for viewing neuronal populations and projections in the intact mammalian brain are needed, but light scattering prevents imaging deep into brain structures. Here, we describe a chemical approach to imaging fixed brain tissue based on Scale, an aqueous reagent that makes biological samples optically transparent but completely preserves fluorescent signals within the clarified structures. In Scale-treated mouse brain, neurons labeled with genetically-encoded fluorescent proteins could be visualized at unprecedented depth in millimeter-scale networks and at subcellular resolution. This improved depth and scale of imaging permitted comprehensive 3-dimensional reconstructions of cortical, callosal, and hippocampal projections whose extent was limited only by the working distance of objective lenses. In the intact neurogenic niche of the dentate gyrus, Scale allowed the quantitation of distances of neural stem cells to blood vessels. These findings indicate that the Scale method will be useful for light microscopy-based connectomics in brain and other tissues.

DYNAMIC PROGRESSION ANALYSIS OF IPS CELL REPROGAMMING AND DIFFERENTIATION BY HIGH-DIMENSIONAL MASS CYTOMETRY

Zunder, Eli R.[1], Lujan, Ernesto[2], Wernig, Marius[2], Nolan, Garry P.[1]

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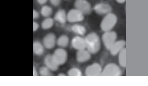
Pluripotent stem cell biology holds great promise for human disease modeling and regenerative therapy. The ability to understand and control the course of iPS cell reprogramming and iPS/ES cell differentiation is fundamental to realizing this promise, but analysis of these processes is complicated by highly heterogeneous cell cultures, in which the cell type of interest is often rare and may be obscured by pooled analysis methods such as mRNA microarray analysis or western blotting. To investigate these processes at the single-cell level, reprogramming MEFs and differentiating iPS/ES cells were analyzed by a CyTOF® mass cytometer (DVS Sciences). Mass cytometry is a novel flow cytometry technique that uses rare earth metal isotopes instead of fluorophores for antibody labeling and detection, here allowing 34 markers of pluripotency, differentiation, cell cycle status, and cellular signaling to be monitored simultaneously at the single-cell level. iPS cell reprogramming was investigated using a Secondary MEF strain with DOX-inducible Oct4, Sox2, Klf4, and c-Myc. After DOX induction, samples were collected every day over the



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course of reprogramming, generating a comprehensive model that describes the transition to pluripotency, including necessary milestones as well as non-productive paths. iPS/ES cell differentiation was investigated by inducing differentiation to the three germ layers with Retinoic Acid (Ectoderm), BMP4 (Mesoderm), or Activin (Endoderm). Samples were collected every day over the course of differentiation to each germ layer, generating a comprehensive set of models that describe the unique cell populations that arise during differentiation as well as the transitions that occur between cell types. To identify and describe the unique populations that exist within high-dimensional mass cytometry data sets, Spanning-tree Progression Analysis of Density-normalized Events (SPADE) was used, an unsupervised approach that identifies unique cell populations in n-dimensional space and arranges them hierarchically into a minimum spanning tree. To identify and describe the transitions that occur between these unique populations over time, the SPADE algorithm was extended to include time information in the tree building process. The cellular state of each population in the Time-SPADE progression tree, revealed by 34 cellular markers of pluripotency, differentiation, cell cycle status, and cellular signaling, identifies key points for small molecule intervention that can be used to interrogate and to control iPS cell reprogramming and iPS/ES cell differentiation. Mass cytometry coupled with Time-SPADE analysis is a powerful new approach that is generally applicable to progression analysis of cell populations in dynamic systems, including hematopoiesis, transdifferentiation, oncogenesis, and cancer evolution, in addition to iPS cell reprogramming and differentiation.

GENERATION AND USE OF A ROSA26-BASED CONDITIONAL AND INDUCIBLE (COIN) ES/IPS CELL SYSTEM TO STUDY CELLULAR REPROGRAMMING AND GENE FUNCTION DURING LINEAGE-DIRECTED DIFFERENTIATION

Haenebalcke, Lieven[1], Goossens, Steven[1], Dierickx, Pieterjan[1], Bartunkova, Sonia[1], D'Hont, Jinke[1], Haigh, Katharina[1], Naessens, Michael[1], Bondue, Antoine[2], Farhang Ghahremani, Morvarid[1], Hochepied, Tino[1], Wirth, Dagmar[3], Haigh, Jody[1]

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Mouse embryonic stem (mES) cell-based transgenesis is a powerful and widely used method to study gene functions in vitro and in vivo in the context of the whole organism. Many different techniques including recombinase mediated cassette exchange (RMCE) reactions and drug-inducible systems have been developed to create customised and complex alleles within the mouse genome. However, these technologies often require time consuming cloning steps and involve inefficient targeting strategies or rely upon random transgene integration into the genome that is prone to inefficient transgene expression or position variegation. These limiting factors prevent such technologies from being widely applicable and more frequently used for studying gene function in mES cells or in hereof derived mice. We have therefore developed a highly efficient system to target conditional or conditional and inducible (COIN) constructs to the ROSA26 locus of mES cells. By combining the conditional Cre/loxP system with or without the inducible Tet-on system and a recombinase mediated cassette exchange (RMCE) system into Gateway-compatible vectors, we can rapidly generate monoallelic gain-of-function constructs that can be targeted to the ROSA26 locus with efficiencies up to 100%. This system allows expression of genes to investigate their biological functions. Based upon this technology we have created a reprogrammable mouse strain that allows iPS cell generation through conditional and inducible expression of the OSKM reprogramming factors from within the ROSA26 locus. After reprogramming these factors were efficiently replaced by other genes of interest, e.g. to enhance lineage-directed differentiation, using a promoter-trap-coupled RMCE reaction. This mES cell technology as well as the ROSA26 iPS mouse model and derived exchangeable iPS cells will be of great value in studying gene functions and their effects on cellular reprogramming and lineage-directed differentiation in future biomedical research.

CULTIVATING A SINGLE CELL VISUALIZATION AND MANIPULATION WORKFLOW: A COMBINED FLUORESCENCE AND ATOMIC FORCE MICROSCOPY APPROACH

Ach, Robert A., Bernick, Kristin, Tom-Moy, May, Scheffer-Wong, Alicia, Yamada, N. Alice

Molecular Cell Biology, Agilent Laboratories, Santa Clara, CA, USA

Fluorescence microscopy is one of the best methods to follow biological events at the single cell resolution, and fluorescence in situ hybridization (FISH) is a powerful technique for studying the structure, organization, and localization of nucleic acids within individual cells. We have combined our ability to select in silico the highest performance metrics for FISH probe design with de novo chemical synthesis of DNA in massively parallel reactions to produce high resolution fluorescent paints for DNA and RNA. Using these oligonucleotide library-derived probes on DNA, human genomic regions as small as 1.8 kb and as large as whole chromosomes can be visualized in both metaphase and interphase cells using the same simple assay protocol. We have also been able to detect the localization of a variety of both coding and non-coding RNAs in fixed cells, using both conventional wide-field fluorescence and structured illumination microscopy. Using probes designed specifically to transcribed vs. non-transcribed regions, we have been able to simultaneously detect DNA and RNA from the same locus in the same single cell. Our oligo FISH methods are also compatible with the co-detection of cellular proteins by immunocytochemistry. Concurrently, we have coupled fluorescence detection of individual cells with atomic force microscopy (AFM) to introduce the ability to interact with specifically labeled cells. AFM-based force measurements indicate that we are able to puncture the cell membrane and enter a specific, fluorescently-labeled cell for interaction or stimulation, followed by live cell monitoring for response. We are working towards combining the ability to fluorescently label nucleic acids and proteins in live cells with AFM measurements to develop a workflow solution for single cell visualization and manipulation.

GLOBAL GENE EXPRESSION ANALYSIS BY SEQUENCING OF CDNA DERIVED FROM SINGLE NUCLEI

Lasken, Roger S.[1], Grindberg, Rashel V.[2], Yee-Greenbaum, Joyclyn[1], McConnell, Michael[3], Novotny, Mark[1], O' Shaughnessy, Andy[4], Georgina Lambert, Georgina[5], Georgina Lambert, Georgina[5], Araúzo-Bravo, Marcos J.[6], Lee, Jun[7], Lin, Xiaoying[8], Galbraith, David[9], Gage, Fred H.[3]

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A recently developed protocol allows cDNA synthesis from a single cell and next generation DNA sequencing to obtain global gene expression profiles (Tang, F., 2009). We have extended this method to single nuclei which contain sufficient steady levels of mRNA to obtain high quality cDNA. To develop this capability, an NPC line was chosen that has cytoplasmic expression of yellow fluorescent protein (YFP). The presence or absence of YFP was used to verify the identity of whole cells and nuclei respectively. Whole transcriptome sequencing for nuclei was comparable to that for single cells. The ability to use tissues as the source of nuclei was also confirmed. Single nuclei were obtained by micromanipulation from homogenized mouse dentate gyrus tissue. cDNA sequencing from the nuclei gave comparable results to those from single cell controls with about 20,000 expressed genes measured. The ability to carry out global transcriptomics from nuclei will enable investigations of brain tissues where highly interconnected neurons would be nearly impossible to isolate intact.

A QUANTITATIVE PROTEOMIC PANORAMA OF CELLULAR REPROGRAMMING

Hansson, Jenny[1], Reiland, Sonja[1], Polo, Jose M.[2], Hochedlinger, Konrad[3], Krijgsveld, Jeroen[1]
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Reprogramming of somatic cells can be induced by the forced expression of a cocktail of a few transcription factors, causing cells to revert to the pluripotent state. It has been demonstrate that these induced pluripotent stem cells (iPS cells) are functionally very similar to embryonic stem cells, however, the molecular programs underlying the reprogramming process are largely unknown. While initial principles have emerged from transcriptome and epigenetic studies, data at the proteome level are lacking. To fill this gap, we have taken a quantitative proteomic approach to determine the timing and magnitude of proteome dynamics across the entire course of reprogramming. This should help to get mechanistic insights into the gain of pluripotency, while creating an opportunity to identify proteins that may define specific (intermediate) cellular states.

Reprogramming was induced in secondary mouse embryonic fibroblasts (MEFs) by expressing Oct4, Klf4, Sox2 and Myc. Cells were isolated by FACS sorting based on Thy, GFP-Oct4 and SSEA expression at 3-day intervals. In total, 6 cellular populations were collected: MEFs, 4 intermediate stages at 3, 6, 9 and 12 days after induction of reprogramming, and fully reprogrammed iPS cells (day 15). For in-depth quantitative proteomic profiling, cellular proteins extracted at these time points were digested and peptides were differentially labeled with stable isotopes followed by fractionation and analysis by mass spectrometry (nanoflow LC coupled to an Orbitrap Velos Pro).

From reprogramming experiments performed in biological duplicate, 7918 proteins were identified and quantified across the 6 sampled time points. Together, this represents one of the biggest proteomic datasets known to date, and for the first time offers a highly detailed view on proteome dynamics during cellular reprogramming. Cluster analysis of protein expression profiles revealed stage-specific expression of many proteins and protein classes, representing biological processes occurring at distinct time points or intervals. For instance, mensenchymal-to-epithelial transition (MET) was strongly induced within the first 3 days, while changes in glycolytic enzymes occurred more gradually. The data cover large numbers of regulatory proteins, including 558 transcription factors, 354 kinases, 111 phosphatases, and 615 proteins associated with the cell cycle. While some of these don't change in expression appreciably, others specifically peak in an early, intermediate or late stage, suggesting that they may act in a stage-specific manner. For instance, several proteins that are known to promote reprogramming (e.g. Sall4, Lin28) have their maximal expression relatively late, and are accompanied by several other transcription factors that may now be postulated to fulfill a similar role. Interestingly, the presence of several proteins was restricted to just one or two stages, possibly representing candidates that drive stage-specific molecular programs. This is of particular interest for proteins selectively expressed in the so far poorly defined intermediate cells. Among the many hundreds of plasma membrane proteins identified in this study, many emerge only after 6, 9 or even 12 days. These are prime candidates to specifically target intermediate cells, providing a valuable tool to isolate these elusive cells for functional and molecular studies. Together, this study provides an unprecedented view on reprogramming, at the protein level.

Concurrent Session IC — Stem Cells & Tissue Engineering

Thursday, June 14, 2012, 1:30 pm - 3:15 pm

FUNCTIONAL TISSUE RECONSTRUCTION: SEED VS. SOIL

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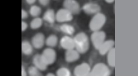
University of Pittsburgh, Pittsburgh, PA, USA

The phenomenon of regeneration is limited to select tissues in most adult mammals, including the bone marrow, epithelium of the skin, and gastrointestinal tract, and the liver. Stem and progenitor cells play obvious key roles in this regenerative process. Although stem and progenitor cells exist in other tissues, the default response to injury involves a proinflammatory response which results in downstream fibrous connective tissue deposition, i.e. scar tissue. The causative factors for the lack of a functional regenerative response is not totally understood but possible reasons include an unfavorable microenvironment at the wound site, the absence of a timely and robust recruitment of appropriate stem, and progenitor cells to the site of injury, and/or an innate immune response phenotype that is strongly biased toward a proinflammatory response as opposed to a regulatory and reconstructive response. Weather the cell based components (seed) or the surrounding milieu (soil) plays the more dominant and determinant role in this process is unknown. Therapeutic strategies directed at controlling either or both hold the potential to alter the default scar tissue response toward a more functional outcome.

HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED KERATINOCYTE PRECURSORS CROSSTALK WITH HAIR INDUCTIVE MESENCHYME AND RECONSTITUTE HAIR FOLLICLES IN VIVO

Veraitch, Ophelia K.[1], Kobayashi, Tetsuro[1], Imaizumi, Yoichi[2], Akamatsu, Wado[2], Amagai, Masayuki[1], Okano, Hideyuki[2], Ohyama, Manabu[1]
[1]Department of Dermatology, Keio University School of Medicine, Tokyo, Japan, [2]Department of Physiology, Keio University School of Medicine, Tokyo, Japan

The hair follicle is a dynamic miniorgan of the skin that self-renews throughout our lives. Its morphogenesis and cyclic regeneration are enabled by intense and cooperative epithelial



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mesenchymal interactions between keratinocytes (KCs) and hair inductive mesenchymal component, the dermal papilla (DP). Previous attempts to regenerate human hair follicles have implied that human embryonic and neonatal KCs more efficiently respond to trichogenic dermal signals than adult KCs. However, supply of such juvenile and plastic KCs is extremely limited. Human induced pluripotent stem cells (iPS) may be a novel and favorable cell source for hair follicle bioengineering due to high proliferative capacity, ethical advantages and the potential to generate juvenile KCs with innate plasticity. In this study, we generated human iPS derived-keratinocyte precursors (KCPs) and investigated whether they represent a promising material to regenerate human hair follicles. Three iPS cell lines (B7, WD39 and WDT2) generated with 4 or 3 Yamanaka factors (Oct3/4, Sox2, Klf4 +/- cMyc) were converted into embryoid bodies (EBs), exposed to retinoic acid and bone morphogenetic protein-4 in floating culture to form cystic EBs. When plated on collagen I-coated dishes and cultured in defined KC medium, all lines of cyctic EBs gave rise to KCP colonies expressing early KC markers keratin 18, p63 and keratin 14. However, only B7 iPS-KCPs further up-regulated KC differentiation markers keratin 1, keratin 10, basonuclin and involucrin in high calcium conditions (1.2mM). To assess the ability to interact with hair inductive mesenchyme, iPS-KCPs were co-cultured with human DP cells. In response to DP signals, each iPS-KCP line differentially increased hair follicle KC markers, keratin 75, LEF1, MSX2 and TRPS1. Interestingly, B7 iPS-KCPs up-regulated keratin 75 and MSX2 more significantly than normal human KCs ($P<0.05$). In addition, DP cells exclusively responded to signals from B7 iPS-KCPs and up-regulated DP biomarkers ALP, LEF1 and BMP4. The potency of B7 iPS to generate KCPs that more efficiently interacted with DP cells than other iPS- KCPs could be partially explained by the observation that B7 iPS cell lines innately differentiated into ectoderm compared to other two iPS cells lines. Finally, either B7 derived iPS-KCPs or human adult KC was co-transplanted with trichogenic mouse dermal cells subcutaneously into nude mice. Strikingly, B7 KCPs but not adult KCs generated large follicular cysts containing complete hair follicle structures, which expressed human bulge markers, keratin 15, CD200 and type 2 iodothyronine deiodinase. This is the first demonstration that human iPS-KCPs can replicate epithelial-mesenchymal interactions in hair follicle homeostasis and contribute to hair follicle regeneration in vivo. The data also emphasizes the importance of pre-assessing the functional aspects of individual iPS lines in order to maximize tissue regeneration efficiency.

A PROSPECTIVE CLINICAL STUDY TO EVALUATE THE SAFETY AND EFFICACY OF AUTOLOGOUS MARROW-DERIVED MESENCHYMAL CELLS INTEGRATED WITH POROUS CERAMICS SCAFFOLD FOR BONE DEFECT AFTER BONE TUMOR REMOVAL

Myoui, Akira[1], **Murase, Tsuyoshi**[2], **Fujimoto, Tetsuho**[2], **Nishikawa, Masatka**[3], **Hashimoto, Nobuyuki**[2], **Tamai, Noriyuki**[2], **Takenaka, Satoshi**[2], **Ueda, Takafumi**[4], **Kakunaga, Shigeyuki**[4], **Ezoe, Sachiko**[1], **Shimokawa, Toshio**[5], **Ohgushi, Hajime**[6], **Yoshikawa, Hideki**[2]

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INTRODUCTION: We performed a prospective clinical study to evaluate the safety and the bone defect repairing efficacy of autologous marrow-derived mesenchymal cells (MMCs) integrated with porous calcium hydroxyapatite (IP-CHA) artificial bone. METHODS: Patients with benign bone tumors or tumor-like conditions were subjected to the current study. Bone marrow aspirates of the patients were cultured in medium supplemented with autologous serum at the Cell Processing Center in the Medical Center for Translational Research, Osaka University Hospital. Attaching cells were introduced into the pores of IP-CHA and further cultured in medium containing dexamethasone to induce osteoblastic differentiation. After resection of the bone tumors, the defects were replaced with autologous MMC/IP-CHA composites. The safety was evaluated by the incidence of adverse events. Primary efficacy endpoint was the improvement of bone defect evaluated by plain X-ray according to the criteria of bone defect repair (Grade 0: osteolysis, 1: no change, 2: minor incorporation, 3: major incorporation). Secondary evaluation endpoints included overall radiographic evaluation, bone mineral density (BMD) evaluated by dual-energy X-ray absorptiometry (DXA) and densitometry on plain X-ray image with aluminum steps, functional evaluation of the affected limb, and comprehensive QOL assessment (SF-36). The observations were made periodically until 48 weeks after surgery. RESULTS: Eleven patients were registered in the study and the protocol treatment was completed in 9 cases. In all 9 cases, prepared MMC/IP-CHAs met all quality specifications including cell surface marker. The median CD105+/34- cell ratio was 92.2%. There were no postoperative local infection and no newly developed neoplasm. Total number of adverse events was 52 and none of them caused clinically important problems. One of the patients was hospitalized for thorough examination of the electrocardiogram abnormality, but it was judged to have no direct causal relation with this protocol treatment. After completion of the observation period, local recurrence of giant cell tumor was confirmed in one case. Since the possibility that the relapse may have been accelerated by this treatment cannot be denied, it must be evaluated carefully in the future. In the evaluation of efficacy, the number of Grade 3 occurrence at 48 weeks after transplantation was six out of nine cases (66.7%, $p = 0.041$). Incidence of improvement (Grade2 or higher) increased with time; 44.4% at week 8, 88.9% at week 48 ($p <0.001$). Among the secondary endpoints, overall radiographic evaluation revealed successful repair in all cases. BMD evaluated by DXA increased with time. The amount of BMD change compared to its initial value (week 1) was significant at week 24 ($p = 0.023$) and week 48 ($p=0.047$). Functional evaluation also showed improvement. However, no significant change was found in SF-36 variables. CONCLUSION: Our study suggests that the transplantation of autologous marrow-derived mesenchymal cells integrated with porous ceramic scaffold is a safe and effective method for the repair of bone defect after the removal of bone tumors. Further investigation is required to elucidate the advantage over conventional therapies.

PERFUSION-DECELLULARIZATION OF PANCREAS AS A SCAFFOLD FOR THE DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS INTO INSULIN-PRODUCING CELLS

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Introduction: Type I Diabetes affects over 1 million people in the United States. While islet transplantation has proved to be a

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promising therapeutic strategy, it is still hindered by lack of donor tissue. Embryonic stem cells (ESC) have emerged as an alternative cell source owing to its virtually unlimited replicative capacity and the potential to differentiate into a variety of cell types, including islet-cells. However, ESC derived islet-cells are currently limited in their yield and functionality. Current differentiation strategies primarily involve various inducer/ repressor concoctions with less emphasis on the substrate. Governed by the understanding that the pancreatic matrix plays a significant role during islet development and maturation, we hypothesize that the pancreatic ECM will significantly enhance the functionality of ESC derived islet cells. To this effect, we propose that perfusion-decellularization of whole pancreas could yield an acellular scaffold suitable for pancreatic differentiation. Materials and Method: Cadaveric pancreata were isolated from adult mice (n=8) and mounted on a perfusion apparatus. Ionic detergent, 0.1% SDS was perfused via the pancreatic duct until tissues were translucent and white in color (24-28 hrs). Acellularity was quantified via DAPI staining and PicoGreen DNA assay. Immunostaining were done to characterize the retention of the ECM protein before and after decellularization of the pancreas. For 2-D in-vitro culture, thick slices (50µm) of acellular matrix were seeded with Beta-TC-6 or hESC (2x105 cells) to examine the proliferation, survival and differentiation (n=3) of the seeded cells. For whole organ recellularization, Beta-TC-6 or hESC (30x106) were introduced into the acellular scaffold via the pancreatic duct in 3 steps, with 20 min interval between each step and then mounted on a perfusion apparatus to allow dynamic culture for 4 days. Both in-vitro and whole organ recell constructs were analyzed using immunostaining, TUNEL, and qRT-PCR. Results and Discussion: Perfusion via the pancreatic duct with 0.1% SDS resulted in complete decellularization of murine pancreas. DAPI staining and DNA quantification confirmed the removal cells and residual DNA. Microstructure and major ECM components such as collagen I, collagen IV, fibronectin and laminin were preserved in acellular pancreas. In the 2-D in-vitro culture, Beta-TC-6 showed higher proliferation when seeded on pancreatic matrix (P<0.05). qRT-PCR of hESC differentiated on 2-D pancreatic matrix showed 52.7±10.8 fold increase in PDX1 (P<0.05), and 92.7±12.7 fold increase in insulin gene expression (P<0.05) compared to Matrigel. In the 3-D whole organ culture construct, cytocompability of the scaffold was determined by IHC - hESC were engrafted (hNuclei marker positive) and low apoptotic cells were detected (<10% TUNEL positive). Engrafted cells were positive for PDX1 and C-peptide from immunostaining - confirming the differentiation of engrafted hESC into insulin-producing cells. Conclusion: Perfusion-decellularization of pancreas efficiently removes cells while retaining ECM protein and microstructure. This perfusable 3-D whole organ scaffold yields functional tissue construct that favored pancreatic differentiation when seeded with hESC. Further proteomic studies are underway to establish a comprehensive and mechanistic understanding of cell-matrix interactions that contributed to the pancreatic differentiation of hESC.

KIDNEY DERIVED MICRO ORGAN MATRICES PROVIDE AN OPTIMIZED ENVIRONMENT TO SUPPORT THE DIFFERENTIATION OF HUM AN EMBRYONIC STEM CELLS TOWARDS A RENAL LINEAGE

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We have developed a system for preparing micro-scaffolds from various organs. Kidney derived micro-scaffolds are particularly relevant because of the complex micro-architecture of the kidney. In the present work we explore the organization and differentiation capacities of Human Embryonic Stem (HES) cells and of Embryoid Bodies (EBs) on kidney derived micro-scaffolds of murine origin. We demonstrate, using High Energy Scanning Electron Microscopy (HESEM) and various staining techniques that the HES remain viable and expand according to the complex structure dictated by the scaffold. Two weeks after seeding dissociated EBs on kidney-derived micro-scaffolds, transcription levels of Renin and of Nephrin were found to be 125 fold and 42 fold respectively compared to the same dissociated EBs grown in standard culture conditions. We also found, in preliminary experiments, a 64 fold increase in expression of Nephrin 17 days post seeding HES cells on kidney-derived micro-scaffolds compared with HES cells grown under normal culture conditions. We believe this approach opens the way for examining differentiation of HES in a more in vivo like system which takes into consideration the natural kidney microenvironment.

PRODUCTION OF FUNCTIONAL ISLETS WITH A THREE-DIMENSIONAL STRUCTURE FROM IPS CELLS IN VITRO

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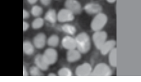
Islets of Langerhans are a pancreatic endocrine compartment consisting of insulin-secreting β cells together with several other hormone-secreting cells and play a central role in glycemic control. While the transplantation of islets is a promising therapeutic option for Type I diabetes that destroys β cells resulting in insulin deficiency, donor shortage is a major problem and a system of preparing a sufficient quantity of functional islets is required. To this end large efforts have been made to generate in vitro functional islets from various cell sources. While some insulin-producing cells or immature pancreatic cells were generated in vitro from ES and iPS cells, it has been difficult to produce islets with proper functions and a three-dimensional (3D) structure. We found that pancreatic cells of mouse embryonic day 16.5, just before forming islets, were able to develop cell aggregates consisting of β cells surrounded by glucagon-producing α cells, a structure similar to murine adult islets. Moreover, the transplantation of these cells improved blood glucose levels in hyperglycemic mice, indicating that functional islets are formed in vitro from fetal pancreatic cells at a specific developmental stage. We also found that tube-like structures were formed in this culture system. The tube-like structures were hollow and composed of a single layer of CK19-positive cells, similar to pancreatic ducts. Interestingly, hormone-producing cells were emerged from those duct-like structures before the formation of islet-like 3D structures. These results suggest that this culture system recapitulates organogenesis of pancreatic ducts and islets. To generate islets from iPS cells, we adopted these culture conditions to the differentiation protocol of mouse iPS cells and developed a two-step



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system to generate islets, i.e. immature pancreatic cells were first produced from iPS cells, and then transferred to culture conditions that allowed the formation of islets from fetal pancreatic cells. These islets exhibited a distinct 3D structure similar to adult pancreatic islets and secreted insulin in response to glucose concentrations. Transplantation of the islets improved blood glucose levels in hyperglycemic mice. We then applied this culture system to human iPS cells and obtained cell aggregates similar to human adult islets that expressed insulin and glucagon.

Concurrent Session ID — Stem Cell Signaling and Niches

Thursday, June 14, 2012, 1:30 pm - 3:15 pm

GUT EPITHELIAL TISSUE MAINTENANCE IN HEALTH AND DISEASE

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Epithelial tissues are the most common site of malignancies in the human body. Although epithelia share many general features, they are uniquely adapted to their specific environment. Gut epithelium is the most dynamic. It is continuously turned over throughout the lifetime of the organism. Once 'born', differentiating cells live for 3 to 5 days and 20 - 50x106 cells are shed from the human gut every minute. This extremely rapid turnover is ensured by the unique organisation of gut tissue and the processes that govern it: stem cells reside in specialised invaginations, crypts, and produce progenitors for the different lineages that populate gut epithelium. Differentiation is accompanied by migration from the crypt base towards the gut lumen where cells are exfoliated. Tumourigenic changes disrupt the normal balance between differentiation and stem cell maintenance and tumours contain increased number of cells positive for stem cell markers. Careful control of stem cell numbers is also important for gut elongation, which proceeds via crypt fission, the bifurcation of crypts initiated from the crypt bottom. We found that fission is accompanied by increasing stem cell numbers. De-regulation of normal stem cell number in tumour growth is also suggested by the fact that crypt fission drives adenoma growth and precancerous tissue undergoes fission more frequently. Determining how stem cells are normally maintained and regulated is crucial for understanding early steps in tumourigenesis. To determine how stem cell maintenance and tissue maintenance are coupled we are using a number of approaches including high- resolution three-dimensional imaging of live and fixed tissue. Specifically, we are investigating the relationship between cell proliferation and crypt fission in normal and precancerous gut tissue. Maintenance of adult stem cells can involve a stem cell 'niche' that contains signals required for stem cell identity. In this case, stem cell number can be regulated by placement of stem cell daughters within the niche. Other mechanisms to regulate stem cell numbers include their ability to divide symmetrically to produce identical daughter cells, either two differentiating or two stem cells, or asymmetrically, to produce one stem cell and one differentiating cell. Evidence for both processes exist. Long-term labelling studies in gut epithelium concluded that stem cell turnover and maintenance follows a pattern in which stochastic stem cell loss by differentiation is compensated for by symmetric self-renewal of neighbouring stem cells. On the other hand, examining mouse and human gut tissue at high resolution in three dimensions showed that stem cells align their spindles perpendicularly to the apical epithelial surface at least 50% of the time, unlike non-stem cells, which tend to align their spindles in parallel or randomly depending on the gut regions examined. In perpendicularly aligned spindles, long-term label-retaining DNA was asymmetrically distributed towards the basally placed daughter suggesting that they represent asymmetric divisions. In pre-cancerous tissue, specifically tissue heterozygous for Adenomatous Polyposis Coli (APC), the most commonly mutated gene in colorectal tumours, stem cell divisions had no preferential alignment or other markers of asymmetry. This correlated with a significant increase in the number of cells in precancerous crypts and illustrates the close relationship between stem cell behaviour and tissue organization.

COMPLEX TIMING OF HUMAN EPIDERMAL STEM CELL FUNCTION BY THE CIRCADIAN MOLECULAR CLOCK

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Epidermal stem cells ensure that skin homeostasis is maintained. In human skin, epidermal stem cells alternate cycles of dormancy and proliferation before committing to differentiation. Stem cell behavior is dictated by cell intrinsic molecular mechanisms coupled to the communication with the surrounding microenvironment (niche) and systemic cues. However, the nature of such communication and its spatiotemporal regulation is still poorly understood. We have previously shown that the circadian machinery fine-tunes the response of murine epidermal stem cells to niche signals, and that perturbation of this clock machinery results in premature epidermal ageing (Janich et al, Nature 2011). We have now studied how the clock machinery modulates the behavior of human epidermal stem cells combining highthroughput transcriptome data and functional in vivo assays. Our results show that the clock machinery establishes a temporal axis of human epidermal stem cell behavior that coordinates their proliferation, protection from UV radiation, onset of differentiation and formation of a proper barrier by the stem cell progeny. Clock-dependent complex successive oscillations in the expression of different cohort of genes provides human epidermal stem cells with temporal functional checkpoints. These consecutive checkpoints allow epidermal stem cells to prevent DNA replication when exposed to UV radiation during the hours of sunlight, and to subsequently respond to proliferative and pro-differentiation extracellular stimuli along a 24 hour period. We provide data indicating that perturbations in this clock mechanism profoundly affects the behavior of epidermal stem cells, with consequences on epidermal homeostasis and neoplastic transformation.

A NOVEL OSTEOCLASTIC NETWORK DETERMINES IN VITRO NICHE FOR MOUSE AND HUMAN HEMATOPOIETIC STEM CELLS

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We recently developed an in vitro/in vivo screening strategy, which revealed 18 nuclear factors that enhance HSC activity (Deneault et al., Cell 2009). Mouse HSCs were kept for 12 days in mini-cultures that included viral producer cells for each tested factor. Interestingly, 4 of the 18 hits identified in this initial screen, i.e., Fos, Tcfec, Hmgb1 and Sfpi1 operated through non-HSC autonomous (NHA) mechanisms: expanded HSCs were not infected with retrovirus. This suggested that the transfected viral producer cells

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(feeder cells) produced membrane-bound or soluble molecules that promote expansion of HSCs introduced in these cultures. We now provide evidence that seven additional factors, i.e., Smarcc1, Vps72, Sox4, Klf10, Ski, Prdm16 and Erdr1 significantly enhance HSC activity through NHA mechanisms, hereafter called "NHA factors". Moreover, we found that Vps72, Fos and Klf10 also promote expansion of human HSCs by NHA mechanisms. Interestingly, we observed that physical contact between HSCs and these engineered support cells was not always necessary, suggesting the presence of secreted molecule(s) in the medium. Expression profiling was next performed using mRNA extracted from feeder cells transduced with each of the NHA factors. Firstly, we shed light on the transcriptional make up and potential convergence of signaling pathways in the engineered feeder cells: our results reveal two independent but interconnected transcriptional regulatory subnetworks. Strikingly, some constituents of the subnetworks, i.e., Sfpi1, Fos, Klf10 and Tcfec (Mitf-related) have previously been shown to play critical roles in the regulation of osteoclasts, which are a myeloid-derived population of cells residing in the HSC bone marrow niche. In addition, all of the NHA factors act in concert to increase Prdm16 expression levels in a range from 2.5- to 54.6-fold. For this reason, Prdm16 clearly holds the position of central hub of the osteoclastic network. However, Tcfec represents the ultimate downstream effector of this pathway as Prdm16 elevates its expression levels by 18.9-fold. In parallel, Prdm16 can also increase the expression of Sfpi1, which in turn can upregulate Tcfec expression up to 874-fold. Moreover, upregulated mRNA targets corresponding to factors that are secreted or associated with the plasma membrane were considered as potential candidate agonists of HSC self-renewal. A high degree of overlap was observed between the sets of proteins produced by feeder cells engineered to overexpress Sfpi1, Fos, Klf10, Tcfec or Ski. These secreted and membrane bound proteins include Ogn, Ptgds, Nckap1l, Rgs16 and Lcn2. Studies are ongoing to characterize the contribution of these newly identified NHA proteins in HSC expansion. Validation of these NHA proteins in human HSC expansion will have a clear potential for translational medicine.

INACTIVATION OF FRIZZLED5, MEDIATING NON-CANONICAL WNT SIGNALING, AFFECTS HSC MAINTENANCE IN THE PERIVASCULAR BUT NOT IN THE ENDOSTEAL NICHES

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A fundamental question underlying hematopoietic stem cell (HSC) maintenance is the signaling and related molecular mechanism. . We previously identified that non-canonical Wnt signaling via Frizzled8 and Flamingo maintains quiescent HSCs in the endosteal niche. Recently, we found that another non-canonical Wnt receptor Frizzled5 (Fz5) is expressed in metabolically active HSCs and αSMA+ perivascular cells in the central marrow, but neither in H2B-GFP label-retaining quiescent HSCs, nor in endosteal cells including sinusoidal cells. Using an Mx1-Cre:Fz5 knockout mouse model, we found a 60% decrease of HSCs isolated from central marrow, but no change in the number of HSCs isolated from endosteum. Functionally, hematopoietic reconstitution was not affected in the primary transplantation, but was substantially decreased (by 80%) in the secondary transplantation. Cell cycle analysis revealed a 60% decrease in the G0 phase of HSCs isolated from central marrow. We also observed a 50% decrease of common lymphoid progenitors and an increase in myeloid progenitor cells. The latter was consistent with the development of myeloproliferative disease 3 months post Mx1-Cre induced inactivation of Fz5. We propose that a certain population of HSCs is maintained in quiescence through Fz5-mediated non-canonical Wnt signaling in the perivascular niche in central marrow.

ESTROGEN AND PROGESTERONE EXPAND AND REJUVENATE MOUSE ADULT UTERINE EPITHELIAL STEM CELLS

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In preparation for implantation and support of an embryo the uterus undergoes massive cellular expansion. The embryo implants in the inner uterine lining, called the endometrium, which is one of the most regenerative tissues in the female body. The endometrium maintains a remarkable lifelong growth capacity, demonstrated by the fact that even a postmenopausal uterus can support embryonic implantation with the co-administration of estrogen and progesterone. The biologic mechanisms for the continuous growth potential of the endometrium had been unknown. Using a combination of in vivo and in vitro growth assays developed in our laboratory, here we provide evidence for the existence of a small subpopulation of adult mouse uterine epithelia with stem-like activity. These cells were capable of forming clonal, self-renewing epithelial spheres in vitro and regenerated into endometrial-like glands in vivo. Uterine epithelial stem cells survived hormonal deprivation and rapidly expanded only when the hormones estrogen and progesterone were administered together. With aging, the regenerative capacity of the uterine epithelia dropped dramatically. Remarkably, co-administration of estrogen and progesterone rejuvenated and expanded the uterine epithelial stem cells in aged mice and increased the regenerative activity of the epithelia to levels observed in neonates. To better characterize the regenerative pool of uterine epithelia, the uterine epithelial stem cells were isolated. Uterine epithelia marked with the cell surface antigenic profile Trop1+CD44+CD49fhiCD90-CD45-CD31-Ter119- contained the majority of both in vitro sphere forming and in vivo gland forming potential. This cell pool encompasses the uterine epithelial stem cells (UtESC). The UtESC underwent multi-lineage differentiation in vivo, demonstrated by regeneration of both Trop1+CD44+ and Trop1+CD44- cells. The UtESC cells were non-quiescent and rapidly cycling in line with their continuous regenerative activity in reproductive years. Despite exquisite hormonal responsiveness, the UtESC did not express estrogen or progesterone receptors, suggesting that the hormonal regulation of these cells occurs through paracrine signaling. We demonstrate the existence of an adult epithelial stem cell pool in the endometrium with life-long dual hormone regulated regenerative capacity. Understanding the mechanisms that govern the growth and differentiation of UtESC will likely have important implications in understanding common diseases

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of the endometrium such as endometriosis, endometrial cancer and infertility.

SIGNALING NETWORKS REGULATING ADULT CARDIAC STEM CELLS

Asli, Naisana S., Xaymardan, Munira, Chandrakanthan, Vashe, Doan, Tram, Harvey, Richard P.
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Recent advances discovering cardiac stem/ progenitor cells in the adult heart have shed a new light on cardiac biology against the old dogma that heart is a post-mitotic organ with no regenerative capacity. We have focused on a population of stem/progenitor cells in the adult heart that have been fully characterized in our previous studies. Here we have addressed possible signaling pathways that are involved in the regulation of cardiac stem cell states and dissected their underlying mechanistic aspects. We have shown that PDGF signaling is involved both in the initial activation as well as the maintenance of cardiac stem cell activity mainly through a Retinoblastoma (Rb)-cdk inhibitor-dependant pathway. We have further shown that PDGF can activate the cardiac stem cells within the niche, when systemically administered to adult mice and subsequently result in an improved cardiac function after induced myocardial infarction, suggestively via induction of a myofibroblast-like intermediate. Using a population of committed cardiac progenitors as a model system, we have further demonstrated that PDGF can mediate the transitions through the stem cell hierarchy, by reprogramming the cells into less committed and more stem cell-like states. We further suggest that the PDGF-mediated reprogramming is governed through induction of an epithelial to mesenchymal transition (EMT), which has recently been shown sufficient to maintain the characteristics of cancer stem cells. We have analyzed the transcriptome of the active and quiescent stem cell populations and identified several signaling pathways including Wnt, BMP, IGF and GPCR together with a number of non-coding microRNAs, which suggestively function within a dynamic network. Elucidation of these pathways will provide insights into the biology of adult stem cells and targets for therapeutic approaches.

Concurrent Session IE — Stem Cells, Injury and Regeneration

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INSIGHTS INTO THE FUNCTION OF ERK SIGNALING IN PLURIPOTENT STEM CELL SYSTEMS FROM AN EVOLUTIONARY VIEWPOINT

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Recent studies have indicated that ERK signaling has important roles in proliferation and maintenance of pluripotent stem cells. Especially, we are interested in the opposing roles of the ERK signal in naïve ES cells and EpiSCs. To maintain naïve ES cells we can shut down ERK signaling by adding ERK inhibitor. Conversely, to obtain EpiSCs, we should add FGF to the culture medium to induce EpiSCs prolifeartion by activating ERK signaling. When we cultured chicken embryonic cells from various developmental stages under conditions containing an ERK inhibitor, we found that ES-like colonies only appeared from embryonic cells before H & H stage 4 (Nakanoh and Agata, in preparation). Similarly, ES-like colonies were also obtained from the embryos of quail and gecko under the same conditions, suggesting that ERK signaling may have a conserved function in the regulation of pluripotent stem cells among vertebrates. Planarians have adult pluripotent stem cells in their mesenchymal space that support both asexual and sexual reproduction, and also regeneration. We are investigating the molecular mechanisms underlying planarian regeneration. Recently, we found that ERK signaling is indispensable for the entry of planarian stem cells into a differentiating stage and for their participation in blastema formation (Tasaki et al., 2011, Development). This property is well correlated with that of ES cells, which can be maintained in the medium containing both ERK and GSK3 inhibitors (2i condition). These observations suggest that the function of ERK signaling in naïve ES cells may have been established at an early stage of the evolution of multicellular organisms. This may provide evidence supporting what Austin Smith's group calls the naïve ES cells, "ground state". We also reported that FGFR-mediated signaling is involved in brain formation from pluripotent stem cells, and that nou-darake may have an important role in causing the accumulation of FGFR-mediated signaling in the head region by its specific expression in the future head region (Cebria et al., 2002, Nature). This suggests that ERK signaling may also regulate the differentiation-fate of the pluripotent stem cells in planarians. Finally, we will discuss the conserved roles of the ERK signal in pluripotent stem cell systems.

UNLIMITED IN VITRO EXPANSION OF ADULT LIVER AND PANCREAS PROGENITORS THROUGH A WNT/ LGR5 REGENERATIVE RESPONSE

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Lgr5 marks adult stem cells in the intestine, skin and stomach, and is a receptor for the Wnt-agonistic Rspondins. Single Lgr5+ stem cells from stomach and intestine form ever-expanding organoid cultures. We now find that Lgr5 is not expressed under physiological conditions in adult liver and pancreas. However, upon in vivo injury, the Wnt pathway became robustly activated and Lgr5 expression appeared in regenerating ducts of liver and pancreas. In vitro, duct fragments from mouse liver and pancreas initiated Lgr5 expression in culture, developing into budding cysts which expanded 10-fold weekly for >40 weeks. The cysts consisted of stem/progenitor cells that could readily be cloned. Clonally expanded liver organoids could be induced to differentiate into functional hepatocytes in culture and in vivo, upon transplantation into FAH-deficient mice. Our culture conditions allow organ-specific adult stem cells to defy the Hayflick limit and may complement Embryonic Stem cell and Induced Pluripotent Stem Cell-based regenerative strategies.

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CRITICAL ROLE OF FGF7 IN REGULATING MOUSE ADULT LIVER STEM/PROGENITOR CELLS AND REGENERATION IN DAMAGED LIVERS

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The liver is a unique organ with a remarkably high potential to regenerate upon injuries. In severely damaged livers where hepatocyte proliferation is impaired, facultative adult liver stem/progenitor cells (LPCs; also referred to as oval cells in rodent models) proliferate and are assumed to contribute to regeneration. An expansion of LPC is often observed in patients with various types of liver diseases. However, the underlying mechanism of LPC activation still remains largely unknown. We recently found that a member of the fibroblast growth factor (FGF) family, FGF7, is a novel and potent regulator of LPCs. In a mouse model of LPC activation by feeding a hepatotoxin 3,5-Diethoxycarbonyl-1,4-Dihydrocollidine (DDC)-containing diet, FGF7 expression was significantly induced in the liver concomitantly with the LPC response. Fgf7 knockout (KO) mice exhibited markedly depressed LPC expansion and high mortality upon toxin-induced hepatic injury. Overexpression of FGF7 in vivo led to the induction of LPCs and ameliorated the liver injury by DDC. We revealed that Thy1+ mesenchymal cells appeared in close proximity to LPCs and produced FGF7, suggesting a role for these cells as a LPC niche in the regenerating liver. To further substantiate the general importance of this growth factor in LPC regulation, we examined its expression and function using other liver injury models. In a liver-specific Tak1 KO mouse, where persistent death of hepatocytes causes chronic inflammation and fibrogenic response in the liver, expansion of Thy1+ cells and induction of FGF7 were clearly detected along with the LPC induction. We also employed common bile duct ligation (BDL), a surgical model for cholestasis-induced liver injury, and found significant expansion of Thy1+ cells and augmented expression of FGF7. Finally, the LPC induction upon BDL was markedly impaired in Fgf7 KO mice. These results together indicate that Thy1+ cells serve as a functional niche for LPCs by providing the critical regulatory factor FGF7 and that this mechanism is well conserved in various liver injuries accompanying LPCs.

DEFINING THE ORIGINS, PROPERTIES AND MOLECULAR CHARACTERISTICS OF SWEAT GLANDS AND THEIR STEM CELLS

Lu, Catherine P.[1], Polak, Lisa[1], Rocha, Ana S.[2], Pasolli, H. Amalia[1], Sharma, Neha[2], Blanpain, Cedric[2], Chen, Shann-Ching[3], Fuchs, Elaine[1]
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Eccrine sweat glands are the most abundant glands of the human body, and their role in producing and secreting sweat is crucial for thermoregulation. Like mammary glands, they originate from epidermal progenitors. However, sweat glands display few signs of cellular turnover, and eccrine cancers occur infrequently. Thus, whether they have stem cells and capacity for tissue regeneration remain largely unexplored. Here we address these issues. We first study the existence of slow-cycling label-retaining cells in the adult sweat gland. We then exploit 8 different promoter-Cre mouse lines to map the development of the straight and secretory coiled duct of the sweat gland. Next we examine the proliferative activity of the adult sweat gland during normal homeostasis and how this changes after skin epidermal wounding and upon myoepithelial-specific and luminal-specific ablation in vivo. We then devise a purification scheme to isolate palmar skin epidermal cells and 4 different populations of cells from the adult sweat glands, which were then subjected to microarray analysis and engraftment. Exploiting molecular differences between sweat and mammary gland progenitors, we discover that these stem cells can generate de novo glandular structures that retain their identity even within a foreign microenvironment. Our findings not only advance our understanding of sweat gland biology, but also reveal its regenerative potential.

PERICYTE-DERIVED SCAR FORMATION FOLLOWING LESIONS TO THE CENTRAL NERVOUS SYSTEM

Goritz, Christian, Dias, David O., Frisén, Jonas
Department of Cell and Molecular Biology, Karolinska Institute, Stockholm, Sweden

Damage to the central nervous system (CNS) often leads to persistent functional deficits. The manifestation of these deficits is believed to be associated with the scar tissue that forms locally at lesions, causing permanent tissue alteration and blocking regeneration.

Studying scar formation after spinal cord injury (SCI), we became interested in the origin and function of the stromal, non-glial, component of the scar. Pericytes (perivascular cells lining most capillaries) have been suggested to be mesenchymal stem cells based on their in vitro differentiation potential and are abundant in CNS tissue. We asked whether pericytes react upon injury and participate in scar formation. In our recently published work (Göritz et al., 2011, Science), we identified a specific pericyte subpopulation, named "type A pericytes", as the major source of scar contributing stromal cells. Type A pericytes are embedded in the vascular wall but proliferate and leave the blood vessel upon injury, differentiating into fibroblast-like cells that deposit extracellular matrix to seal the lesion and form the persistent stromal scar core. With the identification of type A pericytes as the source of the scar stroma, we were now able to address the role of this scar component for CNS regeneration. Using a transgenic strategy to selectively block proliferation of type A pericytes after SCI we could modulate the density of the pericyte-derived scar core. We are going to present data showing a correlation between the density of the stromal scar core and the functional outcome after SCI. Furthermore, exploring the general nature of type A pericyte-mediated scarring, we will present data comparing spinal cord injury with different lesions in the brain.

INJURY-INDUCED EXPRESSION OF THE TRANSCRIPTION FACTOR GATA3 IS A PRO-REGENERATIVE CUE IN ADULT VERTEBRATE BRAIN

Kizil, Caghan[1], Kyritsis, Nikos[1], Dudczig, Stefanie[1], Lehmann, Susann[1], Kroehne, Volker[1], Kaslin, Jan[2], Kempermann, Gerd[3], Echeverri, Karen[4], Brand, Michael[1]
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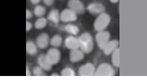
The adult zebrafish central nervous system, unlike mammalian counterparts, has widespread regenerative capacity. Zebrafish can regenerate severe brain injuries, because glial precursor cells act as neurogenic progenitors for replenishing lost neurons. We



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hypothesized that injury-induced regenerative programs in zebrafish brain could underlie the different regenerative capacities between zebrafish and mammals. However, the molecular players of the regenerative response in zebrafish brain are unknown. Here we show that the transcription factor gata3 is necessary for tissue regeneration of the zebrafish. gata3 is expressed only after injury in telencephalon, heart and caudal fin of the adult zebrafish, suggesting a specific involvement in the regeneration programs of different zebrafish tissues. After morpholino-mediated gene knockdown experiments, we found that gata3 is necessary for injury-induced glial cell proliferation and subsequent neurogenesis in the telencephalon, and for the regenerative outgrowth in caudal fin, indicating that gata3 is broadly required for regeneration programs in zebrafish. In the adult mouse hippocampus, gata3 was undetectable under control conditions or after physical exercise-induced neurogenesis. Gata3 is also not detected in non-neurogenic primary rat astrocyte cultures during normal conditions or after scratch-injury. However, overexpression of GATA3 specifically increased the proliferation and neurogenesis of the non-neurogenic astroglia, whereas pro-neural genes such as NEUROG1 and PAX6 are insufficient to stimulate neurogenesis. This indicates that GATA3 is sufficient to elicit a regenerative neurogenesis program that is distinct from the constitutive neurogenesis. Thus, in zebrafish, GATA3 acts as a specific injury-induced pro-regenerative factor, that can kick-start regenerative neurogenesis in mammalian brain from normally non-neurogenic glial precursors. We believe that the association between Gata3 activity after injury and the regeneration response has the potential to be harnessed for therapeutic applications in human neurological disorders or acute injuries in the central nervous system.

Concurrent Session IIA: Neural Stem Cells

Supported by F. Hoffmann - La Roche Ltd.

Thursday, June 14, 2012, 4:15 pm - 6:00 pm

NEURAL STEM AND PROGENITOR CELLS IN HUMAN CORTICAL DEVELOPMENT AND EVOLUTION

Kriegstein, Arnold R., Lui, Jan H., Hansen, David B., Wang, Xiaoqun
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Recent insights gained from studies of the developing cerebral cortex are illuminating potential evolutionary steps that contributed to structural and functional features of the human brain. Radial glial cells (RG), long thought to simply guide embryonic nerve cells during migration, have now been identified as neuronal stem cells in the developing brain. RG cells undergo self-renewing, asymmetric divisions to generate neuronal precursors that can further proliferate in the subventricular zone (SVZ) to increase neuronal number. Unlike the developing rodent cortex, the developing human cortex contains a massively expanded SVZ (OSVZ) that is thought to account for the bulk of cortical neurogenesis. We have begun to characterize the types and locations of progenitor cells responsible for human cortical development. We found that large numbers of radial glia-like cells and intermediate progenitor cells populate the human OSVZ. The OSVZ radial glia-like cells, termed oRG cells, have a long basal process but, surprisingly, do not have basolateral polarity and lack contact with the ventricular surface. Using real-time imaging and clonal analysis, we demonstrate that the oRG cells undergo self-renewing asymmetric divisions to generate daughter neuronal progenitor cells that can further proliferate. The daughter cells undergo multiple rounds of symmetric division before generating neurons, suggesting that they are a transit amplifying cell population. The oRG cells are also gliogenic, supporting their classification as a form of neural stem cell. We have recently found that cells resembling oRG cells are present in mouse embryonic neocortex, and arise from asymmetric divisions of ventricular radial glia. Time-lapse imaging reveals that the cells undergo self-renewing asymmetric divisions to generate neurons directly. This contrasts with human oRG cells that produce neurons indirectly through production of transit amplifying cells. These results suggest that oRG cells are probably present in all mammals and are not a specialization of a larger brain with increased cortical area. Instead, an evolutionary increase in the number of oRG cells and their transit amplifying daughter cells likely amplified neuronal production and contributed to increased cortical size and complexity in the human brain. The diversity of neural stem and progenitor cells observed during human cortical development, consisting of ventricular RG, oRG cells, intermediate progenitors of the inner SVZ, and transit amplifying cells of the OSVZ raise the question of whether a similar diversity of stem and progenitor cells are present during the differentiation of human stem cells toward forebrain neurons. The answer may be important for modeling human neurodevelopmental diseases that affect the cortex ranging from cortical malformations such as microcephaly and lissencephaly to more subtle disorders such as autism and schizophrenia.

IDENTIFICATION OF THE MINIMAL MOLECULAR NETWORK NECESSARY FOR VOLUNTARY AND FORCED NEUROGENESIS

Ninkovic, Jovica[1], Petricca, Stefania[1], Akici, Umut[2], Poot, Raymond[2], Beckers, Johanes[1], Irmler, Martin[1], Götz, Magdalena[1]
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Overexpression of a single transcription factor such as Pax6, Ngn2 or Dlx2 in postnatal astrocytes triggers a neurogenic cascade sufficient to elicit the complete program for functional neurons. To understand the molecular mechanisms of this conversion, we set out to analyze the interactome of the transcription factor Pax6 in NS5 radial glia cells. Our analysis showed direct interaction of Pax6 with the SWI/SNF chromatin remodeling complex. Moreover, we could observe this interaction in neuroblasts freshly isolated from the mouse olfactory bulb. To assess the functional importance of this interaction, we genetically ablated either Brg1, the ATPase unit of the SWI/SNF complex, or Pax6 in the neural stem cell in the SEZ, one of the neurogenic zones in the adult mouse brain. Interestingly, loss of either Pax6 or Brg1 did not alter transient amplifying progenitors or stem cells. However, neuroblasts deficient for the function of either Pax6 or Brg1 converted into glial cells most of which express the proteoglycan NG2 or the transcription factor Olig2, characteristic for oligodendrocytes progenitors. Furthermore, Pax6 but not Ngn2 failed to convert neurosphere-derived glia into neurons in the absence of the Brg1 function. These experiments suggest that the function of Pax6-SWI/SNF is necessary and sufficient for neuronal differentiation. The transcriptome analysis of the Brg1 deficient neuronal progenitors, isolated from the adult brain shortly after loss of Brg1 function, combined with the promoter binding assays unraveled direct targets of the Pax6-SWI/SNF complex mediating neuronal differentiation. We identified Nfib, Sox11 and Pou3f4 to form a cross-regulatory network downstream of Pax6-SWI/SNF complex necessary for the neuronal differentiation. The combination of

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these transcription factors is as efficient as Pax6 in reprogramming neurosphere-derived glia into neurons. Moreover, in support of this network acting down-stream of Pax6, the reprogramming capacity of this cross-regulatory network is independent of Brg1. Taken together, we identified here a molecular network necessary & sufficient for stabilizing the neuronal fate in normal and forced neurogenesis.

DEVELOPMENT OF AN EMBRYONIC STEM CELL-BASED MYELINATION ASSAY

Kerman, Bilal E.[1], **Kim, Hyung Joon**[1], **Mei, Arianna**[1], **Chandross, Karen**[2], **August, Paul R.**[3], **Gage, Fred H.**[1]
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Myelination of axons by oligodendrocytes in the central nervous system and by Schwann cells in the peripheral nervous system is critical for neuronal function and survival. Loss of myelin membrane, as occurs in many neurological disorders such as multiple sclerosis and as a consequence of spinal cord injury, leads to disruption of electrical impulse conductivity, atrophy of neurons and permanent functional deficits. Currently, the myelination process has mostly been studied in vitro with isolated primary cells or in postmortem tissues or in vivo with animal models. Both approaches are restricted by limited number of cells and animals making them difficult for large scale experiments and by variability among individual animals. Embryonic stem cell (ESC) technology allows for a relatively uniform population of a large number of cells. Our goal is to establish an in vitro myelination model, using human and mouse ESC-derived oligodendrocytes and neurons. To this end we developed a novel protocol that yielded over 80% oligodendrocyte progenitor cells (OPCs) starting from mouse ESCs. When co-cultured with mouse ESC-derived neurons these OPCs matured into MBP-expressing oligodendrocytes that wrapped neurites and generated compact myelin. We also showed that isolation of axons using microfluidic chambers in co-cultures improved visualization and quantification of myelination in vitro. We have an ongoing effort to transfer the protocol with mouse ESC-derived oligodendrocytes to human ESCs and induced pluripotent cells (iPSCs). In conclusion, we developed a highly reproducible and scalable myelination assay that provides an opportunity to dissect mechanisms regulating myelination.

NAIVE SANGER HUMAN INDUCED PLURIPOTENT STEM CELLS (SH-IPS) HAVE NEURAL INDUCTION REQUIREMENTS SIMILAR TO MOUSE EMBRYONIC STEM CELLS.

Ryan, David J.[1], **Wang, Wei**[1], **Yang, Jian**[1], **Watts, Colin**[2], **Liu, Pentao**[1]
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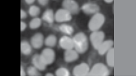
Introduction: In vitro modelling of neural disease and development of autologous cell types for cell based regeneration and tissue repair requires a robust method of neural induction. Current methods of neural differentiation of human embryonic / human induced pluripotent stem cells rely on inhibition of BMP / Activin signalling. However, significant interline variability in neural induction creates a barrier to clinical translation. miR-371-3 has recently been identified as a predictor of neurogenic propensity in response to dual SMAD inhibition. High miR expressing lines were similar to naive mouse embryonic stem cells and were poorly responsive to neural induction. Low miR expressing lines similar to mouse epistem cells were highly responsive to SMAD inhibition. Recent work from our lab has produced naive human induced pluripotent stem cells with biological requirements similar to mouse embryonic stem cells. These cells are independent of Activin / FGF signalling for maintenance and highly express miR-371-3. We sought to investigate the signalling requirements necessary for neural induction of naive human iPS cells. Methods: SH-iPS lines were generated as previously described and maintained in 2i and LIF. Neural induction conditions were refined using 46C SOX1 reporter mouse embryonic stem cells. The optimized induction regime was subsequently applied to SH-iPS cells and neural conversion was quantified. Results: SH-iPS cells maintained in 2i/LIF do not respond to dual SMAD inhibition whereas FGF cultured human iPS cells show extensive SOX1 mRNA induction. Combined FGF induction with single molecule inhibition of BMP / Activin signalling in adherent culture produced >90% SOX1 conversion conversion at 2 weeks. During early neural induction pluripotency markers became undetectable and in female lines XIST transcript increased reflecting X chromosome inactivation and dosage compensation during differentiation. The protocol was highly specific with downregulation of markers related to mesoendoderm and trophectoderm. SH-iPS derived neural stem cells were self renewing in vitro, multipotent and karyotypically normal. Neural stem cells have been efficiently produced from SH-iPS lines generated by integrational and episomal methods of reprogramming. In both methods there are no detectable exogenous factors in the stable iPS cell lines. Conclusion: SH-iPS cells demonstrate naive characteristics and can be differentiated efficiently to neuroectodermal precursors using murine neural induction cues in adherent culture conditions. We propose that miR-371-3 cluster rather than being predictive of overall neurogenic propensity identifies two distinct stem cell states, ES or ground state and epistem cell or primed state with distinct neural induction requirements.

TSUKUSHI MAINTAINS THE GROWTH AND UNDIFFERENTIATED PROPERTIES OF NEURONAL STEM/PROGENITOR CELLS AS A NICHE MOLECULE

Ito, Ayako[1], **Shinmyo, Yohei**[1], **Kaneko, Nahoko**[2], **Hirota, Yuki**[2], **Hatakeyama, Jun**[1], **Yamaguchi, Masahiro**[3], **Shimamura, Kenji**[1], **Sawamoto, Kazunobu**[2], **Tanaka, Hideaki**[1], **Ohta, Kunimasa**[1]
[1]*Kumamoto University, Kumamoto, Japan,* [2]*Nagoya City University, Nagoya, Japan,* [3]*University of Tokyo, Tokyo, Japan*

We have identified a BMP antagonist, Tsukushi (TSK), which is a soluble molecule containing 12 leucine-rich repeats and belongs to the Small Leucine-Rich Proteoglycan family. TSK is expressed in the primitive streak and Hensen's node during chick gastrulation and involved in their formation. TSK is also involved in the neural crest specification of Xenopus embryo by regulating BMP and Delta-1 activities at the boundary between the neural and the non-neural ectoderm. We have generated KO mice lacking Tsukushi (TSK) function and found that TSK inactivation results in expansion of the adult ciliary body and the increased proliferation of the retinal stem/progenitor cells in vitro. Biochemical assays and overexpression experiments in the retina suggest that these effects are dependent on the ability of TSK to modulate Wnt signaling by direct binding to Fzd receptor. In the adult mouse brain, TSK is strongly expressed in the Subventricular Zone and Dentate Gyrus, where brain stem cells are located. Interestingly, TSK-/- mouse shows the enlargement of Lateral Ventricule. By immunochistochemical study, we find that TSK is expressed in the ependymal cell layer. BrdU labelling study shows that the number of BrdU-positive S phase cells is increased in TSK-/- mouse brain. Taken together, we uncover a new crucial role for TSK in maintaining the growth and undifferentiated properties of neuronal stem/progenitor cells as a niche molecule.



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GLOBAL REGULATION OF THE CHROMATIN STATE IN MOUSE NEURAL STEM CELLS DURING THE NEOCORTICAL DEVELOPMENT

Kishi, Yusuke, Fujii, Yuki, Hirabayashi, Yusuke, Gotoh, Yukiko
Institute of Molecular and Cellular Biosciences, the University of Tokyo, Tokyo, Japan

Neurons, astrocytes and oligodendrocytes, three major cell types that consist the central nervous system, are derived from common neural stem cells (NSCs). Whereas NSCs generate neurons in the early stages of neocortical development, they lose the neurogenic potential and generate only glial cells in the late stages. In this study, we found "global" changes of the chromatin state in NSCs during neocortical development in addition to "local" changes at specific gene loci. Chromatins isolated from early-stage NSCs were less condensed compared to those from late-stage NSCs, as revealed by nuclease digestion and salt-extraction analyses, as well as by a fluorescent recovery after photobleaching (FRAP) analysis using histone H1-GFP. We also identified chromatin-binding proteins necessary for the "less condensed" state of the chromatin in early-stage NSCs. Importantly, knockdown of these proteins reduced the neurogenic capacity of early-stage NSCs, and conversely, their overexpression increased neurogenesis and suppressed astrogenesis of late-stage NSCs. These results suggest that global chromatin condensation might contribute to the fate restriction of NSCs during neocortical development.

Concurrent Session IIB — Cell Fate Conversion

Thursday, June 14, 2012, 4:15 pm – 6:00 pm

CONVERTING FIBROBLASTS TO NEURONS WITH A MICRO-RNA CHROMATIN SWITCH

Crabtree, Gerald, Yoo, Andrew, Sun, Alfred
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During normal development an essential switch in BAF (mSWI/SNF) chromatin remodeling complexes occures such that three subunits are removed and replaced by three other neural specific subunits. This switch is directed by mir124/9* which bind to the 3' UTR of BAF53a causing its removal and replacement by neural specific BAF53b at mitotic exit. This switch from the neural progenitor npBAF complex to the neural specific nBAF complex is essential for neral development. Recapitulating this switch in human fibroblasts leads to their conversion to neurons. Remarkably, both excitatory and inhibitory neurons are produced which develop repetitive action potentials, functional vesicles and that release and take up vesicular contents. The production of inhibitory neurons has not been seen before in other way of producing neurons from IPS cells or fibroblasts and attest to the roboustness of this approach and its importance in controlling neural fate determination. We have investigated the underlying mechanism used by BAF complexes in controlling cell fate and find that a significant component depends upon the ability of these complexes to control placement of H3K27Me3 across the genome and the polycomb repressive complexes 1 and 2. In addition, purification of neural progenitors and neurons and subsequent genome wide analysis indicates that the switch of complexes orchestrates the placement of vital signaling pathways and transcription factors needed for defining the differentiated phenotype.

GENERATION OF PATIENT-SPECIFIC MOTOR NEURONS FROM FIBROBLASTS BY DEFINED FACTORS

Son, Esther Y.[1], Ichida, Justin K.[1], Wainger, Brian J.[2], Toma, Jeremy S.[3], Woolf, Clifford J.[2], Rafuse, Victor F.[4], Eggan, Kevin[1]
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The mammalian nervous system is composed of a host of distinct neuronal subtypes, each with its unique phenotype and differential sensitivity to disease. Although specific neuronal types can be isolated from intact rodent embryos or engineered from stem cells, transcription factor-mediated reprogramming might provide a more direct route to generating defined neuronal subtypes. To this end, we sought to transdifferentiate fibroblasts into spinal motor neurons, the neurons that actuate all voluntary movement by synapsing directly with muscle and which are lost in amyotrophic lateral sclerosis (ALS). Specifically, to study the underlying mechanisms of this largely sporadic disease, motor neurons with the exact genotypes represented in the diverse patient population must be made. We first showed that mouse fibroblasts are converted to induced motor neurons (iMNs) by factors involved in the development of motor neurons (Lhx3, Ngn2, Isl1, Hb9), in conjunction with factors that convert fibroblast into induced neurons (Ascl1, Brn2 and Myt1l). The iMNs exhibited morphologies, global transcriptional profile and electrophysiological properties highly similar to embryonic motor neurons. Strikingly, the iMNs formed functional synapses with muscle fibers to cause acetylcholine-dependent muscle contraction and, in the most stringent test of their specific functionality, integrated into the chick embryonic spinal cord when transplanted. Therefore, this approach allows fibroblasts to be converted to a precisely defined, physiologically relevant neuronal subtype with its salient molecular and functional features. When we surveyed the Hox gene profile of the iMNs, a wide range of motor neuron subtypes was represented in the population, including Foxp1+ limb-innervating motor neurons of the lateral motor column (LMC) known to be severely affected in ALS. To directly demonstrate their utility in modeling ALS, we showed that the iMNs recapitulate the non-cell-autonomous and cell-autonomous effects of the ALS-causing SOD1G93A mutation. Interestingly, lineage tracing experiments revealed the absence of highly proliferative Nestin+ intermediate states during reprogramming. This may be advantageous when generating patient-specific motor neurons with ALS-associated, unstable genetic elements, such as hexanucleotide repeat expansions. Importantly, this method has enabled us to generate iMNs from fibroblasts of a cohort of ALS patients with a range of mutations and clinical manifestations. Interestingly, ALS patient iMNs showed higher propensity to fire action potentials than did iMNs made from fibroblasts of healthy subjects, which may be a novel disease-related phenotype. To facilitate the testing of such hypotheses using a more diverse array of ALS genotypes, we have identified a small molecule, a Tgf-beta inhibitor named RepSox, which dramatically enhances the conversion of adult fibroblasts into iMNs. Going forward, iMN reprogramming may be an ideal tool for rapidly surveying more ALS patient-specific motor neurons for disease-relevant phenotypes. By stratifying the patient population into phenotypic classes with clinical significance, a more targeted approach to developing therapeutics may become possible. Applying it to the production of other neuronal subtypes could help tackle a variety of neurodegenerative diseases, and reveal principles that govern the stability and plasticity of cell fate.

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DIRECT CONVERSION OF MOUSE FIBROBLASTS TO SELF-RENEWING, TRIPOTENT NEURAL PRECURSOR CELLS

Lujan, Ernesto[1], **Chanda, Soham**[2], **Ahlenius, Henrik**[3], **Sudhof, Thomas C.**[2], **Wernig, Marius**[3]

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We recently showed that defined sets of transcription factors are sufficient to convert mouse and human fibroblasts directly into cells resembling functional neurons, referred to as "induced neuronal" (iN) cells. For some applications however, it would be desirable to convert fibroblasts into proliferative neural precursor cells (NPCs) instead of neurons. We hypothesized that NPC-like cells may be induced using the same principal approach used for generating iN cells. Toward this goal, we infected mouse embryonic fibroblasts derived from Sox2-EGFP mice with a set of 11 transcription factors highly expressed in NPCs. Twenty-four days after transgene induction, Sox2-EGFP+ colonies emerged that expressed NPC-specific genes and differentiated into neuronal and astrocytic cells. Using stepwise elimination, we found that Sox2 and FoxG1 are capable of generating clonal self-renewing, bipotent induced NPCs that gave rise to astrocytes and functional neurons. When we added the Pou and Homeobox domain-containing transcription factor Brn2 to Sox2 and FoxG1, we were able to induce tripotent NPCs that could be differentiated not only into neurons and astrocytes but also into oligodendrocytes. The transcription factors FoxG1 and Brn2 alone also were capable of inducing NPC-like cells; however, these cells generated less mature neurons, although they did produce astrocytes and even oligodendrocytes capable of integration into dysmyelinated Shiverer brain. Our data demonstrate that direct lineage reprogramming using target cell-type-specific transcription factors can be used to induce NPC-like cells that potentially could be used for autologous cell transplantation-based therapies in the brain or spinal cord.

INDUCTION OF FUNCTIONAL HEPATOCYTES FROM FIBROBLASTS BY DEFINED FACTORS

Huang, Pengyu[1], **Zhang, Ludi**[1], **He, Zhiying**[2], **Ji, Shuyi**[1], **Sun, Huawang**[1], **Xiang, Dao**[2], **Liu, Changcheng**[1], **Hu, Yiping**[2], **Wang, Xin**[3], **Hui, Lijian**[1]

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Differentiated cells are epigenetically plastic as they can be reprogrammed into pluripotent stem cells by nuclear transfer or transcription factor overexpression. Recent studies have revealed that overexpression of lineage-specific transcription factors converts differentiated cells into other lineages without reversion to the stem cell state. Here, we induce mouse mesenchymal fibroblasts directly into functional hepatic (iHep) cells by transduction of three hepatic transcription factors, and inactivation of p19Arf. iHep cells show typical epithelial morphology, express hepatic genes and acquire hepatocyte functions. Importantly, using fumarylacetoacetate hydrolase-deficient mice as a liver injury model, transplanted iHep cells repopulate the liver and rescue the recipients from death by restoring liver functions. Our study thus provides a novel strategy to generate functional hepatocytes for liver engineering and regenerative medicine to treat liver diseases.

IN VIVO TRANSDIFFERENTIATION OF SOX2 EXPRESSING COCHLEAR SUPPORTING CELLS TO AUDITORY HAIR CELLS BY GAMMA SECRETASE INHIBITOR CONTRIBUTES TO HEARING RECOVERY IN NOISE INDUCED DAMAGED MICE

Fujioka, Masato[1], **Mizutari, Kunio**[1], **Hosoya, Makoto**[1], **Bramhall, Naomi**[2], **Ogawa, Kaoru**[1], **Okano, Hirotaka James**[3], **Okano, Hideyuki**[4], **Edge, Albert SB**[2]

[1]Department of Otolaryngology, Head and Neck Surgery, Keio University, School of Medicine, Tokyo, Japan, [2]Department of Otology and Laryngology, Harvard Medical School, Boston, MA, USA, [3]Division of Regenerative Medicine, Jikei University School of Medicine, Tokyo, Japan, [4]Department of Physiology, Keio University, School of Medicine, Tokyo, Japan

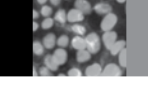
Hearing loss due to damage to auditory hair cells is irreversible because mammalian hair cells do not regenerate. Here, we show that new hair cells are induced by a gamma-secretase inhibitor through the inhibition of Notch signaling and the deafness is ameliorated. Notch inhibition is known to induce ectopic auditory hair cells in the late embryonic stages, but the effect in the adult mammalian cochlea has not been elucidated. We first screened inhibitors for hair cell induction using inner ear-derived neurosphere and selected highly potent compound, LY411,575. In addition to the previously reported ectopic hair cell induction on the neonatal organ culture, we also observed an induction of normotopic hair cell differentiation at the expense of supporting cells in number after specific hair cell-ablation in the organ of Corti explant culture. In vivo prospective labeling of Sox2-expressing cells with a Cre/lox system unambiguously demonstrated hair cell genesis by trans-differentiation of supporting cells after inhibitor treatment. The phenomenon was accompanied with the recovery of hearing in the ears damaged by noise trauma that was measured by auditory brainstem response. Severe side effects observed after oral administration of inhibitor were avoided by local, surgical application to the ear. Our findings indicates manipulating cell fate of peripheral auditory sensory cells in vivo is a novel, feasible therapeutic target against deafness including trans-differentiation of auditory supporting cells to hair cells by pharmacological inhibition of Notch signal.

A LONG NON-CODING RNA ANTISENSE TO EVX1 IS NECESSARY AND SUFFICIENT FOR MESODERM GENERATION

Perkins, Andrew C.[1], **Dinger, Marcel**[2], **Tangermann, Pierre**[3], **Amaral, Paulo**[4], **Kalsbeek, Anton**[5], **Frith, Jessica**[6], **Tallack, Michael**[1], **Ru, Ke-Lin**[7], **Crawford, Joanna**[2], **Gardiner, Brooke**[2], **McMahon, Jill**[8], **McMahon, Andrew**[8], **Mattick, John**[9]

[1]Mater Medical Research Institute, University of Queensland, South Brisbane, Australia, [2]Diamantina Institute, University of Queensland, Brisbane, Australia, [3]Institut für Biochemie, University of Leipzig, Leipzig, Germany, [4]Cambridge University, Cambridge, United Kingdom, [5]Department of Medicine, Erasmus University, Rotterdam, Netherlands, [6]Australian Institute for Biotechnology and Nanotechnology, University of Queensland, Brisbane, Australia, [7]Institute for Molecular Bioscience, University of Queensland, Brisbane, Australia, [8]Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, USA, [9]Garvan Institute of Medical Research, University of New South Wales, Sydney, Australia

Long non-coding RNAs (lncRNAs) are dynamically expressed during development and differentiation of many tissues. There is increasing evidence to suggest they play important roles in epigenetic regulation of gene expression during development and differentiation. We performed a screen for lncRNAs that are dynamically expressed during ES cell differentiation into mesoderm. We identified Evx1as lncRNA, a novel gene that is expressed in an antisense direc-



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tion with respect to Evx1, a homoebox gene at the 3' end of the HoxA cluster. Evx1as comprises 4 exons and a promoter that resides in the first intron of Evx1. Knockdown of Evx1as results in complete failure of the primitive streak wave of differentiation from day 3 of embryoid body (EB) differentiation from ES cells. Evx1, Mixl1, brachyury (T) and other primitive streak and definitive endoderm genes are never activated upon differentiation in the absence of Evx1as. In contrast, ES cell, epiblast (pre-streak), and visceral endoderm expression patterns persist longer than in wildtype, suggesting a specific role for Evx1as in primitive streak determination from epiblast. We also engineered a conditional Evx1as over expression construct into the ROSA26 locus in YFP3.1 ES cells. Conditional activation of Evx1as results in persistent expression of the primitive streak program and persistent growth of early mesoderm cells. RNA-seq data demonstrates global changes in the streak transcriptome and ChIP shows loss of occupancy of active chromatin marks and MLL at streak gene promoters. Thus, Evx1as is necessary and sufficient (in trans) for global regulation of primitive streak gene expression, which sets up the mesoderm and definitive endoderm programs of differentiation.

Concurrent Session IIC — Modeling Human Disease

Supported by F. Hoffmann - La Roche Ltd.

Thursday, June 14, 2012, 4:15 pm - 6:00 pm

MODELING HUMAN NEURODEGENERATION IN HES/IPS DERIVED NEURONS

Zhong, Zhong, Fang, Haiyan, Xu, Xiaohong, Lei, Ying, Zhao, Jing
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One of the promises of human embryonic stem cells is the ability to self-renew with unlimited passages and differentiate into large quantities of specific cell types upon defined culture conditions. For neurodegenative diseases, the iPS cells and their derived neurons have the same genetic makeup as that of the patients and the expanded neuronal culture is expected to have the same biochemical defects or the same vulnerability as the neurons in the patients. This opportunity to model human diseases at the cellular level would enable disease mechanism study and compound screen with unprecedented accuracy of cellular context, and therefore hold promise to improved success rate of target identification and drug discovery. To capitalize this opportunity, a few important problems still need to be solved. First, production of large amount of fully differentiated neurons for consistent performance in compound screen requires at least 6-8 weeks and exact steps of manipulations. Even small deviations of operation procedures can lead to changes of end stage cells. Two cryopreservation steps along the lineage specific differentiation process were introduced to enable scaling up with opportunities to QC a large stock of intermediate products of cell differentiation. Second, human neurons tend to differentiate in clusters with unpredicted efficiency in each of these clusters, leading to unacceptable well-to-well assay data variability. In this study, one additional step of re-plating into screening multi-well plated was introduced to ensure even distribution of mature neurons in a monolayer format appropriate for compound screens. Finally and most importantly, disease specific phenotypes in differentiated iPS neurons are still elusive for most neurodegenerative diseases. In a mix culture enriched with human doparminergic neurons, it was demonstrated a Parkinson Disease associated toxin MPP+ led to cell toxicity specific to the doparminergic neurons. To our knowledge, this is the first demonstration of a scalable cell assay system demonstrating disease related neuronal vulnerability that enables compound discovery and development directed at protecting this disease specific cellular toxicity. In another example, a neuronal population with ~ 50% GABAergic subtype differentiated from an iPS cell line was subjected to the insult by an oligomeric preparation of amyloid β peptides with a half maximal toxicity at 5 uM. A few compound sets were screened in this cellular model of Alzheimer Disease and a number of kinase inhibitors were identified as potent inhibitors of the cellular toxicity elicited by amyloid β oligomers. Interestingly, cdk2 inhibitors showed dose-dependent protection consistent of its potency in kinase inhibition. In this iPS neuron model, detailed characterization of the cycle events suggests that cellular toxicity of amyloid correlates with cellular markers of cell cycle re-entry. It is possible to suggest that blocking of cell cycle reentry specifically at G1/S through cdk2 inhibition affords protection of neurons against amyloid β oligomers' insult.

MISLOCALIZATION OF FUS/TLS IN SPINAL MOTOR NEURONS DERIVED FROM ALS PATIENT-SPECIFIC INDUCED-PLURIPOTENT STEM CELLS

Han, Steve S.W.[1], **Berry, James D.**[1], **Kiskinis, Evangelos**[2], **Davis-Dusenbery, Brandi**[2], **Williams, Luis A.**[2], **Moccia, Robert**[1], **Atwater, Nicholas**[2], **McKenna-Yasek, Diane**[3], **Cudkowicz, Merit E.**[1], **Brown, Robert H.**[3], **Eggan, Kevin**[2]
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Amyotrophic lateral sclerosis (ALS) is an adult-onset, progressive, neurodegenerative disease characterized by the loss of motor neurons leading to death typically within 3-5 years of onset. In the majority of cases, the disease is sporadic and the cause is unknown. However, genetic mutations in several genes, including FUS/TLS and TARDBP, which are ubiquitously expressed DNA-RNA binding proteins, implicate defects in RNA metabolism in ALS pathogenesis. Aberrant cytoplasmic localization of these predominantly nuclear proteins and subsequent inclusion formation typify the neuropathology of familial cases linked to mutations in these genes as well as the majority of sporadic ALS. The subsequent cascade of events leading to motor neuron degeneration is presently unclear though loss of normal nuclear function, toxic gain of function, or a combination of both are possible. Mutations in FUS/TLS, which account for approximately 5% of familial and 1% of sporadic ALS cases, cluster in the extreme C-terminus of the FUS/TLS protein and harbor a nuclear localization signal (NLS). It has been shown that mutations in this region affect, to varying severities depending on the specific mutation, the amount of cytoplasmic mislocalization and propensity of FUS to incorporate into stress granules when overexpressed in cell lines. In the current study, we sought to characterize the cellular localization of FUS/TLS and the dynamics of stress granule formation when expressed at physiologically relevant levels from cultured primary fibroblasts of patients with mutations in FUS/TLS and healthy controls. To further examine these phenotypes in spinal motor neurons, cells preferentially affected in ALS, induced pluripotent stem (iPS) cell lines were generated from patient fibroblasts by the forced expression of SOX2, KLF4, and OCT4 and subsequently directed to differentiate to spinal motor neurons. We report the discovery of a novel mutation in FUS/TLS, a one base-pair insertion leading to frameshift and truncation of the NLS, which was associated with an extremely early onset and rapidly progressive clinical course. Furthermore, this novel truncation muta-

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tion mislocalized FUS/TLS into the cytoplasm and upon cellular stress, completely co-localized with stress granules in primary fibroblasts. In comparison, a homozygous recessive mutation leading to an amino acid change in the NLS, was associated with predominantly nuclear localization of FUS/TLS with modest incorporation into stress granules whereas cells from healthy controls, FUS remained predominantly nuclear and did not co-localize with stress granules. Importantly, we show that the mislocalization of FUS/TLS and alterations in stress granule dynamics occur in motor neurons resulting from the directed-differentiation of FUS patient-specific iPS cells. Thus, aberrant localization of FUS/TLS in iPS-derived motor neurons recapitulates a key pathogenic event in ALS. This cellular model will serve as a valuable tool for ongoing investigations into ALS disease-specific mechanisms.

GLUTAMATE INTAKE UNBALANCE IN RETT SYNDROME NEURAL CELLS

Carromeu, Cassiano, Freitas, Beatriz, Muotri, Alysson
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Rett syndrome (RTT) is a progressive neurological disorder caused by mutations in the X-linked gene encoding MeCP2 protein. RTT patients have a large spectrum of autistic characteristics and are considered part of the autism spectrum disorders population. These individuals undergo apparently normal development until 6-18 months of age, followed by impaired motor function, stagnation and then regression of developmental skills, hypotonia, seizures and autistic behaviors of different levels of intensity. Using neurons derived from female RTT-iPSC, we previously show that MeCP2 expression levels can affect the number of glutamatergic synapses, leading to an impaired neuronal network in vitro. Now, we generated iPSCs from several male RTT patients plus one individual with MeCP2 duplication. Using a gene expression approach, we validated the misregulation of several GABA and glutamate receptors in electrophysiologically active RTT cortical neurons. To distinguish a potential non-cell autonomous effect from glia cells, we developed a new protocol to generate astrocytes. RTT astrocytes also showed defects in glutamate intake, revealing the astrocyte contribution to neurotransmitter unbalance in RTT neuronal networks. Finally, we optimized our system for a high-throughput drug screening using synaptic proteins density as read outs. We found a candidate drug that rescued neuronal phenotypes in human RTT neurons. Preliminary data in a MeCP2 knockout mouse model showed increase BDNF levels in the brain and longer life span for treated animals. The phenotypes observed in the RTT iPSC-derived human neurons provide evidence of an unexplored developmental window, before the disease onset, where potential therapies could be successfully employed. Our model recapitulates early stages of a human neurodevelopmental disease and represents a promising cellular tool for drug screening, diagnosis and personalized treatment.

CONSEQUENCES OF DYSFUNCTIONAL TELOMERE HOMEOSTASIS IN HUMAN PLURIPOTENT CELLS

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Inherited mutations in genes controlling telomere homeostasis underlie dyskeratosis congenita (DC), a disease of impaired tissue maintenance resulting in bone marrow failure, pulmonary fibrosis, epidermal defects and cancer predisposition. Patients suffering from DC have extremely short telomeres, usually bellow the first percentile in length when compared to the rest of the population. Due to difficulties in purifying and maintaining telomerase positive stem cells from patients in culture, tissue stem cells from DC patients have not been directly studied. We have recently described a new model for studying DC pluripotent cells, by reprogramming patients fibroblasts carrying mutations in TERT, the telomerase reverse transcriptase, dyskerin (DKC1) and TCAB1, all of these genes belonging to the telomerase complex. The iPS cells we generated perfectly recapitulated the disease phenotype, with progressive telomere shortening and premature loss of self-renewal. In our present study we 1) expand the focus of our research to different telomere homeostasis genes that are also implicated in human disease 2) study the mechanism that links short telomeres to loss of pluripotency in human iPS cells. Our results indicate that telomere dysfunction due to mutant telomerase leads to the activation of the p53/p21 pathway, and that this activation is related to the premature loss of self-renewal observed in DC iPS cells. The activation of this pathway seems to be restricted to iPS cells carrying mutations in genes that are responsible for the more severe forms of this disease, indicating not only that cardinal features of DC are accurately modeled within pluripotent iPSCs but that telomerase activity and the efficiency of telomere maintenance within iPSCs can predict disease severity. Interestingly we observe that although resulting in a similar phenotype (short telomeres), each mutation acts trough a different mechanism, including telomerase trafficking and activity. We believe that these results clearly establish the importance of iPS cells as a new model to study the consequences of mutant telomerase in human pluripotent cells, which until recently could only be inferred indirectly.

ANALYSIS OF PRIMARY ADIPOCYTE DISORDER: USING HUMAN PLURIPOTENT STEM CELL-DERIVED ADIPOCYTES TO STUDY LIPODYSTROPHIES

Lee, Youn-Kyoung[1,2], **Ahfeldt, Tim**[1], **Peters, Derek**[1,2], **Xia, Fang**[1,2], **Fresen, Max**[1], **Musunuru, Kiran**[1,3,4], **Cowan, Chad A.**[1,2,3]
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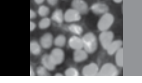
Adipose biology has taken on an urgent importance as it is intimately linked to serious and costly health problems such as obesity, type 2 diabetes, and cardiovascular disease. Serving as an integrator of these metabolic complications, adipose has been studied using both cell culture systems and model organisms. However, the mechanisms of adipocyte regulation are not comprehensively understood, as currently available in vitro or in vivo systems do not fully recapitulate human metabolic processes. To overcome these limitations, we developed a protocol for the reliable and efficient generation of adipocytes from human pluripotent stem cells (PSC). We found that inducible expression of PPARG2 or PPARG2 combined with CEBPB in PSC-derived mesenchymal progenitor cells programmed their development towards a white or brown adipocyte cell fate with efficiencies of 85% to 90%. These PSC-derived adipocytes retained their identity independent of transgene expression, could be maintained in culture for several weeks, expressed mature markers, and exhibited mature functional properties such as lipid catabolism and insulin-responsiveness. When transplanted into mice the programmed



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cells gave rise to ectopic fat pads with the morphological and functional characteristics of white or brown adipose tissue. Thus, these cells allow us to faithfully model human adipose disorders and explore the underlying mechanisms of disease progression. Lipodystrophy is a disorder of adipose tissue characterized by a selective loss of body fat. There are numerous forms of lipodystrophy and these can be divided into either genetic or acquired forms of the disease. Patients with lipodystrophy exhibit metabolic dysfunction such as insulin resistance, glucose intolerance, and accumulation of plasma triglycerides. They often develop diabetes mellitus, hypertriglyceridemia, and early-onset atherosclerosis, which are also found in individuals with morbid obesity. To study the inherited form of this disease, we have established autosomal dominant partial lipodystrophy models by: 1) generating disease-specific human induced PSC from patients harboring specific mutation in the LMNA gene with Dunnigan-type familial partial lipodystrophy (FPLD2), or 2) introducing mutations into the perilipin (PLIN1) gene in PSCs using transcription activator-like effector nucleases (TALENs). While the physiological consequences of LMNA and PLIN1 mutations have been described, very little about the molecular events underlying these diseases is known. In an attempt to better understand the pathophysiology of partial lipodystrophies, we have differentiated PSC harboring these mutations into adipocytes and have found a number of developmental and/or functional differences. The most prevalent type of lipodystrophy is HIV-associated lipodystrophies. These acquired forms occur in HIV-infected patients treated with highly active antiretroviral therapy (HAART). To investigate the pathophysiological mechanism of this form of lipodystrophy, we are testing the impact of HAART in human PSC-derived white and brown adipocytes. Taken together, our human cell-based models of lipodystrophy provide a valuable system for generating a limitless source for human-derived adipocytes to identify novel disease mechanisms.

SELECTIVE INDUCTION OF DISTINCT NKX2.1+ NEURONAL POPULATIONS FROM HUMAN PLURIPOTENT STEM CELLS WHOSE PATTERNING DEPENDS ON TIMING OF SHH EXPOSURE

Maroof, Asif M.[1], **Ying, Shui-Wang**[2], **Tyson, Jennifer A.**[3], **Ganat, Yosif M.**[1], **Keros, Sotirios**[2], **Liu, Rebecca**[1], **Goulburn, Adam**[3], **Widmer, Hans-Ruedi**[4], **Goldstein, Peter A.**[2], **Anderson, Stewart**[3], **Studer, Lorenz**[1]

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Human pluripotent stem cells (PSCs) are a powerful tool for the study of regenerative medicine and their use in modeling various aspects of disease. Cortical interneurons have been implicated in a myriad of neuropsychiatric diseases, including schizophrenia, epilepsy, and autism. While many mechanisms to specify the diverse neuronal cell populations are conserved between mammalian species, several processes have already been described in humans to be evolutionarily divergent. By comparing the mouse and human prosencephalon during embryonic development, we have identified the molecular signatures unique to humans as well as those conserved with rodents. Here we demonstrate that the modification of the timing of sonic hedgehog activation alone is sufficient to direct the specification of multiple ventrally derived neuronal populations, as characterized by the expression of distinct transcriptional profiles together with an NKX2.1-GFP reporter human embryonic stem cell line. These NKX2.1-GFP expressing cells progress in vitro into distinct neuronal precursors that exhibit functional properties, as demonstrated through their physiological characteristics, the expression of specific synaptic targeting proteins, and their ability to migrate extensively in the mouse cortical parenchyma. When placed into a cortical environment, either in vivo or in vitro, these GFP-expressing progenitors give rise to GABA-expressing and CHAT-expressing neurons that receive inputs and can fire action potentials reminiscent of cortical interneurons. In addition, several markers of mature interneurons, such as parvalbumin and somatostatin, are expressed significantly earlier than reported in human development. Therefore, this work demonstrates the use of human PSCs to model aspects of prosencephalic development, which shall be highly informative in our efforts to understand subtleties in human neuronal disease pathology.

Concurrent Session IID — Pluripotent Stem Cells II

Supported by F. Hoffmann - La Roche Ltd.

Thursday, June 14, 2012, 4:15 pm – 6:00 pm

EROSION OF DOSAGE COMPENSATION IMPACTS HUMAN IPSC DISEASE MODELING

Eggan, Kevin

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Although distinct human induced pluripotent stem cell (hiPSC) lines can display considerable epigenetic variation, it has been unclear if such variability impacts their utility for disease modeling. Here, we show that although low passage female hiPSCs retain the inactive X chromosome of the somatic cell they are derived from, over time in culture they undergo an "erosion" of X chromosome inactivation (XCI). This erosion of XCI is characterized by loss of XIST expression and foci of H3-K27-trimethylation, as well as transcriptional de-repression of genes on the inactive X that cannot be reversed by either differentiation or further reprogramming. We specifically demonstrate that erosion of XCI has a significant impact on the use of female hiPSCs for modeling Lesch-Nyhan syndrome. However, our finding that most genes subject to XCI are de-repressed by this erosion of XCI suggests that it should be a significant consideration when selecting hiPSC lines for modeling any disease.

BMP-SMAD-ID AXIS PROMOTES REPROGRAMMING TO PLURIPOTENCY

Hayashi, Yohei[1], **Hsiao, Edward C.**[2], **Sami, Salma**[1], **Lancero, Mariselle**[1], **Schlieve, Christopher R.**[1], **Yano, Koyori**[3], **Nagahashi, Ayako**[3], **Ikeya, Makoto**[4], **Matsumoto, Yoshihisa**[4], **Asaka, Isao**[3], **Toguchida, Junya**[4], **Conklin, Bruce R.**[1], **Yamanaka, Shinya**[1]

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Reprogramming somatic cells to pluripotent stem cells represents an exciting new paradigm in biology and medicine. We developed a technology to generate induced pluripotent stem cells (iPSCs) by transducing defined factors, such as OCT4, SOX2, KLF4, and C-MYC, into somatic cells. These defined factors are transcription factors that regulate gene expression important in self-renewal and pluripotency; however, the efficiency of iPSC

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generation remains very low. Cytokine-induced cell signaling can affect the efficiency of iPSC generation, but the precise effects and mechanisms required for the reprogramming are still unclear. BMP-SMAD signaling is one of the major signal pathway to regulate cell behavior, including stem cell self-renewal and differentiation. On the self-renewal and differentiation of mouse and human embryonic stem (ES) cells and iPSCs, BMP-SMAD signal has opposite effects; for mouse ES/iPSCs, BMP-SMAD signal has positive effects on their self-renewal and inhibits differentiation. Conversely, for human ES/iPSCs, BMP-SMAD signal has negative effects on their self-renewal and promotes differentiation. Thus, it is important and interesting to examine the effect of BMP-SMAD signaling on mouse and human reprogramming to iPSCs. We found that the efficiency of iPSC generation from the human dermal fibroblasts (HDFs) obtained from patients of fibrodysplasia ossificans progressiva (FOP) with the R206H mutation in ACVR1 gene, which leads to hyperactivation of BMP-SMAD signaling, was much higher than that of normal HDFs. The Inhibition of BMP-SMAD signaling by adding Dorsomorphin or LDN-193189 or by transducing SMAD6 or SMAD7 to FOP-HDFs decreased the efficiency of iPSC generation. Conversely, the activation of BMP-SMAD signaling by transducing mutant ACVR1 (R206H) or SMAD1 or by adding BMP4 recombinant protein to normal HDFs in specific periods of reprogramming increased the efficiency of iPSC generation. Furthermore, inhibitor of DNA binding (ID) genes, which are direct targets of BMP-SMAD signaling, increase the efficiency of iPSC generation from normal HDFs and are required for iPSC generation. We also confirmed that the same results were obtained from the reprogramming experiments using mouse fibroblasts. Our results indicate that the BMP-SMAD-ID axis is critical for efficiently generating iPSCs from both human and mouse somatic cells. In addition, our findings suggest that human genetic diseases such as FOP may favor more efficient iPSC generation, and that these pathways could provide methods for improving the efficiency of iPSC generation.

LIN28 INTERACTS WITH DISCRETE BINDING MOTIFS IN MESSENGER RNA AND REGULATES ALTERNATIVE SPLICING THROUGH MODULATION OF SPLICING FACTORS

Wilbert, Melissa L.[1], Huelga, Stephanie C.[1], Stark, Thomas J.[1], Massirer, Katlin B.[1], Chen, Stella X.[1], Liang, Tiffany Y.[1], Lovci, Michael T.[1], Moresco, James J.[2], Kazan, Hilal[3], Vu, Anthony Q.[1], Yates III, John R.[2], Morris, Quaid[3], Yeo, Gene W.[1]

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LIN28 is a conserved RNA binding protein (RBP) important for pluripotency, reprogramming, and oncogenesis. Studies thus far have focused on the roles of LIN28 through its regulation of the let-7 family of microRNAs (miRNA). Our goal was to identify and characterize the network of messenger RNA targets of LIN28 in order to explain its regulatory mechanisms distinct from its ability to block let-7 processing. Using UV cross-linking and immunoprecipitation of the LIN28 protein followed by high-throughput sequencing (CLIP-seq) we have identified discrete LIN28 binding sites on target RNAs in human embryonic stem cells and somatic cells expressing exogenous LIN28. We found that in addition to hundreds of miRNAs, LIN28 directly interacts with thousands of human mRNA transcripts, including LIN28 itself and dozens of splicing regulators. Furthermore, these binding events preferentially occur at instances of a LIN28 binding motif within hairpin loops and other types of unpaired, secondary structures. In a set of targets we confirm that this binding results in an increase in translation of the target mRNA. Finally, splicing-sensitive junction arrays demonstrated that LIN28 over-expression causes widespread downstream alternative splicing (AS) differences. These findings demonstrate a novel mechanism coupling AS events with translational control, specifically for mRNA transcripts directly regulated at the translational level by LIN28. These findings help to explain the ways in which LIN28 contributes to the maintenance of cellular homeostasis via mRNA regulation, and how changes in this control may result in cancer and differentiation.

ELUSIVE NON-MAMMALIAN TRANSGENICS: IN-VIVO PLURIPOTENCY IN NON-MAMMALIAN IPS-LIKE CELLS, INDUCED BY MAMMALIAN GENES

Rossello, Ricardo A.[1], Chen, Chun Chun[2], Dai, Rui[2], Howard, Jason[2], Hochgeschwender, Ute[2], Jarvis, Erich D.[2]

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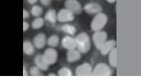
Important issues in biology are addressed in experimental systems other than mammals. Some of these animals have either desired characteristics not found in humans or traits similar to humans that are not found in closely related non-human primates or commonly used laboratory animals. These include vocal learning in parrots and songbirds, widespread adult neurogenesis in non-mammalian vertebrates, and vascularization and organ regeneration in zebra fish. However, stem cells and stem cell based transgenics for some of these species have been hard or impossible to generate. Previously, we showed then that cells for model species spanning 500 million years in evolution (mouse, birds, fish, and fly) could be reprogrammed into an iPS-like state, using the mammalian homologues of the four Yamanaka factors. These cells exhibited morphological transformation, endogenous gene induction, an enhanced proliferative rate, and in-vitro pluripotency, suggesting that the stem-cell state may be highly conserved across non-mammalian vertebrates.

Although these findings were surprising and promising, they had yet to address the proper maintenance of these cells, their potential to differentiate into other cell types and, most importantly, their ability to incorporate in-vivo. Here, we report several new findings that further characterize these mammalian-gene induced non-mammalian bird and fish iPS-like cells. First, full gene expression assays were performed on these cells showing a wide range of not only up-regulated genes, but also and down-regulated ones as a consequence of the mammalian gene transduction. We also showed that the cells, after a 5th passage, silence the exogenous mouse genes, while maintaining their endogenous expression. To assess these and other experiments, we were able to culture cells in thus far optimal conditions, using cytokines, growth factors, and small molecule inhibitors. These conditions vary significantly between mice, bird, and fish cells. After labeling these characterized iPS-like cells with GFP, and making sure they were karyotypically normal, we addressed two key questions. The first one was to determine if these cells could differentiate into a specialized cell type. Our data shows that iPS-like avian cells (songbird, quail, and chicken) can be differentiated into neurons with characteristic morphology, expressing neuronal markers, and producing action potentials. These are the first non-mammalian neurons derived from non-mammalian stem cells. The second question was to see if these cells had the potential to incorporate in-vivo, providing the platform for non-mammalian transgenics. GFP-labeled iPS-like and control GFP labeled fibroblasts were microinjected into both early embryonic chicken and



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zebrafish. Growth of the embryos was stopped at 3 and 6 days post injection. Fluorescent microscopy and immunohistochemistry confirm incorporation of the GFP labeled iPS-like cells, but not the fibroblasts, into both chicken and fish embryos. Furthermore, the cells were able differentiate into distinct cell types within the embryo. These findings are the first we are aware of to (1) generate iPSs for fish and birds, (2) the first to generate neurons from stem cells in non-mammalian species and (3) the first to generate iPS-based chimeras for birds and fish. Taken together, they open the possibility to create elusive transgenic animals, study complex biological traits, and gain new insights into the nature of cell evolution.

ENGINEERING LOCAL MICROENVIRONMENTS RE-ACTIVATES DOWNREGULATED SIGNALING PATHWAYS, LEADING TO THE IDENTIFICATION AND ISOLATION OF NAÏVE MOUSE AND HUMAN PLURIPOTENT STEM CELLS.

Onishi, Kento[1], Tonge, Peter D.[2], Nagy, Andras[2], Zandstra, Peter W.[1]
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Mouse epiblast stem cells (EpiSCs) can revert to naïve mouse embryonic stem cells (mESCs) upon overexpression of transgenes and subsequent culture in stringent mESC media (2iL). Additionally, transgene-free reversion of EpiSCs can occur when response to key cytokines is enhanced. EpiSCs also display characteristics similar to human ESCs (hESCs); an observation that has motivated the search for a human counterpart to mESCs - the naïve hESC. Here we report that robust response to LIF and BMP4 is achieved by controlling the local microenvironment of EpiSCs. Culture on a layer of mouse embryonic feeders (MEFs) provides the minimal necessary control of local cues to allow for transgene-free reversion to occur. We recapitulate this effect in defined conditions by micropatterning (µP) EpiSCs and demonstrate that this leads to an increase both in LIF responsiveness and in endogenous BMP4 signaling. We further demonstrate that both signals are required to drive reversion of EpiSCs to mESCs. Importantly, reverted EpiSCs display typical markers of naïve pluripotency and are able to contribute to chimeras. We next controlled, by µP, the local microenvironment of hESCs and investigated the effects on the reversion to naïve hESCs. Upon µP of hESCs, we observed an upregulation in transcription of Oct4, Klf4, Klf2, Nr5a1, Tbx3, and Dax1 - genes that are highly expressed in ESCs and play key roles in reversion to naïve pluripotency. We constructed a small-scale screen to identify optimal concentrations of LIF, BMP4, and Wnt that are required during µP culture to preserve Oct4 and Nanog expression in hESCs cultured in 2iL. Furthermore, by non-enzymatically dissociating hESCs, we preserved E-cadherin protein expression, closely mirroring the conditions used in EpiSC reversion and allowing for more robust micropatterning of hESCs. Through this, we have been able to identify conditions that lead to the emergence of cells that resemble naïve hESCs. By controlling the response to exogenous LIF, BMP4, and Wnt, we have established a platform on which to investigate the emergence of precursors to naïve hESCs.

MICRORNA-BASED DETECTION OF MRNA NETWORKS THAT REGULATE DE-DIFFERENTIATION TO INDUCED PLURIPOTENT STEM CELLS

Judson, Robert L.[1], Greve, Tobias[2], Lamouille, Samy[2], Blelloch, Robert[1]
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MicroRNAs (miRNAs) are a class of genetically encoded small RNAs that alter cell behavior by inhibiting the translation of mRNA networks. Once evolved, miRNAs are rarely lost, are highly conserved in both sequence and expression, and apply strong selective pressure on target mRNA sequences. MicroRNAs play a major role in cell fate transitions, including the generation of induced pluripotent stem cells (iPSCs) from fibroblasts. This suggests cell fate transitions can be induced through inhibition of the initial cell's transcriptome (via expression of miRNAs) as opposed to direct activation of a destination cell's transcriptome (via expression of transcription factors). Based on these findings, we hypothesize that forced expression of miRNAs that drive these cell fate transitions can be used as a tool to determine which mRNAs and pathways must be suppressed in order to achieve a desired cellular identity. To test this hypothesis, we have begun to systematically identify genes that are targeted by miRNAs during de-differentiation of mouse fibroblasts to iPSCs. We have performed a screen to uncover all microRNAs that can promote iPSC formation. Nine miRNAs were identified including miR-294 and three from the miR-302 cluster, which we had previously identified as strong enhancers of de-differentiation. The remaining five are novel. We next established that at least four of these miRNAs function primarily during the initiation phase of reprogramming, priming the fibroblasts for the maturation phase. We used several experimental and bioinformatic approaches to identify and verify over 100 mRNA targets of two of the initiation-enhancing miRNAs. Using a siRNA-based gene knockdown approach and high-content analysis of time-lapsed images, we identified 36 mRNAs that suppress the cell transition from a fibroblast to an iPSC. Gene ontology analysis revealed highly enriched signaling pathways and cellular processes, several of which we verified as strong regulators of the initiation phase of de-differentiation. These data represent the most extensive functional characterization of a miRNA-mRNA target network to date and highlight dozens of novel genes and pathways that can be manipulated to make somatic cells more susceptible to de-differentiation.

Concurrent Session IIE — Stem Cell Aging & Metabolism

Thursday, June 14, 2012, 4:15 pm – 6:00 pm

EPIGENETIC MECHANISMS OF STEM CELL AGING AND REJUVENATION

Rando, Thomas A.
Stanford University, Stanford, CA, USA

For most tissues, stem cell numbers decline negligibly with age, but there is a nevertheless an age-dependent decline in stem cell functionality. Many molecular, biochemical, and functional features of stem cells have been characterized across a broad range of tissues, and these changes have been assumed to be largely irreversible and inevitable accompaniments of aging. However, in studies both in vivo and in vitro we have demonstrated a reversibility of the functional and, in some cases, molecular characteristics of aged stem cells. Supported by compelling data from studies of heterochronic parabiotic pairings of mice, it is clear

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that the aged phenotype can be modified when aged cells are exposed to a youthful systemic milieu. These findings challenge the fundamental tenet of aging as an irreversible process and raise the question of whether, or to what extent, the aged phenotype is epigenetically determined. We have begun to examine the epigenetic profiles of young, old, and "rejuvenated" old stem cells to attempt to define youthfulness and aging in epigenetic terms. To the extent that aging can be "reprogrammed" back to youthfulness in somatic tissues has parallels to the resetting of the aging clock that occurs with somatic cell nuclear transfer. Elucidating the underlying molecular features, both genetic and epigenetic, of aged stem cells will provide a framework for understand the fundamental molecular mechanisms of aging and the mechanisms by which environmental influences such as those that occur in the setting of heterochronic parabiosis can reverse the mechanisms of aging.

REGULATION OF ORGANISMAL GROWTH AND METABOLISM BY THE STEM CELL FACTOR LIN28

Shinoda, Gen[1], Shyh-Chang, Ng[1], de Soysa, T. Yvanka[1], Zhu, Hao[1], Shah, Samar P.[1], Abo-Sido, Nora[1], Seligson, Marc T.[1], Hagan, John P.[2], Yabuuchi, Akiko[1], Takeuchi, Ayumu[1], Gregory, Richard I.[2], Moss, Eric G.[3], Daley, George Q.[1]

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The RNA-binding protein lin-28 was originally identified in C. elegans as a regulator of developmental timing. We and others discovered that the two paralogues of mammalian Lin28, A and B, regulate the biogenesis of the let-7 family of tumor-suppressor microRNAs, and play critical roles in ES cells, reprogramming, and malignancy. Recently, we also showed that the Lin28/let-7 axis regulates glucose metabolism. However, the physiological mechanisms linking Lin28a/b to adult growth and glucose metabolism remained unclear, especially since Lin28a/b are primarily expressed in the early embryo, but show limited expression in adult tissues. To further define its physiological roles in mammalian development and metabolism, we generated and analyzed both Lin28a and Lin28b knockout (KO) mice. We observed that Lin28a KO leads to persistent growth retardation from embryogenesis till adulthood, whereas Lin28b KO regulates postnatal growth. Interestingly, Lin28a+/-; Lin28b-/- mice showed postnatal growth retardation and delayed onset of puberty compared to Lin28b-/- mice, indicating a dosage effect of Lin28a and Lin28b. Indeed, when we knocked out both Lin28a and Lin28b, we observed embryonic lethality around E11.5. To test the developmental stage in which Lin28a or Lin28b expression is most critical for animal growth, we generated ubiquitin-CreER; Lin28afl/fl and ubiquitin-CreER; Lin28bfl/fl mice, then injected tamoxifen to conditionally delete the floxed alleles at different ages. Intriguingly, we found that embryonic deficiency in Lin28a or Lin28b exerted long-lasting effects on adult growth, whereas neonatal or adult deficiency in Lin28a/b had no effects. Haploinsufficiency of Tsc1 rescued the adult growth phenotype in Lin28b KO mice, relative to Tsc1+/-; Lin28b+/+ mice, indicating that the Tsc1-mTOR pathway acts downstream of embryonic Lin28b to mediate its effects on adult growth. Since the mTOR pathway is a major regulator of glucose metabolism, we performed metabolomics studies on Lin28a KO embryos and glucose tolerance tests on skeletal muscle-specific Lin28b KO mice. Our results suggest that embryonic Lin28a and Lin28b are necessary for normal glucose metabolism starting from embryogenesis, and extend into adulthood. Finally we found that skeletal muscle-specific Lin28b KO mice phenocopied the adult growth retardation of constitutive Lin28b KO mice, whereas hepatocyte- or pancreatic β cell-specific Lin28b KO did not, suggesting that embryonic muscle-specific Lin28b deficiency disrupts normal glucose metabolism during embryogenesis, with persistent effects on growth and metabolism in adulthood. Human genome-wide association studies have recently shown that Lin28b and several let-7 targets are related to human growth, onset of puberty, and diabetes. Our mouse models should be useful for understanding the "Barker hypothesis", which states that an epigenetic memory of embryonic nutritional conditions in utero influences chronic diseases in adulthood, such as type 2 diabetes.

LKB1-DEPENDENT METABOLIC REPROGRAMMING OF MOUSE HEMATOPOIETIC STEM CELLS DURING DEVELOPMENT

Nakada, Daisuke

Baylor College of Medicine, Houston, TX, USA

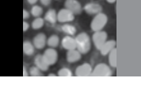
In order to maintain stem cells throughout life and maintain tissue homeostasis, stem cells need to precisely control mechanisms regulating cell growth, proliferation, and maintenance. Metabolic regulation is a fundamental property of cells that regulates cell growth, proliferation, and maintenance, but whether metabolism is a housekeeping function conserved among many cell types and developmental stages, or whether different cell types at different developmental stages have different metabolic regulations is still elusive. Supporting the latter possibility, we recently reported that disrupting the Lkb1-AMPK pathway, which links energy metabolism to cell proliferation, reduced cellular ATP levels and mitochondrial membrane potential in hematopoietic stem cells but not in myeloid progenitors, indicating that metabolism in stem cells differs from that of other progenitor cells. We here show that Lkb1 not only regulates energy metabolism differently between different progenitor populations, but also differently during development. Whereas deletion of Lkb1 from adult hematopoietic stem cells lead to rapid depletion, deletion of Lkb1 from fetal hematopoietic stem cells did not rapidly deplete hematopoietic stem cells. Instead, Lkb1-deficient fetal hematopoietic stem cells were maintained until a specific developmental time point after which they become acutely depleted. The depletion of Lkb1-deficient fetal hematopoietic stem cells was associated with metabolic alterations, which were not observed in other progenitors. These results suggest that a dynamic change in metabolic regulation occurs during fetal hematopoietic stem cell development, which is at least partly regulated by Lkb1.

INTEGRIN AIIB (CD41) IDENTIFIES A MYELOID-BIASED MOUSE HEMATOPOIETIC STEM CELL SUBSET THAT ACCUMULATES WITH AGE

Gekas, Christos, Graf, Thomas

Gene Regulation, Stem Cells and Cancer, Centre for Genomic Regulation (CRG), Barcelona, Spain

Hematopoietic stem cells (HSCs) sustain the life-long production of all blood lineages. However, during aging several hematopoietic alterations take place, such as attenuated lymphoid-cell production, an increased bias towards myeloid lineages and a decline in the functional potential of HSCs. It is now thought that the HSC compartment consists of distinct subtypes that differ in their life-span, cycling status and lineage-bias, and that during aging a specific subset of HSCs becomes selected. However, the driving mechanisms behind this population shift remain poorly



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understood and no surface marker has yet been identified that distinguishes myeloid from lymphoid biased HSCs. Here we describe the identification of integrin alphaIIb (CD41, itga2b, GpIIb) as a possible driver of these processes. CD41 is the classical marker of platelets but is also transiently expressed on emerging fetal HSCs. Whether CD41 marks adult HSCs remains controversial; however, current consensus seems to favor the idea that CD41 in not expressed on real adult HSCs and some current HSC isolation protocols exclude the CD41+ fraction. We found CD41 to be heterogeneously expressed in adult HSCs and to increase gradually with the age of mice to mark most long-term (LT-) HSCs (Lin-Sca1+Kit+ (LSK) CD34-Flt3- or LSK CD150+CD48-) but not progenitors. The CD41+ HSC fraction consisted largely of quiescent cells. CD41:YFP knock-in mice, where CD41 is replaced by the YFP reporter, displayed significant multi-lineage pancytopenia, which was exacerbated with age. In addition, CD41YFP/YFP HSCs showed increased apoptosis and proliferation. Non-competitive transplantations into wild-type recipients showed that these defects were HSC-autonomous. Notably, incubation of bone marrow cells with a CD41 blocking antibody (clone MWReg30) significantly compromised the long-term repopulation ability of wild-type HSCs. Thus, to circumvent this issue we transplanted YFP+ and YFP- LT-HSCs from CD41YFP/+ heterozygous mice, which did not show any hematopoietic defects. Importantly, YFP+ HSCs showed robust multi-lineage long-term repopulation ability, sustained in secondary hosts. In addition, YFP+ HSCs displayed a markedly increased myeloid-bias compared to YFP- HSCs. Finally, microarray analyses revealed distinct expression differences between the CD41+ and CD41- HSC fractions in lineage restricted genes, cytokines and their receptors, metabolic activity and cell-cycle components. In summary we have identified CD41 as a novel marker of a myeloid-biased, quiescent HSC subset which accumulates with age, possibly due to the role of CD41 for the survival and quiescence of HSCs.

PDK PLAYS A CRITICAL ROLE IN MAINTAINING QUIESCENCE OF HEMATOPOIETIC STEM CELLS THROUGH GLYCOLYTIC METABOLIC PROGRAM

Takubo, Keiyo, Suda, Toshio

Dept. Cell Differentiation, Keio University School of Medicine, Tokyo, Japan

Hematopoietic stem cells (HSCs) reside in a hypoxic bone marrow environment, and their metabolic status is distinct from that of their differentiated progeny. HSCs generate energy mainly via anaerobic metabolism by maintaining a high rate of glycolysis. This metabolic balance promotes HSC maintenance by limiting the production of reactive oxygen species, but leaves HSCs susceptible to changes in metabolism including redox status. Here we used capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) analysis to show that mammalian HSCs exhibit a specific metabolic phenotype relevant to central carbon metabolism. Specifically, we found that HSCs generate adenosine-triphosphate (ATP) by anaerobic glycolysis through a hypoxia-inducible factor-1α (HIF-1α) and pyruvate dehydrogenase (PDH) kinases (Pdks)-dependent mechanism. Expression of positive regulators of glycolysis and high LDH activity were observed in HSCs compared to progenitor cells. HIF-1α-deficient HSCs showed attenuated anaerobic glycolysis, decreased ATP generation and compensatory mitochondrial aerobic metabolism. Increased HIF-1α levels seen in VHL-mutant HSCs, in which HIF-1α is stabilized, and their progenitors suppressed mitochondrial mass and increased glycolysis. Expression of Pdk2 and Pdk4, which antagonize aerobic energy metabolism by phosphorylating PDH, was upregulated by HIF-1α. Phosphorylation of PDH was specifically observed in HSCs, but not in the differentiated hematopoietic progenitors and decreased in HIF-1α-deficient HSCs. Overexpression of Pdk2 or Pdk4 in HIF-1α-deficient HSCs restored glycolysis, cell cycle quiescence and stem cell capacity. Because loss of quiescence was evident in HSCs from Pdk2-/-: Pdk4-/- compound knockout mice, cooperative activation of Pdk family member is essential for the phosphorylation of PDH and the suppression of aberrant activation of cell cycle in HSCs. Modulation of Pdk activity in vitro and in vivo by small molecule altered cell cycle quiescence of HSCs, and increased Pdk expression in HSCs resulted in early induction of quiescence during transplantation. These overall data indicate that the metabolic checkpoint by HIF-1α/Pdk axis regulates metabolic status and cell cycle quiescence of HSCs. Also, the modulation of Pdk is a potential target for the artificial stem cell manipulation.

CDC42 ACTIVITY REGULATES HEMATOPOIETIC STEM CELL AGING AND REJUVENATION

Florian, Maria Carolina[1], Doerr, Karin[1], Niebel, Anja[1], Scharffetter-Kochanek, Karin[1], Zheng, Yi[2], Geiger, Hartmut[1]

[1]University of Ulm, Ulm, Germany, [2]Cincinnati Children's Hospital Medical Center and University of Cincinnati, Cincinnati, OH, USA

The global trend in population aging is unprecedented - without parallel in human history. Aging of stem cells is, in combination with other factors, an underlying cause for aging associated diseases and tissue attrition with age. The identification of molecular mechanisms of stem cell aging is a first step towards developing rational approaches to attenuate stem cell aging. In the hematopoietic system, hematopoietic stem cell (HSC) aging is driven by both intrinsic and extrinsic factors and is linked to a decreased immune response, an increase in myeloid disease, late-onset anemia and a reduced regenerative capacity. Cdc42 belongs to the family of small Rho-GTPase and cycles between an active (GTP-bound) and an inactive (GDP-bound) state. Cdc42 is known to regulate actin and tubulin organization, cell-cell and cell-extracellular matrix adhesion and cell-polarity. We previously demonstrated that Cdc42 activity is significantly increased in primitive hematopoietic cells as well as in other tissues of aged mice. Based on this observation we hypothesize that the increased activity of Cdc42 in aged HSCs may be causatively linked to cell-intrinsic aging of HSCs. We first tested the hypothesis by assaying the function of young HSCs that display constitutively elevated Cdc42 activity (HSCs deficient for Cdc42GAP, a highly selective negative regulator of Cdc42-activity). Results demonstrated that young HSCs with elevated Cdc42 activity closely resemble aged HSCs and are significantly distinct from young control HSCs with respect to their repopulation ability, contribution to the B-cell lineage and contribution to the myeloid cell lineage in PB as well as in BM in both primary and secondary recipients. Furthermore, young HSCs with elevated Cdc42 activity, similarly to aged HSCs, contribute significantly more to the pool of LT-HSCs compared to young controls both in primary and in secondary recipients. Thus, chronologically young HSCs with elevated Cdc42 activity are functionally similar to chronologically aged HSCs in competitive transplantation assays, implying a causative role for intrinsically elevated Cdc42 activity in HSCs aging.

Cdc42 activity has been implicated in the regulation of cellular polarity, a phenotype thought to be critical for proper stem cell function. We therefore determined the polarity status of aged LT-HSCs by single cell immunofluorescence staining. Interestingly, young HSCs localize Cdc42, tubulin and several other polarity

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proteins in a highly asymmetric way, while aged HSCs are mainly apolar with respect to these proteins, implying apolarity as a novel phenotype and prospective marker of aged HSCs. We finally tested whether inhibition of the elevated Cdc42 activity in aged HSCs might attenuate phenotypes associated with HSC aging. Hence, we treated ex vivo sorted aged HSCs with a highly selective Cdc42 inhibitor to reduce Cdc42 activity in aged HSCs to the level measured in young cells. Decreasing Cdc42 activity reverts apolar aged HSCs into polar HSCs and rejuvenates aged HSCs functionally, as they differentiate in vivo more readily into lymphoid cells and maintain, compared to untreated aged HSCs, high regenerative capacity upon secondary transplants. Therefore, our data imply a novel and critical mechanistic role for Cdc42 activity in the establishment of HSC polarity and in HSC aging and identify Cdc42 activity as a pharmacological target for rejuvenating cell intrinsic stem cell aging.

Plenary IV — Genomics and Epigenomics of Stem Cells

Supported by Fluidigm Corporation

Friday, June 15, 2012, 9:00 am - 11:30 am

CHARTING THE MAMMALIAN EPIGENOME

Ren, Bing
University of California, San Diego and Ludwig Institute for Cancer Research, La Jolla, CA, USA

The DNA sequence of an organism is a blueprint of life, but it alone is insufficient to explain the phenotypic variations among individuals and the diverse gene expression programs in different cell types. Covalent modifications to DNA and histone proteins also govern the function and activity of the underlying DNA sequences. Such chemical changes are collectively referred to as the epigenome. A key question in biology is how epigenetic variations contribute to cell fate determination and cellular identity. Here, I will describe results from a comprehensive survey of chromatin modification states and DNA methylation patterns in multiple human cell types, including the fibroblast cells, the H1 human embryonic stem cell line and H1-derived neural progenitor cells, mesendoderm cells, mesenchymal stem cells and trophoblast cells. Our analysis reveals widespread differences of epigenomic landscapes between the pluripotent and lineage-committed cell types. Importantly, we observe that cellular differentiation is frequently accompanied by a decrease of non-CpG methylation content, a spreading of repressive chromatin marks and the appearance of large domains of DNA exhibiting partial methylation (PMD). Interestingly, these large domains selectively affect genes involved in developmental pathways, suggesting an important link between the epigenetic state and developmental programs in mammals.

TET PROTEINS AND 5-METHYLCYTOSINE OXIDATION

Rao, Anjana, Huang, Yun Nancy, Ko, Myunggon, An, Jungeun
La Jolla Institute for Allergy & Immunology, La Jolla, CA, USA

TET family enzymes convert 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) and additional oxidized products in DNA. Tet1 and Tet2 are Oct4-regulated enzymes that sustain 5hmC in mouse embryonic stem cells (ESCs) and are induced concomitantly with 5hmC during reprogramming of fibroblasts to induced pluripotent stem cells. Somatic TET2 mutations are frequently observed in myeloid neoplasms in humans. I will describe our recent studies on the functions of Tet proteins and 5hmC in mouse ES cells, haematopoietic stem cells and other precursor cell types.

SYSTEMS BIOLOGY OF STEM CELLS

Ng, Huck-Hui
Dept of Biological Sciences, National University of Singapore, Singapore, Singapore

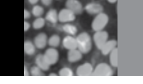
Embryonic stem (ES) cells are characterized by their ability to self-renew and remain pluripotent. Transcription factors have critical roles in the maintenance of ES cells through specifying an ES-cell-specific gene expression program. Deciphering the transcriptional regulatory network that describes the specific interactions of these transcription factors with the genomic template is crucial for understanding the design and key components of this network. To gain insights into the transcriptional regulatory networks in ES cells, we use chromatin immunoprecipitation coupled to ultra-high-throughput DNA sequencing (ChIP-seq) to map the locations of sequence specific transcription factors. These factors are known to play different roles in ES cell biology. Our study provides new insights into the integration of these regulators to the ES cell-specific transcription circuitries. Collectively, the mapping of transcription factor binding sites identifies new features of the transcriptional regulatory networks that define ES cell identity. Using this knowledge, we investigate nodes in the network which when activated, will jump-start the ES cell-specific expression program in somatic cells.

Outstanding Young Investigator Award Winner

STEM CELLS DURING EPITHELIAL HOMEOSTASIS AND CANCER INITIATION

Blanpain, Cedric
IRIBHM, Université Libre de Bruxelles, Bruxelles, Belgium

Stem cells (SCs) ensure the homeostasis and repair of adult tissues throughout the lifetime of the animals. Our lab develops new genetic approaches to study epithelial SC from the skin epidermis as well as from different glandular epithelia. I will discuss how lineage-tracing experiments can be used to define the fate of epithelial SC during morphogenesis, homeostasis, tissue regeneration, and tumor initiation. I will present new approaches combining clonal analysis and mathematical modeling to define the proliferation dynamic acting in the interfollicular epidermis to maintain the homeostasis of the skin barrier and to repair skin injuries after wounding. I will present how we used genetic lineage tracing experiments and clonal analysis to define the cellular hierarchy of the mammary gland during development, adulthood and pregnancy. We found that the mammary gland initially developed through the presence of multipotent progenitors, but its postnatal development is ensured by the presence of both luminal and myoepithelial lineage restricted unipotent SCs that display extensive renewing capacities at the clonal level. I will present new data suggesting that the prostate epithelium also develops through the presence of multipotent progenitors that become progressively lineage restricted during adult life, suggesting that the switch from multipotency to unipotency during postnatal life could be a landmark of glandular epithelia. Finally, I will discuss the role of tissue specific SC during cancer initiation. For the vast majority of cancers, the cell at the origin of tumor ini-



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tiation is still unknown. Two epithelial skin cancers are frequent in human populations: the basal cell carcinoma and the squamous cell carcinoma. I will present how we used new genetic lineage tracing approaches to identify the cells at the origin of these two types of cancer in mice and demonstrated that expression of differentiation markers in tumor is not necessarily predictive of the cancer cell of origin. We have now developed novel strategy to isolate by flow cytometry oncogene targeted cells at different time following oncogene expression and determined the molecular changes associated with tumor initiation and malignant progression. The functional relevance of these genes in mice and the relevance of these findings to human skin cancers will be presented.

Concurrent Session IIIA: Hematopoietic Stem Cells

Friday, June 15, 2012, 1:30 pm - 3:15 pm

GLYCOLYTIC METABOLISM IN HEMATOPOIETIC STEM CELLS

Suda, Toshio

Keio University School of Medicine, Tokyo, Japan

Hematopoietic stem cells (HSCs) are sustained in a specific microenvironment known as the stem cell niche. Adult HSCs are kept quiescent during the cell cycle in the endosteal niche of the bone marrow. Normal HSCs maintain intracellular hypoxia, stabilize the hypoxia-inducible factor-1α (HIF-1α) protein and generate ATP by anaerobic metabolism. In HIF-1α-deficiency, HSCs became metabolically aerobic, lost cell cycle quiescence, and finally exhausted. An increased dose of HIF-1α protein in VHL mutated HSCs and their progenitors induced cell cycle quiescence and accumulation of HSCs in the BM (Cell Stem Cell, 2010). Restored glycolysis by pyruvate dehydrogenase kinases (PDKs) ameliorated cell cycle quiescence and stem cell capacity. HSCs directly utilize the hypoxic microenvironment to maintain their cell cycle by HIF-1α-dependent metabolism by down-regulating reactive oxygen species (ROS). The abnormal hematopoiesis was also detected in PDK2 and PDK4-double KO mice. In this presentation, we will discuss the importance of oxygen homeostasis and energy metabolism for maintenance of HSC function and long-term self-renewal.

POLYCOMB REPRESSIVE COMPLEX 2 IS ESSENTIAL FOR THE MAINTENANCE AND DIFFERENTIATION OF MOUSE HEMATOPOIETIC STEM CELLS

Xie, Huafeng[1], Nguyen, Minh[2], Orkin, Stuart H.[1]

[1]Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA, [2]Children's Hospital Boston, Boston, MA, USA

Though polycomb repressive complex 2 (PRC2) is known to play important roles in several embryonic and adult stem cell types, its role in hematopoietic stem cells (HSCs) remains elusive. To uncover whether PRC2 governs HSC maintenance and/or differentiation we generated a conditional knockout mouse of Eed, a core subunit gene of PRC2 (Eedfl/fl). These animals were crossed to VavCre transgenic mice, to specifically inactivate Eed in all hematopoietic cells including HSCs. EedFl/FlVavCre embryos developed without apparent abnormality and had largely normal HSC numbers in the fetal liver. However, within a few days after birth the mutant pups developed pancytopenia and succumbed to bacterial infection. FACS analysis revealed that compared to wild type controls, mutant pups had more phenotypically-defined long-term (LT)-HSCs (Lin-CD48-CD150+Sca1+ cells), despite a higher percentage of mutant HSCs undergoing apoptosis. At the same time, Eed mutant pups had significantly reduced numbers of mature blood cells, which was due to failure of mutant HSCs to efficiently differentiate into intermediate progenitor cells. To investigate the importance of EED for adult LT-HSCs, we crossed EedFl/Fl mice to the inducible Mx1Cre mouse strain. Bone marrow cells from EedFl/FlMx1Cre mice or EedFl/wtMx1Cre mice were mixed with wild type competitor cells and transplanted into recipient mice at a 2:1 ratio. Upon injection of poly-IC to induce Cre expression, for EedFl/FlMx1Cre marrow this ratio dropped to about 0.5:1 after one month and 0.2:1 after 3 months while it remained unchanged at about 2:1 after transplantation of EedFl/wtMx1Cre cells. Direct evaluation of phenotypically-defined LT-HSCs in bone marrow 4 months after poly-IC injection revealed a ratio of 0.05:1, suggesting a direct effect of Eed loss on the blood stem cell pool. To gain insights into the molecular mechanism of Eed action, we analyzed the global gene expression profiles of purified LT-HSCs from EedFl/FlVavCre pups. This revealed the upregulation of components of the Wnt signaling pathway and of genes associated with apoptosis compared to wild type cells. Among the list of top 50 most highly up-regulated genes in mutant HSCs were also several genes whose over-expression had been shown to increase stem/progenitor cell frequency and hamper differentiation such as Sox7, HoxC4, Muc1 and established PRC2 targets such as CDKN2a. Together, our data suggest that PRC2 has a vital role in HSC maintenance and differentiation. Specifically, PRC2 appears to suppress a set of genes that counteract differentiation and stimulate self-renewal, which prevents these cells to differentiate into more committed progenitors cells and leads to (transient) HSC expansion in newborn mice. In the adult, Eed loss ultimately leads HSC exhaustion by an as yet to be defined mechanism.

MYELOID LEUKEMIA FACTOR 1 AFFECTS HEMOPOIETIC STEM CELL DEVELOPMENT.

Winteringham, Louise N., Nababan, Tara, Beaumont, Jennifer, Klinken, Peter

Western Australian Institute for Medical Research, University of Western Australia, Perth, Australia

The hemopoietic system is an excellent system to study the process of stem cell self renewal and differentiation. Although significant progress has been made in understanding how hemopoietic stem cells become functioning blood cells, the precise regulation of these events is yet to be fully elucidated. Regulation of hemopoietic stem cells (HSC) is complex and requires coordinate control at many levels including transcription, post-translational modification of proteins, as well as RNA based regulation. In addition, epigenetic changes have been implicated in the regulation of HSC. Myeloid Leukemia Factor 1 (Mlf1) was first identified as a gene involved in a t(3;5) translocation associated with acute myeloid leukemia (AML). Significantly, wildtype Mlf1 is also up-regulated in approximately 20% of patients with AML. Mlf1 is most highly expressed in HSC but is down-regulated during differentiation into mature blood cells. We identified Mlf1 independently as a gene involved in the spontaneous reprogramming of erythroid cells to the myeloid lineage in vitro. Subsequently, we demonstrated that ectopic expression of Mlf1 promotes maturation of myeloid progenitors, while inhibiting erythroid differentiation. Taken together, these data suggest that Mlf1 may be involved in regulating HSC, and may play a role in hemopoietic lineage determination. To investigate the role of Mlf1 in hemopoiesis we have used two mouse models : (i) Vav.Mlf1 transgenic mice, which express Mlf1 under the control of the vav promoter in all hemo-

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poietic cells including stem cells, and (ii) Mlf1-/- mice. Utilizing the SLAM family of cell surface markers for flow cytometry, we have examined the effect of Mlf1 expression on HSC. Our data show that up-regulation of Mlf1 in the transgenic mice decreased the HSC and as well as progenitor compartments - GMP (Sca1- ckit+ CD34+ FcγRhi),CMP (Sca1- ckit+ CD34+ FcγRlo) and MEP (Sca1- ckit+ CD34- FcγR-). Adult transgenic mice have reduced circulating lymphocytes but increased monocytes and neutrophils. In addition, there was a reduction in erythroid progenitors (CD71+, Ter119+) and abnormal erythroblasts (megaoblasts) were present in the bone marrow.

In contrast, Mlf1-/- mice have increased HSC. Further, following exposure to 5-flurouracil (5-FU), Mlf1-/- mice were able to repopulate their HSC compartment more effectively. These observations indicate that Mlf1 does play an important role in the regulation of HSC. In mice, definitive hemopoiesis begins at day E 11 in the fetal liver with HSC giving rise to mature circulating blood cells, including enucleated erythrocytes. Using microarray, we examined E11.5 fetal liver cells to determine how Mlf1 ablation affected gene expression. Interestingly, GSEA analyses of genes up-regulated in the absence of Mlf1 revealed a significant enrichment for genes targeted by members of the polycomb repressor complex 2 (PRC2), as well as genes that have a H3K27me3 mark (conferred by PRC2) in their promoter. These data suggest that MLF1 may have a role in the epigenetic regulation of hemopoiesis and if aberrantly expressed could contribute to leukemia by influencing the ability of HSC and progenitors to differentiate.

A LARGE-SCALE REVERSE GENETIC SCREEN IN ZEBRAFISH IDENTIFIES THE CHROMATIN FACTORS REQUIRED FOR HEMATOPOIETIC STEM CELL SPECIFICATION

Kathrein, Katie L., Huang, Hsuan-Ting, Huang, Yue-Hua, Barton, Abby, Gitlin, Zachary, Song, Anhua, Zhou, Yi, Zon, Leonard I.
Hematology/Oncology, Children's Hospital Boston, Boston, MA, USA

Long-term hematopoietic stem cells (HSCs) are capable of self-renewal and differentiation into all mature hematopoietic lineages. Cell specific transcription factors interact with co-factors to orchestrate chromatin structure and facilitate gene expression. To generate a complete compendium of factors that establish the epigenetic code in HSCs, we have undertaken the first large-scale in vivo reverse genetic screen targeting chromatin factors. To accomplish this task, we have designed antisense morpholinos for 430 zebrafish orthologs of conserved human chromatin factors. Each morpholino was injected at three doses, providing a view of gene function similar to an allelic series. For each dose, we injected into 100 wildtype embryos, and the resultant morphants were analyzed by whole embryo in situ hybridization at 36 hours post fertilization for expression of two HSC specific genes, c-myb and runx1, which are expressed in the aorta, gonad, mesonephros region in the developing blood stem cells. This allowed a dose of morpholino to be defined in which morphologic changes did not occur, and yet stem cell number or function was compromised. The morphants were categorized into five groups based on changes in HSC marker expression, ranging from no change, to mild, intermediate, or complete reduction in expression or an increase in expression. 35 morpholinos caused a complete or near complete knockdown of HSC marker expression, while five morpholinos were found to increase HSC marker expression. Of the morpholinos that change HSC formation, several genes known to be essential for HSC self-renewal and maintenance were identified. For example, knockdown of Mll or Dot1, which are also present in leukemia fusion proteins, fail to specify HSCs, as indicated by a 90-95% reduction in expression of the HSC markers in 75% of embryos tested per dose. Reduced expression of six polycomb family members results in a decrease in HSC marker expression ranging from 75-95%. Many of the remaining hits represent factors with no previous function ascribed in hematopoiesis, though some are components of known chromatin remodeling complexes, such as the Hat1 and Hbo1 complexes. As ubiquitous knockdown of chromatin factors could interfere with vascular development and the establishment of proper arterial identity, a crucial upstream event for HSC formation, we subsequently analyzed morphants with the most robust HSC phenotypes using two vascular markers: kdr for overall vasculogenesis and ephrinb2a for arterial formation. We found that of the 30 morpholinos tested, only 6 showed reduced overall vascular or arterial marker staining in 75% or more of morphants, suggesting that the majority of morphants with HSC phenotypes are specific to HSC formation. Our work has been compiled into a web-based database that will be made publicly available. Within this database, users can search by gene names and aliases, chromatin domain names and human or zebrafish genes. This will link to all experimental data, including experimental design, materials, protocols, images, and all further analyses of the 30 most robust morphants. Our large-scale genetic analysis of chromatin factors involved in HSC development provides a comprehensive view of the programs involved in epigenetic regulation of the blood program, offering new avenues to pursue in the study of histone modifications in HSCs and for therapeutic alternatives for patients with blood disorders and leukemia.

POTENTIAL APPLICATION OF AN IMMORTALIZED ERYTHROCYTE-PRODUCING CELL LINE DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

Hirose, Sho-ichi[1], Takayama, Naoya[2], Nakamura, Sou[2], Kato, Takashi[3], Nagasawa, Kazumichi[3], Sameshima, Tadashi[1], Nakauchi, Hiromitsu[4], Eto, Koji[2]
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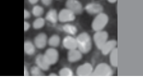
Blood transfusion therapy, essential for fatal anemia and thrombocytopenia, is dependent on only blood donation. However social and scientific reasons including lowered birthrate and increased aged population or viral contamination by influenza etc. lead to the lack of donor blood. In that regard, we have been developing in vitro generation system of functional platelets from human embryonic stem cells (hESCs)- or induced pluripotent stem cells (hiPSCs) (Blood, 2008; J Exp Med, 2010). We further developed system based upon the new concept that an immortalized megakaryocyte (a precursor of platelets) cell line can be created from hESCs or hiPSCs by "self-replication" at the megakaryocyte progenitor level. We previously found that over-expression (O/E) of c-MYC in hematopoietic progenitors (HPCs) derived from hiPSCs showed a transient growth of immature megakaryocytes followed by induction of INK4A locus genes, leading to rapid apoptosis and senescence. Following studies on the mechanism of MYC-mediated megakaryopoiesis, we have determined the condition for "self-replication" of immature megakaryocyte using gene combination of c-MYC and BMI1 O/E (negative regulator for both INK4A and ARF genes) (Nakamura et



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al. 2011 ASH plenary presentation: Blood, 2011 abstract). Earlier studies regarding hematopoietic differentiation suggested that megakaryocytes might be generated from megakaryocyte-erythrocyte progenitors. In addition, it is reported that c-MYC is closely associated with erythroblast proliferation (Guo Y et al. Blood, 2009). In parallel with the development of an immortalized megakaryocyte progenitor cell line, we thus screened genes to create self-replication of erythroblasts in the presence of erythropoietin, and found that c-MYC and BCL-XL (negative regulator of caspase family) transduction against HPCs derived from hESCs increased numbers of Glycophorin A (GPA)+ erythroblasts in an exponential manner for over six months, which we named as immortalized erythrocyte progenitor cells (iERYPCs). Created several cell lines were pro-basophilic erythroblasts that had a large nucleus and basophilic cytoplasm. By using an inducible expression vector system with c-MYC and BCL-XL, the iERYPCs were able to differentiate into orthochromatic erythroblasts with heme synthesis and chromatin condensation. While it has been reported that reduced expression of GCN5, a member of histone acetyltransferase (HAT), is related with chromatin condensation during erythroid maturation (Jayapal SR et al. J Biol Chem, 2010), we confirmed this similar mechanistic evidence as well. Quantitative PCR analysis revealed that erythroid maturation factors, GATA1 and endogenous BCL-XL, were up-regulated along with heme synthesis. Most important point was that the sequential maturation parameters in iERYPCs were corresponding to those of erythroblasts derived from human cord blood hematopoietic stem cells. With regard to "functionality", iERYPCs showed normal oxygen dissociation curve pattern although not completely corresponded to peripheral erythrocytes. Moreover, after transfusion of iERYPCs into immunodeficient NOG mouse models, enucleated human erythrocytes were detectable for a maximum of five days in circulation. This surprising result strongly indicated iERYPCs matured in vivo. We accordingly propose that establishment of an immortalized ERYPC line could potentially provide a stable supply of erythrocytes for transfusion therapy.

PROGRAMMING THE ENDOTHELIAL-TO-HEMATOPOIETIC STEM CELL TRANSITION IN LY6A AORTIC CELLS DURING DEVELOPMENT OF THE MOUSE HEMATOPOIETIC SYSTEM

Dzierzak, Elaine, Yamada-Inagawa, Tomoko, Vink, Chris S., Solaimani, Parham, Kaimakis, Polynikis, de Pater, Emma, Speck, Nancy A.
Erasmus MC Erasmus Stem Cell Institute, Rotterdam, Netherlands

Hematopoietic stem cells (HSC) are the source of all blood cells in the adult. The first HSCs (as potent as adult bone marrow HSCs) are generated in the aorta-gonad-mesonephros (AGM) region at midgestation in the mouse embryo. AGM HSCs are generated following the anatomical appearance of clusters of hematopoietic cells closely associated with the lumenal wall of aorta and vitelline/umbilical arteries. The relationship of HSCs to these clusters and the identification of the precursors to HSCs have been established through genetic studies, and also real-time vital and 3D confocal imaging. Remarkably, HS/PCs arise directly from a subset of endothelial cells of the dorsal aorta in a natural transdifferentiation event. These hemogenic endothelial cells and the emerging HS/PCs are characterized by the expression of Ly-6A (Sca-1) GFP, as shown in a transgenic HSC marker model we generated previously. New high resolution imaging data reveals flat endothelial cells becoming Ly-6A GFP positive, Ly-6A GFP+ endothelial cells beginning to bulge into the lumen and round Ly-6A GFP+ hematopoietic cells closely adherent to the endothelium. We have quantitated these cellular events and performed extensive FACS analysis with endothelial and hematopoietic markers. These results indicate that Ly-6A GFP expression is the most specific marker described to date for hemogenic endothelial and emerging HSCs. The Ly-6A GFP marker allows their discrimination from non-hemogenic endothelium and earlier hematopoietic cells and progenitors. This is supported by data from the Ly-6A directed expression of CBFβ in CBFβ knockout embryos - specific rescue of HSCs, but not erythroid-myeloid progenitors was observed (Chen et al. CellSC, 2011, 9(6):541-552). To identify the genetic program of hemogenic endothelial cells and HSCs, we developed an efficient cell sorting method based on Ly-6A GFP and c-kit expression in the CD31 expressing population of the E10.5 aorta. RNA sequencing has been performed on small numbers of these cells for analysis of the complete transcriptome. Several known hematopoietic transcription factors and some uncharacterized factors have been identified. We are closely analysing Gata2, since it is expressed to low levels in non-hemogenic endothelial cells, several fold higher levels in hemogenic endothelial cells and many fold higher levels in emerging HS/PCs. Interestingly we found that early hematopoietic progenitors are Gata2 independent. To explore the differences in genetic programs in Gata2 dependent and Gata2 independent hematopoietic progenitors and to understand the function of Gata2 in HSC generated we created a Gata2 Venus marker mouse model. In parallel, Gata2 conditional knockout embryos are being analysed to determine in what cells Gata2 is required (endothelial and/or hematopoietic). The results of these studies will be presented

Concurrent Session IIIB — Epigenetics of Stem Cells

Friday, June 15, 2012, 1:30 pm – 3:15 pm

TUDOR DOMAIN PROTEINS AND PIWIS FUNCTION IN NUAGE FOR PIRNA AMPLIFICATION IN STEM CELLS AND GERMLINE CELLS

Anand, Amit[1], **Lim, Robyn S. m.**[2], **Kai, Toshie**[2]
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The piRNAs provide defense against transposons in animal gonads. In Drosophila ovaries, distinct piRNA pathways involving different components function in somatic and germline cells. The germline piRNAs arise from bidirectional clusters and are amplified by ping-pong cycle. We report kumo, a conserved yet component of the germline piRNA pathway in Drosophila. Kumo is a tudor domain protein that localizes to nuage; a unique structure present in germline cells, proposed as a processing site of germline piRNAs where Piwi family proteins are also localized. kumo is required for localization of all other examined piRNA pathway components to nuage, suggesting that kumo is required as upstream component for nuage organization. kumo is also important for production of sufficient cluster and ping-pong derived germline piRNA, but not for somatic piRNAs. In addition to nuage, Kumo also localizes to the nucleus in the germarium and promotes the production of longer precursor transcripts from the germline piRNA clusters. Our data suggest that kumo is important for the production of germline piRNAs by orchestrating organization of nuage and promoting transcription from the germline piRNA clusters in the Drosophila. In the vertebrates and ecdysozoans investigated thus far, Piwi proteins and piRNAs are highly en-

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riched in the germline. Interestingly, Piwi proteins are expressed in the multipotent or totipotent stem cells of other bilaterians and in non-bilaterian animals, implying a wider role of such genes throughout the animal kingdom than previously thought. We have generated antibodies against two Piwi orthologs, Piwi1 and Piwi2, in the basal metazoan, Hydra. Both Piwi proteins are specifically expressed in the interstitial stem cells_multipotent stem cells that give rise to somatic and germline lineages_and localize to perinuclear foci which are largely reminiscent of the nuage. Both Piwi1 and Piwi2 foci progressively diminish in size and intensity in differentiating intermediates of the interstitial cell lineage and are not observed in fully differentiated cells. Our preliminary data suggests that piwi2 is involved in the maintenance of interstitial stem cells.

RNF12 ACTIVATES XIST AND IS ESSENTIAL FOR X INACTIVATION IN FEMALE MOUSE EMBRYONIC STEM CELLS

Barakat, Tahsin Stefan, Grootegoed, J.Anton, Gribnau, Joost
Reproduction and Development, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands

X chromosome inactivation (XCI) in placental mammals is a mechanism which equalizes dosage of X-linked genes between females and males. This crucial process for female development is first initiated around the two-to-four cell stage in the pre-implantation embryo in an imprinted way, in which the paternal X chromosome is inactivated. In the inner cell mass (ICM) of the developing blastocyst, the inactivated X chromosome is reactivated, allowing a short window in time in which a double dosage of X-linked gene products is tolerated. Upon further development, random XCI is initiated in the post-implantation embryo, thereby coupling initiation of XCI to differentiation. Female mouse embryonic stem (ES) cells, which are derived from the ICM, are therefore characterized by two active X chromosomes, and undergo XCI upon differentiation, making them a unique in vitro model. Initiation of chromosome wide silencing of the X chromosome is regulated by the up-regulation of the non-coding RNA Xist, which is negatively influenced by its antisense partner Tsix. In undifferentiated cells, the core pluripotency factor network consisting of Nanog, Oct4 and Sox2 directly and indirectly suppress Xist up-regulation. We have previously shown that Xist up-regulation is triggered by a stochastic mechanism, in which the X-linked gene Rnf12 acts as an X-linked activator of XCI, allowing XCI initiation only in females. The encoded RNF12 (RLIM) is an E3 ubiquitin protein ligase, known to be involved in down-regulation of LIM homeodomain transcription factors. Over expression of RNF12 results in ectopic XCI, and homozygous Rnf12-/- ES cells fail to undergo XCI upon differentiation, showing that Rnf12 is a key factor in the XCI initiation process. Here we further decipher the XCI initiation mechanism, by showing that Rnf12 interacts with the pluripotency factor network and initiates XCI by degradation of Rex1. Rnf12 and Nanog expression is mutually exclusive in ES cells, providing evidence that Rnf12 is negatively regulated by the pluripotency factor network itself. Here we report the results of targeted deletions of pluripotency factor binding sites in the Rnf12 promoter, providing novel data on the regulation of Rnf12 during XCI initiation. Our pre- and post-implantation embryo data reveal that Rnf12 is also crucial for XCI initiation in vivo. Furthermore, large scale deletions of other X-linked loci in ES cells previously suggested to be crucial for XCI regulation, including the X-pairing region and the non-coding gene Jpx, provide evidence that X chromosome pairing is not essential for XCI. Instead, Rnf12 together with its interplay with the pluripotency factor network regulates XCI during mouse development.

COMPARATIVE EPIGENOMICS

Xiao, Shu[1], Xie, Dan[1], Cao, Xiaoyi[1], Yu, Pengfei[1], Xing, Xiaoyun[2], Chen, Chieh-Chun[1], Musselman, Meagan[1], Xie, Mingchao[2], West, Franklin[3], Lewin, Harris[1], Wang, Ting[2], **Zhong, Sheng**[1]
[1]University of Illinois at Urbana-Champaign, Urbana, IL, USA, [2]Washington University, St. Louis, MO, USA, 3University of Georgia, Athens, GA, USA

Despite the explosive growth of genomic data, functional annotation of the regulatory sequences remains difficult. Here we introduce 'comparative epigenomics' - interspecies comparison of epigenomes - as a novel approach for annotation of the regulatory genome. We measured in human, mouse, and pig pluripotent stem cells the genomic distributions of nine epigenomic marks, four transcription factors, and transcribed RNAs. We made the unexpected observation that epigenomic conservation was strong in both fast-evolving and slowly evolving DNA sequences, but not in neutrally evolving sequences. In contrast, evolutionary changes of the epigenome and the transcriptome exhibited a linear correlation. We suggest that the conserved co-localization of different epigenomic marks can be used to discover regulatory sequences. Indeed, seven pairs of epigenomic marks thus identified exhibited regulatory functions during differentiation of mouse embryonic stem (mES) cells into mesendoderm cells. In particular, the H2A.Z-H3K4me3 pair marked a novel class of poised promoters, and the H3K36me3-H3K4me1 and H3K36me3-H3K27ac pairs marked novel classes of active enhancers in mES cells. Thus, comparative epigenomics reveals regulatory features of the genome that cannot be discerned from sequence comparisons alone.

DNA METHYLATION DYNAMICS IN STEM CELLS AND DEVELOPMENT

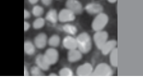
Meissner, Alexander
SCRB, Harvard University, Cambridge, MA, USA

Cytosine methylation in mammals is an epigenetic modification that is largely restricted to CpG dinucleotides and serves multiple critical functions including stable repression of target promoters, maintaining genomic integrity, establishing parent-specific imprinting patterns, and silencing endogenous retrotransposon activity. In somatic tissues, CpG methylation exhibits global patterns based on relative CpG density: it is unmethylated in localized CpG islands at housekeeping or developmental promoters, and hypermethylated at non-regulatory CpGs distributed elsewhere in the genome. This landscape is relatively static across all somatic tissues that have been examined to date on a genome scale, where the majority of methylated CpGs are pre-established and inherited through cell divisions. Generally, only a small fraction of CpGs switch their methylation levels as part of an orchestrated regulatory event. By contrast, DNA methylation is much more dynamic during mouse germ-cell and pre-implantation development. I will present recent insights gained through genome-scale mapping of DNA methylation in human pluripotent stem cells and murine development.

MOLECULAR SIGNATURES OF HUMAN INDUCED PLURIPOTENT STEM CELLS HIGHLIGHT SEX DIFFERENCES AND CANCER GENES

Anguera, Montserrat, Lee, Jeannie T.
Molecular Biology, Massachusetts General Hospital, Boston, MA, USA

Although human induced pluripotent stem cells (hiPSC) have enormous potential in regenerative medicine, their epigenetic variability suggests that some lines may not be suitable for hu-



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man therapy. There are currently few benchmarks for assessing quality. Here we show that X-inactivation markers can be used to separate hiPSC lines into distinct epigenetic classes and that the classes are phenotypically distinct. Loss of XIST expression is strongly correlated with upregulation of X-linked oncogenes, accelerated growth rate in vitro, and poorer differentiation in vivo. Whereas differences in X-inactivation potential result in epigenetic variability of female hiPSC lines, male hiPSC lines generally resemble each other and do not overexpress the oncogenes. Neither physiological oxygen levels nor HDAC inhibitors offer advantages to culturing female hiPSC lines. We conclude that female hiPSC may be epigenetically less stable in culture and caution that loss of XIST may result in qualitatively less desirable stem cell lines.

DEVELOPMENT OF A NOVEL TECHNIQUE TO VISUALIZE A COMBINATION OF HISTONE MODIFICATIONS

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Histone modifications are known to play important roles in epigenetic regulation of cell type-specific gene expression, and the crucial importance of their combination is also shown by accumulating evidence. Especially, the combination of histone H3 lysine 4 trimethylation (H3K4me3) and histone H3 lysine 27 trimethylation (H3K27me3), named bivalent modification, is found at development-related genes in pluripotent stem cells, such as embryonic stem cells (ESCs), and is believed to keep development-related genes poised to respond to differentiation stimuli. However, a technique to detect the presence of a specific combination of histone modifications in a single cell has not been developed. In this study, we applied the in situ proximity ligation assay (in situ PLA), an imaging technique of protein-protein interaction, to detect bivalent modifications. Specific visualization of bivalent modifications was confirmed using i) mouse ESCs, which are known to contain bivalent modifications, ii) Suz12 knockout ESCs, which lack global H3K27me3 and thus bivalent modifications, and iii) mouse embryonic fibroblasts (MEF), which have few bivalent modifications. In the wild-type ESCs, the fluorescence signals showing colocalization of H3K4me3 and H3K27me3 were observed, and the average number of signals was 15.2/nucleus. On the other hand, the average number of signals was 0.9/nucleus and 3.4/nucleus in Suz12 knockout ESCs and MEF, respectively. These data showed that bivalent modification was visualized by in situ PLA technique.

Establishing a new method, we took advantage of it to analyze individual cells in a heterogeneous sample at an early stage of ESC differentiation. ESCs treated with retinoic acid for two days showed phenotypic variation in the morphology of ESC colonies, and decrease of Oct-4 mRNA expression was not completed. In contrast, no or little fluorescence signals of bivalent modifications were observed at this time point. This data showed that the epigenetic layer of differentiation was completed at two days in a highly coordinated manner, while the phenotypic layer of differentiation took place in a variable manner. In conclusion, we were able to develop an imaging technique to detect bivalent modifications in individual cells using in situ PLA technology. The visualization of bivalent modifications is expected to have a wide range of applications, such as detection of cancer stem cells and tissue stem cells.

Concurrent Session IIIC — Stem Cell Therapies

Supported by International Society for Cellular Therapy (ISCT)

Friday, June 15, 2012, 1:30 pm - 3:15 pm

STEM CELL THERAPIES FOR AGE-RELATED MACULAR DEGENERATION: THE CHALLENGES AHEAD

Coffey, Pete
Institute of Ophthalmology, University College London, London, United Kingdom and NRI, UCSB, USA

Our goal is to replace cells essential for "seeing" lost by disease at the back of the eye. We aim to repair and regenerate the aged diseased eye using human embryonic or induced pluripotent stem cells which have been transformed into the cells affected in AMD: retinal pigment epithelium. The cells will be surgically implanted into a clinical population of AMD patients. Clinical trials have already been initiated both in the US and UK. In this presentation, I aim to discuss the challenges ahead in order for these therapies to become licensed therapies.

RE-ESTABLISHMENT AND GENETIC CORRECTION OF STEM/PROGENITOR CELLS FROM MUSCULAR DYSTROPHIES VIA REPROGRAMMING OF AUTOLOGOUS CELLS

Tedesco, Francesco Saverio[1], Gerli, Mattia F.M.[1], Benedetti, Sara[1], Hoshiya, Hidetoshi[2], Cassano, Marco[3], Ungaro, Federica[2], Perani, Laura[2], Tagliafico, Enrico[4], Sampaolesi, Maurilio[5], Torrente, Yvan[6], Oshimura, Mitsuo[7], Broccoli, Vania[8], Cossu, Giulio[1]
[1]Dept. of Cell and Developmental Biology, University College London, London, United Kingdom, [2]Division of Regenerative Medicine, San Raffaele Scientific Institute, Milan, Italy, [3]School of Life Sciences, Ecole Polytechnique Fédérale, Lausanne, Switzerland, [4]Department of Biomedical Sciences, University of Modena and Reggio Emilia, Modena, Italy, [5]Stem Cell Interdepartmental Institute, KU Leuven, Leuven, Belgium, [6]Department of Neurological Science, University of Milan, Milan, Italy, [7]Department of Biomedical Science, Tottori University, Yonago, Japan, [8]Division of Neurosciences, San Raffaele Scientific Institute, Milan, Italy

Mesoangioblasts are stem/progenitor cells derived from a subset of skeletal muscle pericytes expressing alkaline phosphatase. They have been shown to naturally contribute to skeletal muscle growth and regeneration and, most importantly, to ameliorate muscular dystrophies (currently incurable diseases) in different animal models upon systemic delivery. This preclinical evidence of safety and efficacy allowed their current clinical translation into a phase I/II clinical trial based upon allogeneic transplantation for Duchenne muscular dystrophy (DMD) children (EudraCT no. 2011-000176-33). However, human mesoangioblasts have a finite lifespan and the need to obtain billions of cells to treat all the skeletal muscles of an adult patient challenges their proliferative potency. Moreover, we show here that patients affected by limb-girdle muscular dystrophy 2D (LGMD2D, characterized by α-sarcoglycan deficit) have a reduction of this subset of pericytes and hence mesoangioblast could not be derived for cell therapy. Therefore, we reprogrammed LGMD2D fibroblasts and/or myoblasts to induced pluripotent stem cells (iPSCs) and developed a protocol for the derivation of mesoangioblast-like cells from them. These cells can be expanded and genetically corrected with a novel muscle-specific lentiviral vector expressing human α-sarcoglycan. Notably, upon xenotransplantation into ad hoc generated α-sarcoglycan-null immunodeficient mice, they gener-

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ate myofibers expressing α-sarcoglycan and upon intra-specific transplantation they also cause functional amelioration of the dystrophic phenotype. Finally, we extended this strategy to other forms of muscular dystrophy and gene correction. Indeed, we recently provided the first evidence of therapeutic efficacy of stem cell-mediated transfer of a human artificial chromosome in a mouse model of DMD and report here efficacy of this strategy also with human iPSC-derived DMD mesoangioblasts. This approach may be useful for muscular dystrophies that show a reduction of resident progenitors and provides evidence of pre-clinical safety and efficacy of disease-specific iPSCs.

FUNCTIONAL ENGRAFTMENT OF COLON EPITHELIUM EXPANDED IN VITRO FROM A SINGLE ADULT LGR5+ STEM CELL

Nakamura, Tetsuya[1], Yui, Shiro[2], Sato, Toshiro[3], Nemoto, Yasuhiro[2], Mizutani, Tomohiro[2], Zheng, Xiu[2], Ichinose, Shizuko[4], Nagaishi, Takashi[2], Okamoto, Ryuichi[1], Tsuchiya, Kiichiro[2], Clevers, Hans[5], Watanabe, Mamoru[2]

[1]Department of Advanced Therapeutics for GI Diseases, Tokyo Medical and Dental University, Tokyo, Japan, [2]Department of Gastroenterology and Hepatology, Tokyo Medical and Dental University, Tokyo, Japan, [3]Department of Gastroenterology, Keio University School of Medicine, Tokyo, Japan, [4]Research Center for Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan, [5]Hubrecht Institute, Utrecht, Netherlands

Advancement in culture method to grow normal intestinal stem cells in vitro is expected to accelerate the adult stem cell therapy for human gastrointestinal diseases. However, in order to exploit the potentials of cultured stem cells as a source for regenerative medicine, further refinement of culture technologies and validation of their tissue regeneration capacity would be essential. We were particularly interested in testing whether cell-based therapy using colonic stem cells would be feasible to repair damaged colonic tissues. First we developed a novel culture method that maintains Lgr5+ colonic stem cells in vitro (TMDU method). Colonic crypts from normal adult mice were three-dimensionally cultured in serum-free medium supplemented with a combination of growth factors. The crypt cells formed a round cystic structure consisting of epithelial monolayer of multilineage cells and could be propagated without losing their properties. Importantly, expression of Lgr5 was significantly up-regulated and then constantly maintained for a long time period. The expansion of Lgr5+ stem cells was also confirmed by using the colonic cells obtained from knock-in mice in which Lgr5+ cells can be visualized. Furthermore, it was shown that the cultured cells retain their physiological capability of differentiating into secretory lineages by Notch signal inhibition in vitro.

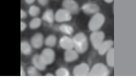
Next we tested if the cultured colonic stem cells could regenerate damaged colon epithelium in vivo. GFP+ colon organoids were transplanted into the lumen of mouse colon superficially damaged by DSS treatment. Surprisingly, transplanted cells readily integrated into the colonic tissues covering the area that lacked epithelium, and accelerated the recovery of recipients from acute colitis. At 4 weeks post-transplantation, the donor-derived cells constituted single-layered epithelium forming self-renewing GFP+ crypts that were functionally and histologically normal. Moreover, successful, long-term engraftment was observed even with the transplantation of organoids that were derived from a single Lgr5+ colon stem cell after extensive in vitro expansion. These data for the first time demonstrate the feasibility of colon stem cell therapy based on in vitro expansion of a single adult colonic stem cell. The present study would build a basis for developing a novel approach in regenerative medicine for patients with severe gastrointestinal epithelial injuries in humans.

HUMAN MÜLLER STEM CELLS CAN BE INDUCED TO DIFFERENTIATE INTO RETINAL NEURAL PHENOTYPES IN VITRO AND EXERT FUNCTIONALITY IN VIVO UPON TRANSPLANTATION.

Limb, G. Astrid[1], Becker, Silke[1], Jones, Megan F.[1], Singhal, Shweta[2], Cottrill, Phillippa B.[1], Khaw, Peng T.[1], Jayaram, Hari[1]

[1]Institute of Ophthalmology, Univ College London, London, United Kingdom, [2]Ophthalmology, Singapore National Eye Centre,, Singapore, Singapore

Background: Neural degeneration is the leading cause of blindness in prevalent ocular conditions such as glaucoma, age related macular degeneration and congenital retinal diseases. At present there are no treatments to restore vision and neural cell replacement by stem cells offers a hope to conserve or improve visual function. This study investigated the ability of adult human Müller stem cells to differentiate into enriched populations of retinal ganglion cells and photoreceptor cells as a source of retinal neurons for potential use in cell-based therapies to treat retinal disease. Methods: Factors known to modify transcription factors of neural retinal progenitors, including FGF2, retinoic acid, insulin growth factor, γ-secretase inhibitor and Wnt inhibitors were used to induce neural differentiation of human Müller stem cells in vitro. Protein and gene expression of markers of retinal neurons such as Isl1, Brn3b, HuD, rhodopsin, recoverin and interphotoreceptor binding protein (IRBP) were examined to identify enriched populations of cells expressing retinal ganglion cell (RGC) or photoreceptor (PR) cell markers in vitro. Enriched populations RGC were transplanted onto the inner retinal surface of RGC depleted eyes (by NMDA), whilst enriched populations of PR were transplanted into the subretinal space of P23-H rhodopsin knockout rats affected by PR degeneration. Anatomical integration and functional effects of the transplanted cells were assessed by immuno-histochemistry and electroretinography respectively. Results: Human Müller stem cells differentiated into enriched populations of RGC upon Notch and Wnt down regulation. This was accompanied by upregulation of genes and protein expression of the RGC markers Isl1, HuD and Brn3b. Furthermore, upregulation of the transcription factors CRX and Nr2e3 by a combination of agents, including insulin growth factor, was accompanied by upregulation of genes and proteins of the PR markers recoverin, interphotoreceptor binding protein (IRBP) and rhodopsin. Transplantation of enriched RGC populations into RGC depleted rat retina caused a partial recovery of the scotopic negative response in the ERG (a marker of RGC function), whilst subretinal injection of PR enriched populations into P23H rats caused a significant improvement of the A-wave of the ERG (a marker of PR function). Immunohistochemical examination of the transplanted retinae showed migration of the transplanted cells into the retina and their expression of the correspondent neural markers in situ. Conclusions: Our findings indicate that human Müller stem cells can be induced to differentiate in vitro into enriched populations of cells expressing specific retinal neural phenotypes. Upon experimental transplantation, selective neural populations migrated into the correspondent retinal cell layers affected by disease models, and improved retinal function as judged by ERG responses. The data suggests that human Müller stem cells may constitute a potential source of cells for retinal therapies.



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IN VITRO MODELS OF ENHANCED HUMAN MESENCHYMAL STEM CELL HOMING TO BRAIN TUMORS

Smith, Christopher L.[1], **Lin, Benjamin**[1], **Gupta, Amrita**[1], **Quiñones-Hinojosa, Alfredo**[2], [1]

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Introduction: Brain cancer is a devastating ailment, leading to more than 1,300 deaths in the United States each year. Glioblastoma (GB) is the most common and aggressive type of primary brain cancer in adults. Despite the use of standard treatments, such as radiation, chemotherapy, and surgical resection, GB displays characteristic recurrence, followed by swift fatality within two years. In the search for more effective therapies, recent studies have specifically targeted multipotent adult mesenchymal stem cells (MSCs) to brain tumor (glioma) microenvironments. MSCs have obtained significant interest as drug delivery tools due to their intrinsic tropism for tumors in vivo. The ability of MSCs to breach the blood brain barrier (BBB) provides the unique potential to treat brain disorders, namely brain cancer. However, delivery of MSCs to specific tissues, especially to the brain, has faced some limitations in efficiency for reasons not fully understood. We hypothesize that modulation of MSCs can enhance their delivery to the brain. Methods: Our studies employ commercial and primary-cultured human adipose-derived mesenchymal stem cells (hAMSCs), which benefit from less invasive isolation than bone marrow-derived cells. We characterized these cells through marker expression (flow cytometry) and differentiation analyses. Utilizing novel micro- and nanotechnology, e.g. microfluidics and patterned cell substrates, we developed intricate in vitro models to analyze the multi-step process by which hAMSCs reach tumors. Our experiments have specifically investigated the putative MSC homing steps of firm adhesion, transendothelial invasion, migration, and chemotaxis. We analyzed effects on these processes by soluble factors in glioma-conditioned medium (GCM), and by the extracellular matrix (ECM) proteins, fibronectin and laminin. GCM was harvested following two-day culture of monolayers of human GB cells in serum-free medium. Results: We have established the identity of adipose cells from primary and commercial sources as mesenchymal stem cells. We demonstrated expression of established mesenchymal markers (CD70, 90, 103) and lack of endothelial markers (CD31, 45) by hAMSCs. In addition, we observed their multi-lineage differentiation to adipocytes, osteocytes, and chondrocytes. By preconditioning hAMSCs through pre-exposure to soluble and immobilized proteins, we have observed enhanced homing in vitro. hAMSC adherence to blood vessel endothelium, measured by numbers of flowing cells that attach to endothelial monolayers, is improved following pre-exposure to GCM and fibronectin ($p \leq 0.05$). After this preconditioning, hAMSCs also demonstrate enhanced transendothelial invasion towards GCM through a model of the BBB ($p \leq 0.05$). Other results show enhanced migration (measured by speed, alignment, and persistence) of hAMSCs pre-exposed to GCM and laminin ($p \leq 0.05$). In addition, hAMSC chemotaxis towards GCM gradients intensifies following GCM preconditioning ($p \leq 0.05$). Conclusions: Our results suggest that specialized culturing methods can enhance specific elements of the MSC homing process, which may lead to in vivo applications. This approach mirrors previous reports that have improved engraftment to other target tissues, e.g. the heart. Uncovering methods to improve tissue-specific MSC localization could revolutionize drug delivery for various diseases.

STEM CELL-BASED THERAPIES - PRODUCT OR PRACTICE?

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The regulation of stem cell-based therapies is challenging in several respects. Existing frameworks, best suited to mass-produced pharmaceutical products and devices, must be adapted to novel cell and tissue products. At the same time, defining and enforcing the boundaries between legitimate innovations in treatment and irresponsible or exploitative marketing of unproven therapies have preoccupied many scholars and policy makers.
Underlying these concerns is a basic distinction that shapes the ways in which stem cell-based therapies are regulated and by whom: the distinction between a medical product and a medical practice. In many cases, treatment involves both a product (e.g. a drug or device) and its use or application as a form of medical practice. Distinct forms of regulation apply to products, on one hand, or practice, on the other; other forms of regulation apply to both. In some countries, the power to regulate products and practices is held by different levels of government (e.g. federal regulation of medical products and state regulation of medical practice in the United States). In order to better understand the distinction between products and practices, this paper briefly examines two areas of law where the distinction has been defined and used: patent law and off-label use. In patent law, some countries draw a distinction between medical products (e.g. drugs and devices), which are patentable, and methods of medical treatment, which are not. In off-label use, a distinction is drawn between the approval of a product for certain indications, and the freedom of practitioners to use it for other indications. The paper then identifies types of stem cell-based therapies that fall on different points of the spectrum between products and practices. For example, "off-the-shelf" allogeneic stem cell-based products are similar to other regulated products such as vaccines or other biologics. At the other end of the spectrum, an autologous stem-cell transplant used in cancer treatment would be simply a form of medical practice. In between, therapies involving manipulation of cells and tissues to treat individual patients, such as autologous stem cell therapies, iPSC-based therapies, and custom-made engineered tissues, lie at the boundary between products and the practice of medicine. Some have argued that product regulation is inappropriate for autologous and other custom therapies that fall at the boundary between products and practices. The basic premise of this paper is that effective regulation, which appropriately balances protection of consumers or patients and freedom to exercise responsible clinical judgment, should apply equally regardless of whether a therapy involves a distinct product that is regulated as such, or only a form of medical practice. A critical review of the forms of regulation that apply to products and practice suggests that some areas of regulation need to be strengthened in order for this goal to be achieved.

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Concurrent Session IIID — Growth Factors Controlling Stem Cell Behavior

Friday, June 15, 2012, 1:30 pm - 3:15 pm

MULTIPLE ROLES FOR HEDGEHOG-GLI SIGNALING IN DIVERSE ADULT STEM CELL POPULATIONS

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The secreted factor sonic hedgehog (SHH), which mediates signaling through three GLI transcription factors, is critical for organ development and enhances proliferation of adult neural stem cells. Human mutations that reduce SHH signaling cause a variety of developmental defects, whereas inappropriate activation of signaling contributes to cancer. Of clinical importance, inhibition of HH signaling using small molecules or gene ablation can reduce stem cell proliferation or tumor incidence and growth. To study the in vivo functions of the GLI effectors of HH signaling, we generated an array of mouse conditional and knock-in alleles. A universal rule for GLI function is that HH signaling modifies the GLI2 and GLI3 proteins, and the critical consequence is that a GLI2 activator is induced and the GLI3 repressor is attenuated. Moreover, high level HH signaling also modifies GLI3 into a weak activator that together with GLI2 induces transcription of Gli1. Thus, SHH regulates the activity of each Gli gene in a distinct manner. Recently, we have been focusing on whether resident stem cells in adult organs can be stimulated by SHH to expand and replenish damaged tissues. We have used expression of Gli1 to identify cells being stimulated by HH signaling, and genetic inducible fate mapping (GIFM) to determine whether GLI1+ cell populations in adult tissues are stem cells. Our premise is that stem cells that are marked using GIFM should continue to produce progeny for the lifetime of the mouse. Indeed, in the adult forebrain, Gli1-expressing cells marked at 2 months of age continue to produce olfactory bulb neurons throughout the life of the mouse. Both GLI2 and GLI3 act downstream of SHH in maintaining these stem cells. In the skin, we found Gli1 is expressed in the upper portion of the bulge (containing epithelial stem cells), and unexpectedly sensory neurons that project to the skin secrete SHH. Furthermore, the nerves are required to maintain the upper bulge stem cells in a plastic state able to both contribute to wound healing and change their fate into skin interfollicular stem cells. We are also studying the role of SHH in regulating mesenchymal stem cells in the prostate, an ideal system since stem cell driven regeneration can be stimulated following castration. We have found that Gli1 is expressed in mesenchymal stem cells that contribute to multiple rounds of regeneration of the prostate. Thus, SHH signaling is involved in stem cell biology in many adult organs.

CONVERSION FROM MOUSE EMBRYONIC TO EXTRAEMBRYONIC STEM CELLS REVEALS ROLES FOR FGF SIGNALLING AND PRIMITIVE ENDODERM TRANSCRIPTION FACTORS

Cho, Lily T.[1], Wamaitha, Sissy E.[1], Tsai, Jason I.[2], Artus, Jérôme[3], Sherwood, Richard I.[4], Pedersen, Roger A.[1], Hadjantonakis, Anna-Katerina[3], Niakan, Kathy K.[5]
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The inner cell mass of the mouse pre-implantation blastocyst is comprised of epiblast progenitor and primitive endoderm cells of which cognate embryonic (mESC) or extra-embryonic (XEN) stem cell lines can be derived. Importantly, each stem cell type retains the defining properties and lineage restriction of their in vivo tissue of origin. Recently, we demonstrated that XEN-like cells arise within mESC cultures. This raises the possibility that mESCs can generate self-renewing XEN cells without the requirement for gene manipulation. We have developed a novel approach to convert mESCs to XEN cells (cXEN) using growth factors. This approach highlights an important function for Fgf4 in cXEN cell derivation. Paracrine FGF-signalling compensates for the loss of endogenous Fgf4, which is necessary for establishment, but not maintenance of cXEN cells. We confirm that primitive endoderm-specific genes Gata4 and Gata6 are necessary for cXEN cell derivation and that while XEN-like cells emerge from Sox17 and Pdgfra mutant cells, they cannot be maintained as stable cell lines. Our cXEN protocol also demonstrates that distinct pluripotent stem cells respond uniquely to differentiation promoting signals. We find that epiblast stem cells (EpiSCs) derived from the post-implantation epiblast are refractory to cXEN establishment, consistent with the hypothesis that EpiSCs represent a pluripotent state distinct from mouse ESCs. cXEN cells can also be derived from mESCs cultured with Erk- and Gsk3-inhibitors (2i) and LIF, similarly to conventional mESCs. In all, these findings suggest that mESC pluripotency includes the capacity to give rise to both extra-embryonic and embryonic lineages.

COORDINATED CONTROL OF CODING AND LONG NON-CODING RNA DURING ENDODERMAL DIFFERENTIATION

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Oct4, Sox2 and Nanog are the core transcription factors that regulate protein coding genes in embryonic stem (ES) cells, but control of long non-coding (lnc) RNA in ES cells remains poorly understood. Furthermore, how coding and lncRNA transcription is coordinated has yet to be investigated. Here we describe the regulatory linkage between protein coding and long non-coding genes in human ES cells based on analysis of global run-on sequencing (GRO-seq), RNA sequencing and chromatin immunoprecipitation and sequencing (ChIP-seq). We further show how this coordinated linkage changes during TGF-beta-mediated endodermal differentiation.

GLI3 MEDIATED HEDGEHOG INHIBITION AUGMENTS AND CONTROLS ADULT VERSUS EMBRYONIC HEMATOPOIETIC PROGRAMMING OF HUMAN STEM CELLS

McIntyre, Brendan A.S.[1], Rampali, Shravanti[1], Ramos Meija, Veronica[1], Mechael, Rami[1], Shapovalova, Zoya[1], Avdic, Vanja[1], Lee, Jong-Hee[1], Schnerch, Angelique[1], Alev, Cantas[2], Sheng, Guojun[2], Bhatia, Mickie[1]
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Applications of differentiated cells derived from human pluripotent stem cells (hPSCs) require effective programming that generates adult vs. embryonic lineage specific cell types. Here, using hPSCs, we reveal correlations between Hh signaling, and the development and progression of hematopoiesis throughout

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human ontogeny. Both chemical- and gene targeting-mediated (in)activation of Hh signals augmented hematopoietic fate from hPSCs, but also induced adult hematopoietic specification. Hh inhibition in hPSCs mediated cleavage of the Hh effector Gli3R via direct association with members of the polycomb repression complex-2 to selectively occupy embryonic vs. adult globin loci. Our study reveals an unprecedented role for Hh signaling in epigenetic regulation of differentiation that allows hPSCs to overcome intrinsic embryonic programs and adopt adult hematopoietic cell fate.

MESENCHYMAL STEM CELLS FROM PRIMARY BREAST CANCER TISSUE PROMOTE CANCER PROLIFERATION AND ENHANCE MAMMOSPHERE FORMATION

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Mesenchymal stem cells (MSCs) play a critical role in promoting cancer progression. However, it remains unknown whether MSCs are located in breast cancer tissues and correlated with tumor proliferation. In this study, we successfully identified MSCs from primary breast tumor tissues via flow cytometry analysis and multi-lineage differentiation assays. They showed homogenous immunophenotype, and possessed tri-lineage differentiation potential (osteoblast, adipocyte and chondrocyte).When co-transplanted with cancer cells in a xenograft model in vivo, the breast cancer MSCs increased the volume and weight of tumors considerably. We observed that breast cancer MSCs stimulated mammosphere formation in the transwell co-culture system in vitro. This effect was significantly suppressed in the presence of EGF receptor inhibitor. Furthermore, our data showed that EGF secreted from breast cancer MSCs could promote mammosphere formation via PI3K/Akt signaling pathway. Our results confirmed the presence of MSCs in primary breast cancer tissues, they provide a favorable microenvironment for tumor cell proliferation in vivo, and partially enhance mammosphere formation via EGF/EGFR/Akt pathway.

MOUSE EMBRYONIC STEM CELL RENEWAL BY AFFINITY TARGETED PARACRINE STIMULATION

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Stem cell growth and differentiation is controlled by intrinsic and extrinsic factors. The latter includes growth factors, which are conventionally supplied in vitro in media exchanged daily. Here we exemplify the use of Biodegradable Encapsulates for Affinity Targeted (BEAT) paracrine stimulation as a novel approach to sustain the growth and pluripotency of mouse embryonic stem cells (mESCs). Two different biodegradable nanoparticle formulations, a Poly(lactide-co-glycolid (PLGA) polyester and a novel hydrogel-based liposomal system (Nanolipogel, NLG), were used to encapsulate and provide temporally and spatially controlled release of Leukemia Inhibitor Factor (LIF). To achieve targeting specificity, an antibody to the oligosaccharide stage-specific early antigen (SSEA1) was conjugated to particle surfaces by avidin-biotin cross-linkage. BEAT-PLGA and NLG particles consisted of ~107 particles per mg of encapsulant with a diameter of 200 ± 50 nm. LIF was concentrated at approximately 1 ng/mg equating to 10-7 ng or 2,400 molecules of LIF per particle. The kinetics and magnitude of LIF release from each formulation were similar and constant over 4 days. Particles encapsulating a pH sensitive dye that only fluoresces upon internalization were first used to determine the specificity of affinity targeted delivery to mESC on mouse embryonic fibroblasts. This established that exposure to particles once at 10 µg/ml overnight could achieve mESC specific labelling. BEAT particle mediated delivery of LIF before and after passaging was evaluated in the context of mESC clonal outgrowth at low density (50 cells/cm2) on 0.1% gelatin. Treatment was in a basal medium consisting of GMEM supplemented with 10% fetal calf serum and other nutrients (GMEM-NS) and followed by daily exchange for 6 days of only the basal medium. After 7 days of culture there was no significant difference in cell colony yield and the proportion of Alkaline Phosphatase positive colonies between the optimised treatment with NLG-LIF particles and the positive control consisting of daily exchange of GMEM-NS containing solubilised LIF at 25 µg/ml. There were also no differences between NLG-LIF particles and solubilised LIF in support of growth rate, metabolic activity by WST-1 assay, and cell substrate interaction by Electrical Cell Impedance Sensing. NLG-LIF particles stimulated a two-fold increase in STAT-3 phosphorylation within 24 hours as compared to no LIF, whereas solubilised LIF stimulated a 300-fold increase within 10 min. PLGA-LIF particles were inferior to NLG-LIF and soluble LIF but still better than no LIF or empty particles across all of the aforementioned. Over 7 passages, the optimised treatment with NLG-LIF particles supported consistent mESC growth and expression of pluripotency markers (Oct4, Nanog, Sox2, SSEA1) and yielded cells which retained a normal karyotype and endoderm, ectoderm and mesoderm lineage potential following injection under the kidney capsule of 129/Ola mice. By virtue of particle mediated delivery of LIF this was achieved using 1.74 x 104-fold less LIF than its conventional supply in medium exchanged daily. Our study exemplifies a novel paradigm for stem cell culture, which emphasises affinity targeted paracrine stimulation of cells. This provides new opportunities to control stem cell renewal and differentiation in vitro for research and to improve the efficiency and cost-effectiveness of stem cell manufacturing for industrial and clinical applications.

Concurrent Session IIIE — Genomic Integrity

Friday, June 15, 2012, 1:30 pm - 3:15 pm

GENETIC AND EPIGENETIC INTEGRITY OF REPROGRAMMED HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells can be reprogrammed from adult cell types by somatic cell nuclear transfer (SCNT) or specific activation of a defined set of transcription factors. The clinical utility of such pluripotent stem cells depends on the complete reprogramming of the epigenome without compromising the integrity of the genome. We previously showed that human induced pluripotent stem cells (hiPSCs) contain an average of six mutations in the protein coding regions that are not present in the bulk populations of their fibroblast progenitors. We have extended the characterization of the somatic mutation load in hiPSCs derived from four additional somatic cell types: human umbilical vein endothelial cells (HUVECs), astrocytes and neural

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stem cells (NSCs). A similar level of mutation load was observed, regardless of the somatic cell types and reprogramming efficiencies. Interestingly, human pluripotent stem cells reprogrammed by SCNT from human fibroblasts also carry a comparable somatic mutation load. Quantitative analysis of cDNA showed that the mutated alleles expressed at the similar levels as the wild-type copies in hiPSCs. A selected set of 17 mutated genes were evaluated on their potential of promoting reprogramming, based on gain-of-function and loss-of-function experiments. We did not find evidence that these mutations facilitate the acquisition of pluripotency. To characterize the completeness of epigenetic reprogramming to a pluripotent state, we compared the DNA methylation profiles using targeted bisulfite sequencing among a panel of hiPSCs, somatic progenitors and human embryonic stem cell (hESC) lines. We observed 1.4-3.3% of CpG sites with aberrant methylation. Roughly half of such sites exhibit the methylation patterns similar to the somatic progenitors (epigenetic memory), whereas the other half has abnormal patterns neither similar to the somatic progenitors, nor to the other hiPSCs and hESCs (epigenetic mutations). A subset of genomic regions that exhibit common aberrant methylation among multiple hiPSC lines were tested for their potential functional effects on gene expression, and all tested genes exhibited significant differences in gene expression. Such epigenetic and transcriptional abnormalities also persist during differentiation. A set of 67 genes were identified that can generally distinguish hiPSCs from hESCs based on either epigenetic or transcriptional signatures. Taken together, our work showed that human pluripotent stem cells generally carry somatic point mutations with yet no evidence of functional consequences, whereas the epigenetic aberrations appear to exhibit more detectible functional effects.

WHOLE GENOME VIEW OF DNA SEQUENCE VARIATIONS IN HUMAN IPS CELL LINES GENERATED WITH NON-INTEGRATING PLASMID VECTOR EXPRESSION

Cheng, Linzhao[1], **Hansen, Nancy F.**[2], **Zhao, Ling**[2], **Du, Yutao**[3], **Zou, Chunlin**[1], **Donovan, Frank X.**[2], **Chou, Bin-Kuan**[1], **Zhou, Guangyu**[3], **Ye, Zhaohui**[1], **Chandrasekharappa, Settara C.**[2], **Yang, Huanming**[3], **Mullikin, James C.**[2], **Liu, Pu Paul**[2]

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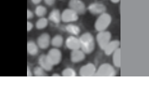
The induced pluripotent stem cell (iPSC) technology holds great promise for human stem cell biology and regenerative medicine, but the reprogramming processes and the resulting iPSCs remain incompletely characterized. Specifically, it is not clear how many changes occur at the genome level during reprogramming. With recently available technologies such as single nucleotide polymorphism (SNP) array and exome sequencing, several recent studies reported first glimpses of genetic abnormalities in human iPSCs derived from fibroblasts. Nucleotide substitutions, copy number variation (CNV) changes, and other chromosomal aberrations, which are either pre-existing or generated during reprogramming, may be selected for iPSC induction and/or expansion. In addition, the origin of starting somatic cell types may influence the quality of derived iPSCs. A whole-genome sequencing (WGS) analysis is necessary to assess potential alterations in the entire nuclear and mitochondrial genomes. In this study, we conducted WGS analysis with the HiSeq2000 technology to determine the DNA sequences of 3 human iPSC lines (passage 15-25 in culture) and their parental somatic cells. The first two iPSC lines, BC1 and BCT1, were derived from marrow CD34+ cells of a male adult donor after 4 days in culture (Chou et al., 2011), by either a single episomal vector pEB-C5 or by a combination of pEB-C5 plus a second vector transiently expressing SV40 T antigen. The third iPSC line, E1, derived from cultured marrow mesenchymal stromal cells (MSCs, 15 days in culture) of the same donor, was also induced with episomal vectors. We achieved 48-59x high-quality sequence coverage of the iPSC lines as well as the parental CD34+ cells and MSCs. The vector sequence was undetectable in all 3 deeply sequenced iPSC lines. We identified 1058-1808 heterozygous single nucleotide variants (SNVs) in each iPSC line. Importantly, none of these iPSC-associated SNVs are shared between the 3 iPSC lines; neither did we observe any clustering of these variations in specific chromosomal regions. Only a single SNV was found in a variable region of the mitochondrial genome in one of the 3 iPSC lines. For SNVs in coding regions (6-12 per iPSC line), ~50% of them are synonymous changes. The SNVs resulting in non-synonymous changes did not cluster in genes related to common functional pathways such as recurrent mutations found in cancers. The WGS with deep-depth and paired-end read mapping data also provides us with a new way to assess CNV changes at a high resolution. We used 3 prediction algorithms for CNV detection, RDXplorer, CNVseq and BreakDancer, on the 3 pairs of fully sequenced DNA samples (iPSCs vs. respective parental somatic cells). No tenable examples of CNV differences between an iPSC line and pair-wise sequenced parental cells were found by more than one of the three methods. We further used the standard SNP array with 2.5 million probes to confirm the lack of CNV variations after reprogramming. Our data thus suggest that episome-mediated reprogramming is not inherently mutagenic during integration-free iPSC induction, and such derived iPSC cell lines have low incidence of DNA sequence variations. The 3 fully sequenced iPSC lines derived from two different cell types of the same person would provide valuable references or standards with DNA sequence information for future studies of genomic integrity and epigenomics (such as DNA methylation) of iPSCs before and after differentiation.

HIGH PREVALENCE OF EVOLUTIONARILY-CONSERVED AND SPECIES-SPECIFIC GENOMIC ABERRATIONS IN MOUSE, RHESUS AND HUMAN PLURIPOTENT STEM CELLS

Ben-David, Uri, Benvenisty, Nissim

The Hebrew University, Jerusalem, Israel

Mouse pluripotent stem cells (PSCs) are the best studied pluripotent system, and are often regarded as the "gold standard" to which human PSCs are compared. However, while the genomic integrity of human PSCs has recently drawn much attention, mouse PSCs have never been systematically evaluated in this regard. The genomic stability of mouse PSCs in culture is a matter of profound significance, as it can affect their pluripotency, differentiation and tumorigenicity. Moreover, a comparison of the typical genomic aberrations between mouse and human PSCs may shed light on the human aberrations as well. We thus thoroughly analyzed the genomic integrity of 325 samples of mouse PSCs, using gene expression profiles from 48 studies. This extensive data set included 127 induced pluripotent stem cell (iPSC) samples, 154 embryonic stem cell (ESC) samples, and 44 epiblast stem cell (EpiSC) samples. Genomic aberrations were found to occur frequently in mouse embryonic stem cells (ESCs) of various mouse strains, and in mouse iPSCs of all cell origins and derivation techniques. Aberrations in iPSCs accumulated already at early passages, and could take over the culture within several passages. Four hotspots of chromosomal aberrations were detected: full



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trisomy 11 (with a minimally recurrent gain in 11qE2), full trisomy 8, and deletions in chromosomes 10qB and 14qC-14qE. The most recurrent aberration in mouse PSCs, gain 11qE2, turned out to be fully syntenic to the common aberration 17q25 in human PSCs. Analysis of chromosomal aberrations in 74 samples of rhesus macaque PSCs further demonstrated the evolutionary conservation of this gain. The anti-apoptotic gene Survivin resides within this aberrant region in mouse, rhesus and human, and is over-expressed in the aberrant cell lines of all species, suggesting it plays a key role in the recurrence of this aberration. Gene expression comparison of orthologous genes between the syntenic regions also revealed novel genes that may promote these aberrations and account for the evolutionary conservation. Interestingly, other recurrent aberrations were found to be species-specific. This specificity did not seem to derive from distinct developmental stages of mouse and human PSCs, since mouse EpiSCs and human PSCs did not share syntenic aberrations. Importantly, we detected chromosomal aberrations in several recently published cell lines, which were considered as normal and were thus used in comparison studies of mouse PSCs. These aberrations may jeopardize the interpretation of such studies, as they seem to have contributed to the reported observations, thus emphasizing the need to carefully and frequently monitor the genomic integrity of PSCs. In summary, we found that PSCs from all species are exposed in culture to selection pressures that lead to the acquisition of non-random genomic aberrations; whereas ESCs and iPSCs from the same species tend to acquire the same aberrations, some of these aberrations are evolutionarily-conserved and others are typical to a specific species, reflecting distinct selection pressures, culture conditions and/or genome organization.

CONTROL OF MOUSE EMBRYONIC STEM CELL STATE BY CHROMOSOME MAINTENANCE PROTEINS

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Fundamental insights into the transcriptional control of embryonic stem cell state, including the regulatory circuitry underlying pluripotency, have emerged from studying the roles of transcription factors such as Oct4, Sox2 and Nanog and chromatin regulators such as Polycomb. Recent genetic screens have identified additional components of the core regulatory circuitry in embryonic stem cells (ESCs), including Mediator, cohesin and the cohesin/condensin loading protein Nipbl. A model for global ESC gene control has emerged where Oct4 and other enhancer-bound factors recruit the Mediator, which recruits Nipbl, which in turn recruits cohesin, thus producing DNA loops that bring active enhancers in proximity to promoters. More recently, we have found that two additional Structural Maintenance of Chromosome (SMC) complexes, condensin I and condensin II, are also loaded at active enhancers and promoters by Nipbl in ESC. Condensin II is loaded on active promoters across chromosome arms during interphase, and condensin I is loaded at these sites during early mitosis. These results reveal novel links between control of the pluripotency gene expression program and chromosome structure during various stages of the cell cycle. In addition, these results shed new light on the mechanisms involved in Cornelia de Lange Syndrome, which is most frequently due to mutations in the cohesin/condensin loading protein Nipbl.

THE ROLE OF DORMANT REPLICATION ORIGINS IN MAINTAINING MOUSE EMBRYONIC STEM CELL GENOME STABILITY DURING SELF-RENEWAL AND DIFFERENTIATION

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Maintenance of genomic integrity is especially important for stem cells, as they are the source of tissue replenishment. By necessary, stem cells self-renew with minimal mutations throughout life. To ensure genome integrity, the genome must be replicated accurately, completely, and only once during S phase of the cell cycle. DNA replication initiates from numerous replication origins. These origins need to be licensed prior to S phase, a process that involves the binding of Mcm2-7 heterohexamers to DNA. During S phase, Mcm2-7 drive replication forks by acting as a helicase to unwind double stranded DNA. However, there are 10-20 times more Mcm2-7 complexes loaded onto DNA than the actual number of replication origins used. The excess Mcm2-7 normally remain dormant and will initiate additional replication forks only when primary replication forks are slowed or stalled. In this way, complete genome replication is maintained. Without dormant origins, prolonged stalling of replication forks will elicit fork collapse and chromosome recombination, which can result in genome rearrangement. Given the fact that replication forks constantly stall during unperturbed S phase and the importance of genomic integrity in stem cells, we set out to investigate whether there is a specific requirement of dormant origins in stem cells using mouse ES cells as a model. We demonstrated that dormant origins indeed exist in mouse ES cells and promote complete genome replication in ES cells that were under replicative stress. When partially depleted of dormant origins, ES cells were able to proliferate, but they were hypersensitive to replication inhibitors and underwent apoptosis. In addition, differentiation of ES cells to multiple lineages was compromised. Our results reveal a differential requirement of dormant origins during ES cell self-renewal and differentiation in order to cope with replication stress. Altogether, our study reveals an important role of dormant origins in maintaining ES cell genomic stability during self-renewal and differentiation.

CRITICAL ROLE OF BRCA1 DURING THE SPECIFICATION OF PROSPECTIVE MOUSE ADULT HAIR FOLLICLE STEM CELLS

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Stem cells (SC) are responsible for tissue regeneration and repair, fuelling the replacement of damaged or dead cells. Using mouse epidermis as a model, we have recently defined the molecular mechanisms by which adult SC respond to DNA damage. We found that multipotent hair follicle (HF) bulge SC exhibit increased resistance to DNA damage-induced cell death by higher expression of anti-apoptotic Bcl2 and transient p53 stabilization, as a result of a faster DNA repair activity mediated by a higher non-homologous end joining (NHEJ). Since distinct DNA repair mechanisms are used at the different stages of the cell cycle, we explored the possibility that the relative importance of NHEJ versus homologous recombination (HR) vary during the ontogeny and the activation stage of HF SC. Conditional deletion of Brca1, a key factor that dictates the choice between HR and NHEJ, in the skin epidermis during morphogenesis resulted in a hairless

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phenotype. We found that the prospective adult multipotent HF SC are exhausted during HF morphogenesis due to the high level of DNA damage and apoptosis that occurred in hair follicle transit amplifying cells. Deletion of p53 or inhibition of apoptosis during HF morphogenesis initially rescues the lack of HF SC and the hairless phenotype of Brca1 deficient epidermis. Interestingly, while HF SC seems exquisitely sensitive to Brca1 deletion, the interfollicular epidermis and sebaceous gland are almost unaffected in the absence of Brca1. The molecular mechanisms underlying the critical and unique role of Brca1 in prospective multipotent HF SCs and the role of Brca1 in quiescent and active adult HF SCs will be presented.

Plenary V — Tissue Engineering

Friday, June 15, 2012, 4:00 pm - 6:00 pm

BUILDING HEPATIC TISSUES FOR DISEASE MODELING AND THERAPY

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ABSTRACT NOT AVAILABLE AT TIME OF PRINTING

GENERATION OF FUNCTIONAL ORGANS FROM PLURIPOTENT STEM CELLS: TOWARD THE NEXT GENERATION OF REGENERATIVE MEDICINE

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Recent development of iPS cell technology has enabled generation of pluripotent stem cells (PSCs) from individual patients, opening up the way to regenerative medicine using the patient's own PSC-derived cells. However, current stem cell therapy mainly targets diseases that can be treated by cell transplantation, such as Parkinson's disease or retinal regenerative diseases. Faced with absolute deficiency of donor organs to treat patients with organ failure, regenerative medicine has as one of its ultimate goals to generate organs using the patient's own PSCs and to transplant those organs into the patient. We recently demonstrated in mouse the generation of functionally normal rat pancreas by injecting rat PSCs into Pdx1-/- (pancreatogenesis-disabled) mouse embryos, providing proof of principle for organogenesis from xenogenic PSCs in an embryo unable to form a specific organ (Kobayashi et al., Cell. 2010). To apply this principle to generate human organs, we need to use larger animals such as pigs. However, to make organ-deficient large animals and to supply them in large numbers are two difficult issues that need to be addressed. To do so, we generated pig fetuses genetically lacking pancreata. We then investigated whether blastocyst complementation could generate pancreata in pancreatogenesis-disabled pigs as it can in pancreatogenesis-disabled rodents. Pancreatogenesis-disabled pig embryos prepared by somatic cell nuclear transfer using cells from genetically apancreatic pig fetus were complemented with blastomeres from cloned pig embryos expressing huKO fluorescent protein to produce chimeric pigs. As in rodent models, these chimeric pigs had pancreata and survived to adulthood. The pancreata formed in chimeric pigs were about the size of human pancreas, functioned normally, and were composed of huKO-positive donor-derived cells. These adult chimeric pigs provided a pool of sperm that can produce large numbers of apancreatic pig embryos. Demonstration of generation of a functional organ from PSCs in pigs is a very important step toward generation of human organs from individual patients' own PSCs in large animals. In addition, production of many organ-deficient large animals now appears within reach.

SYNTHETIC HYDROGELS AS DYNAMICALLY TUNABLE STEM CELL CULTURE NICHES

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A better understanding of the physical and biomolecular cues in the stem cell niche has led to a growing interest in the development of material systems for improved 3D culture environments, as well as delivery vehicles to promote cell survival and differentiation. As a result, hydrogels based on both protein components (e.g., collagen and Matrigel) and highly-tunable synthetic chemistries (e.g., PEG) have evolved to address many of these needs. However, as advances in real-time tracking of dynamic cellular functions have emerged, complementary approaches to alter the surrounding extracellular environment in a user-defined and highly-controlled fashion are extremely limited. Such materials systems would have the potential to significantly improve our understanding of how stem cells receive information from their microenvironment and the role that these dynamic processes may play in biological questions related to their differentiation. Towards the goal of developing a dynamically tunable scaffold, we recently reported an approach for in situ hydrogel property manipulation with light, allowing intimate control of a cell's microenvironment in both time and space. These photoactive hydrogels afford unique user-defined manipulation of the biochemical and biomechanical nature of the extracellular microenvironment. This talk will present several examples where user-triggered changes in the material environment can be used to study the maintenance of pluripotent mouse embryonic stem (ES) cell state, direct differentiation of ES cells to distinct neuronal subtypes, and manipulate axon guidance using ES cell derived neurons.

CELL SHEET ENGINEERING: CURRENT CLINICAL APPLICATION AND PREPARATION OF 3D TISSUES

Okano, Teruo
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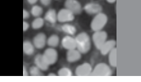
Our research has been focused on constructing a novel form of co-culture consisting layered tissue structure. For our goal, we first developed unique tissue culture dishes which equipped their inner-bottom surface coated with the temperature-responsive polymer poly(N-isopropyl acrylamide) (PIPAAm). The "intelligent surface" of these dishes possessed the hydrophobicity similar to regular tissue culture polystyrene dishes at 37 ºC. However, the surface reversibly became hydrophilic at a lower temperature and spontaneously released the cultured cells as a single layer without the need for trypsin or EDTA, thus leaving the cell layer with extracellular matrix (ECM) intact. All the cultured confluent cells were harvested as a single contiguous cell sheet from the temperature-responsive culture dishes and readily applied to other biological and non-biological surfaces, thanks to the intact ECM. We here propose this novel system of cells and cell-layers arrangement called "cell sheet engineering." Human clinical studies had been initiated for cell sheet engineering therapy using oral mucosal cell sheet for treatment of cornea epithelium deficient disease and recovery from endoscopic submucosal dissension



Detailed Program and Abstracts — Friday, June 15

surgery for esophageal epithelial cancer, and we also succeeded in treating cardio-myopathy using myoblast cell sheet. Applications only required primarily two dimensional manipulations of the cultured cells without prevascularized networks, which would be essential for the development of biological structures to treat or replace dysfunctional organs and tissues in human patients, the next stage of cell sheet tissue engineering. While they were vital, there were no known feasible methods to introduce effective vascular networks to sustain the fundamental functions of the regenerated organs and tissues, e.g., the liver and capillary blood vessels. Therefore, a new strategy was required, and the construction of cell sheets which had more than one type of cells became necessary in attempt to create the desired prevascular networks in three dimensional biological constructs. In order to overcome the challenges of developing crucial and functional prevascular networks, copolymers with different phase transition temperatures were coated on the surface of culture dishes to produce a patterned dual phase thermo-responsive cell culture surface using electron beam polymerization method and porous metal masks. On the patterned surface of dishes, site-selective adhesion and growth of hepatocytes and endothelial cells yielded a patterned co-culture layer based on the hydrophobic-hydrophilic surface chemistry regulated by simple temperature change. In addition, the recovered co-cultured cell sheets could be modified and multilayered, and other types of cells could even be inserted between the cell sheets. The experimental results demonstrated that this method could provide a unique approach for the development of organ-like structures with the essential vascular networks found in normal human organs. As a unique technology utilizing in vivo vascularzation potential, triple-layered-constructs were repeatedly transplanted into rat subcutaneous tissue with an adequate time-interval for blood vessel formation. Multi-step transplantation at 1 or 2-day intervals gave synchronously beating-thick-myocardial tissues with blood vessels. Furthermore, 10 repeated transplantation gave approximately 1 mm thickness, cell-dense-beating-myocardial tissue, corresponding to 30-layered cell sheets. After being fabricated over a surgically connectable artery or vein, multilayered grafts were blood-perfused from the thick artery or vein, and were ectopically-transplantable with direct vessel anastomoses. These cultured-cell-sheet-integration methods overcame long-standing barriers for producing thick, vascularized myocardial tissues and would be applicable to many types of tissues. To imitate in vivo environment, the production of media-perfused microvascular beds in vitro and the transplantation of layered rat cardiac cell sheets over the bed was succeeded. In vitro vascular network formation in 3-D tissues should be a breakthrough technology in regenerative medicine and contribute to future organ engineering.

Plenary VI — RNA Control of Stem Cell Behavior

Saturday, June 16, 2012, 9:00 am - 11:25 am

LINKING RNA TO HUMAN HEALTH AND DISEASE

Cabili, Moran[1], Sun, Lei[2], Trapnell, Cole[1], Goff, Loyal[1], Lodish, Harvey[3], Rinn, John[4]

[1]Broad Institute of MIT and Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA, [2]Whitehead Institute of MIT, Cambridge, MA, USA, [3]Broad Institute of MIT and Harvard University, Cambridge, MA, USA, [4]Broad Institute of MIT, Stem Cell and Regenerative Biology, Harvard University and Whitehead Institute of MIT, Cambridge, MA, USA

In the 50 years since RNA was identified as a central component in the flow of genetic information, it has become increasingly clear that RNA is more than a mere messenger and instead performs vast and diverse functions. Numerous studies have revealed that the mammalian genome is pervasively transcribed, giving rise to many thousands of non-coding transcripts including a class of long intergenic noncoding RNAs (lincRNAs). Raising the question of what do lincRNAs do? To address this question we have developed a 'guilt by association' to 'predict' lincRNA functions leading to hypothesis driven experimentation. Our guilt by association method pointed to a clear connection of lincRNAs and numerous cellular pathways ranging from pluripotency, cancer, adipogenesis to parasitology. Experimental perturbation experiments have unraveled a myriad of functional roles for lincRNAs in these pathways. Together, these results point to key regulatory roles for lincRNAs across diverse biological pathways and diseases, with a common theme of interfacing with and modulating protein regulatory complexes.

LIN28 SHAPES THE TRANSLATIONAL LANDSCAPE IN EMBRYONIC STEM CELLS

Kim, V Narry, Cho, Jun, Chang, Hyeshik, Kwon, S. Chul

Seoul National University, Seoul, Korea, Republic of

Lin28 plays a conserved role in developmental transition and contributes to the formation of induced pluripotent stem cells. At the molecular level, Lin28 inhibits let-7 microRNA biogenesis by directly interacting with the precursors of let-7 microRNAs. Lin28 has also been reported to enhance translation of select mRNAs. In order to obtain an unbiased view of the molecular function of Lin28, we carried out RNA crosslinking-immunoprecipitation followed by Illumina sequencing (CLIP-seq) and identified the targets of Lin28 in mouse embryonic stem cells. Our data confirm the specific interaction of Lin28 with the terminal loop of precursor let-7, and further show that a large number of mRNAs are bound to Lin28. Using ribosome profiling, we discover that translation of Lin28-bound mRNAs is increased in Lin28-depleted cells, without a significant change in mRNA levels. Notably, Lin28 preferentially targets and suppresses mRNAs that are destined for endoplasmic reticulum. Our current genome-wide analyses reveal an unexpected role of Lin28 as a translational suppressor of proteins in the ER secretory pathway.

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NANOS2-MEDIATED RNA REGULATION IN GERM CELL DIFFERENTIATION

Saga, Yumiko[1], **Suzuki, Atsushi**[2]
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In mammals, sexual differentiation of germ cells is induced after their colonization with somatic cells in the embryonic gonads. One of most important events for male germ cell development is the expression of Nanos2, encoding an RNA binding protein, since male germ cell pathway is completely disrupted in the absence of Nanos2. In addition, NANOS2 is also involved in the maintenance of spermatogonial stem cells via mainly suppressing differentiation of stem cells during spermatogenesis. The major issue to be addressed is the molecular mechanism involved in the NANOS2 function. We have recently shown that Nanos2 directly interacts with components of CCR4-NOT deadenylation complex thereby target RNA would be degraded. The possible target RNAs are identified as NANOS2-interecting RNAs. The next critical question is how NANOS2 recognizes target RNAs since NANOS2 itself exhibits no RNA specificity. We now find that NANOS2 makes another protein complex except for CCR-NOT proteins. Among them we paid attention to an RNA binding protein Dnd1, which is already known as a key molecule during germ cell development. I like to discuss the possible mechanism based on the recent our findings.

TRANSCRIPTIONAL REGULATORY CIRCUITS INVOLVED IN THE DYNAMIC EQUILIBRIUM BETWEEN CANCER STEM CELLS AND THEIR NON-STEM CANCER CELL COUNTERPARTS

Struhl, Kevin
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Transient activation of Src oncoprotein can mediate an epigenetic switch from immortalized breast cells to a stably transformed line that forms self-renewing mammospheres that contain cancer stem cells (CSCs). Src triggers an inflammatory response mediated by NF-κB that directly activates Lin28 transcription and rapidly reduces the level of let-7 miRNAs. Let-7 inhibits IL6 expression, thus resulting in higher levels of IL6 than achieved by direct activation by NF-κB. IL6-mediated activation of STAT3 is necessary for transformation, and IL6 activates NF-κB, thereby completing a positive feedback loop. Furthermore, STAT3 activates transcription of the miR-21 and miR-181b miRNAs, and each of these is sufficient to activate the epigenetic switch. This positive feedback loop is enhanced in CSCs as compared to non-stem cancer cells in the same population. Thus, inflammation activates a positive feedback loop that involves NF-κB, Lin28, Let-7, IL6, STAT3, miR-21, and miR-181b that maintains the epigenetic transformed state for many generations in the absence of the inducing signal. The miR-200 family has a selective role in CSC function, and it directly targets Suz12 and (to a lesser extent) Bmi1, which are subunits of the PRC2 and PRC1 polycomb complexes. Loss of miR-200 during CSC formation results in increased expression of Suz12, increased Suz12 binding and H3-K27 tri-methylation at the E-cadherin (CDH1) promoter, and transcriptional repression of CDH1. Expression of miR-200b or depletion of SUZ12 blocks the formation and maintenance of mammospheres, and the transcriptional signature of the miR-200b-SUZ12-CDH1 pathway is observed in metastatic human breast tumors. Thus, regulation of polycomb by the miR-200 family complexes is critical for CSC function, self-renewal, and cancer. Using a genetic screen, we identify miRNAs that inhibit CSC growth and are down-regulated in CSCs. Aside from the miR-200 family, these include miR-15/16, miR-103/107, miR-145, miR-335 and miR-128b. Interestingly, these miRNAs affect common target genes that encode Bmi1 and Suz12 components of the polycomb complexes as well as DNA-binding transcription factors Zeb1, Zeb2, and Klf4. Conversely, expression of the CSC-modulating miRNAs are inhibited by Zeb1 and Zeb2. There is an inverse relationship between the levels of CSC-regulating miRNAs and their respective targets in samples from triple-negative breast cancer patients, providing evidence for the relevance of these interactions in human cancer. In addition, combinatorial overexpression of these miRNAs progressively attenuates the growth of CSCs derived from triple-negative breast cancers. These observations suggest that CSC formation and function are reinforced by an integrated regulatory circuit of miRNAs, transcription factors, and chromatin-modifying activities that can act as a bi-stable switch to drive cells into either the CSC or the non-stem state within population of cancer cells.

INHERITANCE AND REPROGRAMMING OF HETEROCHROMATIN WITH SMALL RNA

Martienssen, Rob
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Plants and fission yeast provide excellent model organisms to investigate how epigenetic information is propagated to daughter cells, and possess a wealth of epigenetic phenomena including transposon regulation, heterochromatic silencing, and gene imprinting. We are investigating heterochromatic silencing and RNAi in the fission yeast Schizosaccharomyces pombe and the plant Arabidopsis thaliana. In plants we have found that RNAi is important in determining germ cell fate as well as in transposon regulation, while in fission yeast, RNAi and retrotransposons mediate silencing by controlling DNA replication and recombination. Parallels between these mechanisms may account for the role of RNA in epigenetic inheritance in higher organisms.

Concurrent Session IVA: Stem Cells in Organ Development

Saturday, June 16, 2012, 1:30 pm - 3:15 pm

TISSUE SPECIFIC TRANSLATIONAL PROFILING DURING ZEBRAFISH HEART REGENERATION

Poss, Kenneth D., **Fang, Yi**, **Gupta, Vikas**, **Karra, Ravi**, **Wahlig, Taylor**, **Holdway, Jennifer**
Department of Cell Biology and Howard Hughes Medical Institute, Duke University Medical School, Durham, NC, USA

By contrast with adult mammals, zebrafish regenerate cardiac muscle after major injury. In recent studies, we used genetic fate-mapping to reveal that this regeneration occurs through activating proliferation of pre-existing cardiomyocytes at sites of injury. Yet, the molecular mechanisms by which injury activates cardiomyocyte proliferation remain elusive. Here, we have used new technologies to identify cardiac cell type-specific gene expression profiles during heart regeneration. Our findings indicate key injury responses that enable cardiomyocyte proliferation and new muscle regeneration.

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APOPTOSIS-DEPENDENT LINEAGE TRACING FROM CRYPT BASE COLUMNAR CELLS IN THE MOUSE INTESTINE

Zhu, Yunhua, Bulavin, Dmitry
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Emerging lineage tracing data support the existence of two pools of adult stem cells in the mouse intestine. While new evidence suggests that the Lgr5-positive crypt base columnar (CBC) cells give rise to lineage tracing in the intestine and may be intestinal stem cells (ISCs), the long-standing paradigm is that the ISCs are in position +4. Here, we investigate the potential cross-talk between these two cellular compartments. We show that lineage tracing from a significant fraction of Lgr5-positive CBC cells is dependent upon tamoxifen-induced apoptosis of neighboring +4 cells. In turn, suppression of this apoptosis in several mouse genetic backgrounds substantially reduced lineage tracing from Lgr5-positive cells in the mouse intestine. Our data argue that lineage tracing from CBC cells relies on apoptosis of +4 cells and thus supports the idea that +4, but not CBC cells are ISCs.

HGF/C-MET SIGNALING PATHWAY GOVERNS SYNCYTIOTROPHOBLAST STEM CELL MAINTENANCE AND CELL POLARITY DURING PLACENTAL DEVELOPMENT

Ueno, Masaya[1], Lee, Lydia[1], Chhabra, Akanksha[1], Sasidharan, Rajkumar[1], Wang, Ying[1], van Handel, Ben[1], Jiang, Meisheng[1], Mikkola, Hanna[2]
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The mammalian placenta serves as the structural interface for nutrient and waste exchange for the developing fetus. The labyrinth layer, which is composed of highly branched fetal vasculature and maternal blood spaces, is responsible for efficient substance exchange between maternal and fetal circulation. Although defects in feto-maternal substance exchange may result in intra-uterine growth restriction (IUGR) and/or spontaneous abortion, molecular mechanisms that orchestrate development and function of the placenta are largely unknown. Trophoblasts, a cell type unique to the placenta, play an important role in implantation, endocrine function and feto-maternal exchange. Trophoblasts can be divided to various subtypes based on histological characters and specific markers; however, all trophoblasts share a common origin in the trophectoderm of the blastocyst. Although multipotent mouse trophoblast stem (TS) cell lines can be derived from trophoectoderm, TS cells disappear completely before E8.75 (11 somite pair stage), suggesting that yet uncharacterized, partially committed stem/progenitor cells give rise to the definitive placenta. Loss of hepatocyte growth factor (HGF)/c-Met signaling has been associated with placental defects, although its specific function in placentation is unknown. HGF or c-Met germline KO (g-KO) embryos exhibit growth retardation and markedly reduced size of the placenta. In the placenta, HGF is secreted by mesenchymal cells, while the receptor, c-Met, is expressed in the trophoblasts. Using trophoblast-specific lentiviral gene targeting in vivo, we demonstrate that tissue specific c-Met gene inactivation in placental trophoblasts (t-KO) recapitulates the conventional c-Met knockout placental phenotype as well as IUGR. Microarray analysis of c-Met deficient trophoblasts indicated that c-Met signaling acts on multiple levels to govern placental development and function. Our data revealed a direct requirement of HGF/c-Met signaling in sustaining proliferation and differentiation of EpCamhi putative syncytiotrophoblast stem cells (SynTS cells), while other trophoblast subtypes were unaffected. Our data further suggested that c-Met signaling is required to maintain the expression of GCM1, a transcription factor essential for syncytiotrophoblast differentiation. Furthermore, while differentiated syncytiotrophoblasts normally form epithelium and exhibit unique cellular polarity, loss of c-Met signaling disrupted polarity in syncytiotrophoblasts. Surprisingly, loss of c-Met signaling in trophoblasts also resulted in abnormal lipid accumulation and upregulation of leukotriene synthesis, which triggered inflammation and macrophage infiltration in c-Met deficient placentas. The cell autonomous requirement of c-Met signaling in regulating these processes in trophoblasts was confirmed by blocking c-Met signaling in vitro in TS cells. Thus, our study is the first to show that compromised development labyrinth trophoblasts due to loss of HGF/c-Met signaling leads to defective proliferation and differentiation of SynTS cells and establishment of cellular polarity in syncytiotrophoblasts. Furthermore, our data links abnormal development of syncytiotrophoblasts to disruption of lipid transport and induction of inflammation in the placenta, offering new insights to the etiology of common pregnancy complications.

DIRECT OBSERVATION OF THE BEHAVIORS OF UNDIFFERENTIATED SPERMATOGONIA IN MOUSE TESTIS

Hara, Kenshiro, Yoshida, Shosei
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In the mammalian testis, numerous spermatozoa are continuously produced throughout the reproductive period. These accurate and flexible spermatogenesis stands on the persistence of stem cells that harboring the activity of both self-renewal and production of progeny that differentiate into sperm. Purpose of this study is to understand how spermatogenic stem cells behave in steady-state. Our recent study implies that the population of GFRα1-expressing Asingle (As) cells are the best related to the actual stem cells that support normal steady-state spermatogenesis in adult mouse testis. However, the behavior of GFRα1+ spermatogonia has not directly been observed. One of the biggest questions raised here is whether GFRα1+ As cells are immobile and anchor at specific position like the germline stem cells in Drosophila male and female gonads. To approach this, we have performed live-imaging analyses of mouse testis harboring GFRα1-expressing cells labeled with GFP. Interestingly, it has been revealed that GFRα1+ As spermatogonia are actively moving: some are migrating over the seminiferous tubules, while others are screaming along the blood vessels. This is an anatomically and physically surprising phenomenon because GFRα1+ spermatogonia are closely surrounded by differentiating spermatogonia and Sertoli cells on the basement membrane of seminiferous tubules. Furthermore, it has also been observed that Sertoli cells are immobile in the seminiferous tubules by live-imaging analysis of GATA1-GFP transgenic mouse testis, indicating GFRα1+ As spermatogonia migrate actively among immobile somatic cells. Active migration of GFRα1+ As strongly suggests that mouse spermatogenic stem cells may not be tethered to a definitive microenvironment in contrast to Drosophila germline stem cells.

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SECRETED FRIZZLED RELATED PROTEIN 2 MEDIATES FORMATION OF BONE AND THE HEMATOPOIETIC MICROENVIRONMENT BY MULTIPOTENT BONE MARROW STROMAL CELLS (SKELETAL STEM CELLS)

Robey, Pamela G.[1], Yoshizawa, Sayuri[1], Kuznetsov, Sergei A.[1], Mishra, Prasun[2], Holmbeck, Kenn[1], Berendsen, Agnes D.[1], Phillips, Matthew D.[1], Burbach, Nathan J.[3], McClendon, Britney N.[1], Cherman, Natasha[1], Merlino, Glenn[2], Balakumaran, Arun[1], Sworder, Brian J.[4]

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Bone marrow stromal cells (BMSCs) are a population of fibroblastic reticular cells, a subset of which is composed of multipotent skeletal stem cells (SSCs, also known as "mesenchymal stem cells") with the capacity to recreate bone, marrow adipocytes and hematopoiesis-supportive stroma. As such, they are central mediators of skeletal homeostasis. We established colonies (originating from Colony Forming Unit-Fibroblasts) by plating a single cell suspension of bone marrow from a single human donor at low density. After ex vivo expansion of individual colonies, we determined their differentiation capacity by vivo transplantation with a ceramic scaffold. In this series of clones (N=24), 5 (20.8%) formed fibrous tissue (FT), 16 (67%) formed bone only (B), and 3 (13%) formed bone, marrow adipocytes and supported hematopoiesis, and thus were multipotent (MP). These frequencies are similar to previous reports. mRNA isolated from clones just prior to transplantation was also analyzed by microarray (Affymetrix). Unsupervised hierarchical clustering revealed that FT, B and MP clones did not separate into three distinct groups (some B clones were interspersed with FT clones and MP clones). No two clones were the identical, although highly related to one another. Subsequent analysis compared FT clones to MP clones, which did separate into two distinct groups, and revealed that SFRP2 was highly expressed in MP clones compared to FT clones (verified by qPCR). The role of SFRP2 in the bone marrow microenvironment is unknown; therefore, we analyzed Sfrp2 deficient (KO) mice to evaluate their skeletal status. By microCT analysis, KO long bones were mildly osteopenic [thinner cortices and reduced trabecular bone compared to wildtype (WT)]. Colony forming efficiency, the closest approximation of the number of SSCs in the BMSC population, was reduced in the KO by ~40% compared to WT (6.6+2.1/105 vs. 11.0+3.7/105, p<0.03). Non-clonal BMSC strains were also established, with no difference in growth rate between KO and WT. However, when transplanted in vivo with collagen scaffolds, KO BMCS formed ossicles with thinner cortices, increased marrow adipocity and decreased hematopoiesis in comparison to those formed by WT BMSCs. These data indicate that Sfrp2 KO BMSCs display not only a decreased capacity for bone formation, but also a decreased ability of SSCs (or decreased numbers) to recreate the hematopoietic microenvironment, a seminal feature of SSCs. In vitro, Sfrp2 deficiency in BMSCs (using both KO BMSCs and siRNA knockdown of Sfrp2 in WT BMSCs) caused reduced expression of osteogenic transcription factors (Runx2 and Osx) and reduced calcium accumulation. There was also decreased phosphorylation of the Wnt coreceptor, Lrp6, and decreased expression of the Wnt downstream target gene, Axin 2. Addition of recombinant mouse Sfrp2 restored Runx2, Osx and Axin 2 expression, as well as phosphorylation of Lrp6. From these data, it appears that: 1) Sfrp2 is associated with maintaining the multipotency of SSCs within the BMSC population, as demonstrated by its high expression in MP clones, and reduced ability of KO BMSCs to support hematopoiesis in vivo, and 2) Sfrp2 is a positive regulator of osteogenesis mediated by canonical Wnt signaling, based on the fact that its deficiency lead to decreased osteogenesis in vivo and in vitro. These data indicate that Sfrp2 has multiple roles that depend on the differentiation stage of stem/progenitor BMSCs in the bone marrow microenvironment.

A MOUSE MODEL FOR IN VIVO QUANTITATIVE MONITORING OF WNT/B-CATENIN SIGNALLING IN SKIN REVEALS HAIR CYCLE DYNAMICS IN SOX18 MUTANT MICE

Hodgson, Samantha S.[1], Francois, Mathias[2], Khosrotehrani, Kiarash[1]

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Wnt signalling through its canonical pathway promotes hair follicle development and differentiation via the stabilization of nuclear beta catenin and triggers proliferation of epithelial progenitor cells within the hair follicle. Sox18 is a SRY transcription factor that is expressed in the dermal papilla and is thought to specify hair follicle type. We aimed to generate a model for in vivo quantitative monitoring of beta catenin activity in the skin and follow Wnt signalling in sox18 deficient mice displaying hypotrichosis, hypoonychia and lymphoedema. We generated mice harbouring a luciferase reporter gene under the control of beta catenin binding sites TCF/LEF inducible promoter to track hair follicle cycles in live mice by in vivo bioluminescence imaging. In adults, hair plucking and cyclosporine induced beta catenin activity and hair growth. The validated model was used to track hair follicle cycles in mice harbouring TCF/LEF transgene reporter activity and a sox18 dominant negative mutation displaying a thin ragged coat lacking zigzag and auchene hairs. In normal reporter mice, beta catenin activity was seen in the skin from P1 and moved in rostro-caudal waves, peaking at P9 with >400 fold increase in luciferase signal compared with newborns during hair development. Mice with the sox18 dominant negative mutation displayed similar waves of beta catenin activity during hair development and the first hair cycle, however beta catenin activity was significantly reduced and delayed, peaking around P14 during hair follicle morphogenesis and reaching telogen approximately 3 days later than WT mice. Histological analysis revealed both telogen and anagen hair follicles in P26 and 6wk old mice when luciferase signal was absent, suggesting populations of unsynchronized follicles in sox18 dominant negative mice. At least a portion of these follicles however appear to cycle in a beta catenin dependant manner. In conclusion, wnt/b-catenin activity and hair cycling in sox18 deficient mice are patially disconnected. A lower level of wnt pathway activation in the absence of functional sox18 suggests a key role for this pathway in the observed phenotype.

Concurrent Session IVB — Self Renewal Mechanisms

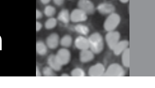
Saturday, June 16, 2012, 1:30 pm - 3:15 pm

TRANSCRIPTION FACTOR CONTROL OF TRANSITIONS IN PLURIPOTENT CELL STATES

Chambers, Ian, Karwacki-Neisius, Violetta

University of Edinburgh, Edinburgh, United Kingdom

Embryonic stem (ES) cells are defined by two key characteristics; the ability to differentiate into cells of all three germ layers, a property referred to as pluripotency and the ability to undergo apparently symmetrical self-renewing cell division, essentially indefinitely. Pluripotent cell identity is governed by the action of a



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gene regulatory network centred on Oct4, Nanog and Sox2, three transcription factors that bind to closely localised sites in ES cell chromatin. Binding sites for each are present in the Oct4, Nanog and Sox2 genes and these sites are generally considered to reflect positive feedback interactions that stabilise expression of the three genes. However, Nanog is expressed heterogeneously in ES cells with some Oct4-expressing undifferentiated ES cells completely lacking Nanog. The mechanisms giving rise to this heterogeneous expression pattern are unknown. Here we show that Oct4+/- ES cells express Nanog homogeneously. Consistent with a model in which differentiation proceeds through Nanog-negative cells, ES cells expressing reduced Oct4 levels are delayed in their differentiation kinetics. However, both heterogeneous Nanog expression and rapid differentiation kinetics can be restored by elevation of Oct4 expression towards wild-type levels. Therefore, Nanog and Oct4 are linked through a feedback control mechanism that is finely tuned in wild-type cells to enable the stem cell population to simultaneously self-renew and respond to differentiation cues.

AN RNAI SCREEN UNVEILS A NEW PLAYER IN MOUSE SKIN STEM CELL SELF-RENEWAL AND LONG-TERM REGENERATION IN VIVO

Chen, Ting, Beronja, Slobodan, Heller, Evan, Stokes, Nicole, Fuchs, Elaine
Rockefeller University, New York, NY, USA

Adult stem cells (SCs) sustain tissue maintenance and regeneration throughout the lifetime of an animal. They often reside in specific signaling niches that orchestrate the stem cell's balancing act of transitions from quiescence to cell cycle re-entry in order to fulfil a demand for tissue regeneration. How SCs self-renew long-term to maintain this remarkable capacity for tissue regeneration is still poorly understood. Here, we use RNA interference (RNAi)-based loss-of-function screening as a powerful approach to uncover transcriptional regulators governing SC self-renewal and long-term regenerative potential. Hair follicle SCs provide an ideal paradigm, since they've been purified and characterized from their native niche in vivo, and can be maintained and passaged long-term in vitro without losing stemness. Focusing on the nuclear proteins/transcription factors enriched in SCs versus progenies, we screen ~2,000 hairpin shRNAs for their impact on long-term stem cell self-renewal in vitro. To address the physiological relevance of our findings, we select one of the candidates surfacing in the screen. By conditionally ablating this gene in vivo, we show that tissue regeneration during homeostasis is dramatically delayed. Devising an in vivo assay for long-term SC self-renewal, we then show that when challenged with repetitive bouts of regeneration, SCs prematurely exhaust their potential in vivo and become depleted in the absence of this gene. Dissecting mechanism, we discover that this gene acts as an intrinsic rheostat to control pSmad1/BMP signalling at the heart of the transition between SC quiescence to self-renewal in hair follicles. Our results validate the RNAi screen and underscore its power in unearthing new players governing SC long-term self-renewal and tissue-regenerative potential.

ACTIVATION OF UNLIMITED SELF RENEWAL ON A CELL TYPE SPECIFIC ENHANCER PLATFORM

Soucie, Erinn[1], Weng, Ziming[2], Fenouil, Romain[1], Vu Manh, Thien-Phong[1], Dubreuil, Patrice[3], Andrau, Jean-Christophe[1], Ferrier, Pierre[1], Sidow, Arend[2], **Sieweke, Michael H.**[1]
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Genome wide comparison of enhancer regions between different cell types has revealed that enhancer distribution is highly cell type specific and represents a reliable epigenetic signature of cellular identity. Accordingly reprogramming of differentiated cells into self-renewing pluripotent cells is associated with a complete conversion of enhancer distribution into an ES cell like repertoire. We have recently shown that MafB and c-Maf deficiency activates unlimited stem cell-like self-renewal in differentiated functional macrophages by a process requiring the reprogramming factors c-Myc and KLF4 (Aziz et al., Science326 :867(2009). We now investigated whether self renewal activity in differentiated cells is compatibble with maintaining cell type specific enhancer identity. Based on genome wide distribution of promoter distal H3K4me1 epigenetic marks and binding sites of the lineage determining transcription factor PU.1 we demonstrate that self-renewing macrophages maintain a cell type specific enhancer profile with high fidelity, showing fewer differences to normal macrophages than is evident between different macrophage subtypes. However, when analyzing the activity of these enhancers by determining their H3K27 acetylation status and co-occupation by the histone acetylase p300, we observed an increased number of active enhancers in Maf-DKO macrophages. Importantly, these active enhancers were significantly enriched proximal to genes with functionally annotated self-renewal activity. Interrogation of the active H3K27ac+ enhancer profile of other self-renewing cells such as embryonic stem (ES) and neural stem/progenitor cells (NPC) identified a network of genes associated with active enhancers that was shared with Maf-DKO self-renewing macrophages. Interestingly the large majority of these active enhancers were pre-marked by lineage specific H3K4me1/PU.1 enhancer marks but were negative for H3K27 acetylation and p300 in quiescent macrophages. Taken together, our results support a model wherein mature cells only realize part of their enhancer potential and maintain poised enhancers associated with self-renewal genes, whose re-activation is possible without reshuffling of the lineage specific enhancer signature. This suggests that large scale cellular amplification of cells required for regenerative medicine and drug screening applications may be achieved on an existing cell type specific enhancer platform without the need for profound and repeated changes in the enhancer organization that are associated with current reprogramming and differentiation approaches.

THE RNA DECAY PATHWAY, NMD, PROMOTES THE STEM-LIKE STATE

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Nonsense-mediated decay (NMD) is a highly conserved regulatory pathway that was originally identified as a quality control mechanism by virtue of its ability to rapidly degrade aberrant transcripts with premature stop (nonsense) codons. Recently, our laboratory and others discovered that NMD also directly regulates the stability of large subsets of normal transcripts. This discovery raised the possibility that NMD is not only a RNA surveillance mechanism but also a regulator of normal physiological events. Here, we provide several lines of evidence that NMD promotes the stem-like state. First, overexpression of the core NMD factor, UPF1, in mouse P19 embryonal caricinoma cells prevented their differentiation in response to retinoic acid (RA). Second, RNA interference (RNAi)-mediated depletion of UPF1 was sufficient to elicit the downregulation of self-renewal markers (OCT4, NANOG,

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and c-myc) and the upregulation of cellular proliferation inhibitor proteins (AXIN2 and MAPK6) in both P19 cells and mouse embryonic stem (ES) cells. Third, this UPF1-mediated response is conserved, it was also observed in human ES and epidermal keratinocytes. Fourth, UPF1 downregulation is a normal event during epithelial skin differentiation, based on our finding that basal layer cells expressed dramatically higher levels of UPF1 than do differentiated cells. Fifth, differentiation of human ES cells into endoderm downregulated the level of UPF1 and two other NMD factors. Finally, genome-wide analysis of 452 independent pluripotent human cell lines (ES and induced pluripotent stem cells [iPS]) revealed that several factors essential for NMD are significantly upregulated as compared to their level in 254 non-pluripotent human cell lines. These results, coupled with our published finding that NMD preferentially directs the decay of transcripts encoding differentiation proteins (Bruno et al. Mol. Cell 42:500 [2011]), lead us to propose that NMD collaborates with transcriptional repression mechanisms (such as that mediated by polycomb-group proteins) to repress the expression of differentiation proteins and thereby promote the stem-like state.

MIR-181A MEDIATES THE CONTROL OF MURINE EMBRYONIC STEM CELL SELF RENEWAL

Arnold, Christopher P., Ghezelayagh, Talayeh, Wang, Song, Chen, Chang-Zheng
Department of Microbiology and Immunology, Stanford University, Stanford, CA, USA

Identification of the regulatory components that affect stem cell self-renewal may shed light on how to manipulate stem cell proliferation and differentiation ex vivo, thus facilitating therapeutic uses of stem cells. Though much has been learned about the roles of stem cell specific factors, little is known about those factors that are suppressed for optimal stem cell self-renewal. We recently identified a set of miRNAs, termed stem to progenitor transition miRNAs (SPT-miRs), that mark the transition from quiescent self-renewing stem cells to highly proliferative and non-self-renewing transient amplifying cells in both blood and muscle tissues of mice. Up-regulation of SPT-miRs coincides with the loss of self-renewal potential and an increase in proliferation. Premature expression of SPT-miRs in murine hematopoietic stem cells and embryonic stem cells (ESC) negatively impacted their self-renewal and/or differentiation, suggesting suppression of SPT-miRs may be critical for optimal stem cell self-renewal. Interestingly, we found that perturbations that dampened ESC self-renewal, such as serum withdrawal, PI3K inhibition, and shRNA knockdown of Id molecules, caused strong up-regulation of miR-181a. In support of a role for miR-181a in regulating ESC self-renewal, ectopic expression of mir-181a-1 in ESCs reduced ESC self-renewal, whereas conditional deletion of either mir-181ab1 or mir-181ab2 alleles potentiated ESC self-renewal in serum-free medium. Further, we observed that perturbing miR-181a expression modulated the basal level Erk signaling and lengthened the G1 phase of the cell cycle through targeting Lin28a and Spry4 without significant effects on ESC pluripotency and apoptosis. Finally, we demonstrated that serum component such as BMPs may support ESC self-renewal via suppression of miR-181a. Collectively, these results reveal a novel signal network controlled by miR-181a that plays critical roles in modulating ESC self-renewal.

NME6 AND NME7 IS ESSENTIAL FOR MOUSE EMBRYONIC STEM CELL RENEWAL

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Embryonic stem cells (ESCs) are characteristic by its cell renewal, oncogenicity, and pluripotency. Kinases and phosphatases are well-recognized for their importance in proliferation, differentiation, and apoptosis. However, the roles of kinase and phsophatase in ESCs remain elusive. Thus we used 4801 shRNAs against 929 kinases and phosphatases to simultaneously pinpoint the pivotal kinases and phosphtases for ESC renewal and pluripotency. Among the 929 genes targeted by 4801 shRNA, 27 genes induced morphological change were identified as the potential targets. Two of the candidate genes, Nme6 (non-metastatic cells 6, protein expressed in nucleoside-diphosphate kinase) and Nme7 (non-metastatic cells 7, protein expressed in nucleoside-diphosphate kinase), are essential for ESC renewal and pluripotency were studied in depth. Interestingly, Oct4, Nanog, Klf4, c-Myc, telomerase, Dnmt3B, ERas and Nanog were reduced in both Nme6- and Nme7- knockdown ESCs. This indicates that Nme6 and Nme7 can control multiple genes important for ESC renewal. The differentiation markers of ectoderm, endoderm, and mesoderm are also activated upon the depletion of Nme6 or Nme7. The knockdown of either Nme6 or Nme7 abolishes the embryoid body formation ability and reduces the oncogenicity of ESC. The overexpression of Nme6 or Nme7 restores the embryoid body formation ability in the culture absence of leukemia inhibitory factor. Our finding suggests the crucial roles for Nme6 and Nme7 in ESC renewal.

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PLACENTAL DERIVED STEM CELLS: A THERAPEUTIC PLATFORM FOR THE TREATMENT OF AUTOIMMUNE DISEASE

Hariri, Robert J.
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PDA-001 (Cenplacel-L) is a novel, placentally-derived progenitor cell phenotype which has both immunomodulatory and cytotrophic activities and has been evaluated as a therapeutic in the treatment of autoimmune diseases including Crohn's Disease. The derivation, development and clinical experience with this product will be described with specific focus on the unique properties and clinical behavior.

ANTIGEN-SPECIFIC T CELL INDUCTION FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

Nishimura, Toshinobu[1], Kaneko, Shin[1], Tachikawa-Kawana, Ai[2], Eto, Koji[3], Nakauchi, Hiromitsu[1]
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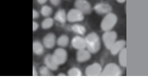
T cells are central players in the adoptive immunity. They survey and recognize abnormal cells such as cancers and virus infected



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cells, and fight such abnormalities to eradicate them from the body as well as regulate other cells of the immune system. The cause of the abnormality is fragmented into the pieces of peptides, and they are presented on cell surface by major histocompatibility complexes (MHCs) as antigen peptides. T cells recognize such an antigen by the T-cell receptor (TCR). Each TCR has a unique conformation at its binding site so that it can fit to the 3D conformation composed of an antigen and an MHC. Therefore, the specific recognition of the target antigen is accomplished in each T cell, and the integration of the antigen-specificity creates the way to handle all pathogens derived from inside and outside of the body. Here we demonstrated the generation of iPS cells from a single antigen-specific T cell, and the subsequent T cell differentiation from this point onwards. At first, we established a highly expanded antigen-specific CD8+ T cell clone (H25-4) from peripheral blood cells of an HIV-1-infected patient. H25-4 was reprogrammed to pluripotency, inheriting the TCR gene rearrangements to the genomic DNAs of the derived T-iPS cells. Next, we differentiated T-iPS cells into CD8 single-positive T cells again, and then we confirmed the CD8+ T cell differentiation by gene expression profiles. Such re-differentiated CD8+ T cells exerted cytotoxic T cell functions in the same antigen-specific manner as the original T cell clone H25-4 had done, because the expressed TCR was identical to the one that H25-4 had expressed. Interestingly, re-differentiated CD8+ T cells became more expandable and had longer telomeres than the H25-4 clone. In other words, passage through the T-iPS cell state rejuvenated these highly expanded clones. Adoptive immunotherapy is a promising approach to fight cancers or chronic viral infections with cytotoxic T cells expressing antigen-specific TCRs. However, its effectiveness is diminished by the exhaustion of antigen-specific T cells during ex vivo expansions. To overcome the obstacle, we established here a basis for the rejuvenation of CD8+ cytotoxic T cells, which could be utilized for T cell infusion therapy to eliminate malignancies. Besides, the concept of T cell rejuvenation may also be applicable to CD4+ helper or regulatory T cells to control desired or undesired immune reactions in cases such as malignancies, chronic viral infections, autoimmune diseases, or transplantation related immune disorders. Several biological and technical challenges may lie ahead, but the data presented in this work could open new avenues toward T cell therapies that would supply unlimited numbers of rejuvenated antigen-specific T cells and restore the normally functioning immune system in a patient, cooperating with iPS cell technology.

DEVELOPMENT OF NATURAL KILLER CELLS FROM HUMAN PLURIPOTENT STEM CELLS USING DEFINED CONDITIONS SUITABLE FOR CLINICAL TRANSLATION

Kaufman, Dan S., **Ni, Zhenya**, **Bendzick, Laura**, **Bock, Allison**, **Knorr, David**
University of Minnesota, Minneapolis, MN, USA

Previous studies by our group have demonstrated derivation of natural killer (NK) cells from human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs). These hESC and iPSC-derived NK cells have potent ability to kill diverse tumor cell types and HIV-infected cells both in vitro and in vivo (using xenograft models). Clinical studies at the University of Minnesota have shown the NK cells isolated from peripheral blood of haplo-identical donors are an attractive source of lymphocytes for adoptive immunotherapy and can achieve durable remissions in patients with poor-prognosis acute myelogenous leukemia (AML). However, use of hESC or iPSC-derived NK cells may have further advantages. For example, starting with a homogeneous starting cell population to produce NK cells can allow development of a universal, "off-the-shelf" product for anti-tumor immunotherapy, rather than needing to obtain NK cells from donors on a patient-specific basis. The previous studies by our group utilized hESCs and iPSCs in a stromal cell co-culture system to derive hematopoietic progenitor cells (CD34+CD45+ cells) that can then produce CD45+CD56+ NK cells in a secondary culture system. Here, we have now used a completely defined, spin embryoid body (spin-EB) approach potentially suitable for clinical scale-up. In these spin-EB cultures, defined numbers of undifferentiated hESCs or iPSCs are first aggregated in 96 well plates by centrifugation (3000 cells per well) in serum-free media containing only the cytokines SCF, BMP4, and VEGF. Under these Stage 1 conditions, high frequencies of hematopoietic progenitor cells that express CD34 (55.9 ± 6.4%), CD45 (26.2 ± 6.6%), and CD43 (41.8 ± 9.51%) develop and expand over 6-11 days. After this time, EBs are directly transferred (without dissociation or sorting) to Stage 2 cultures with EL08 stromal cells or stroma-free conditions in serum-free media containing NK cell-initiating cytokines (IL15, SCF, FLT3L, IL7, and IL3). We find that these Stage 2 cells generated with or without the stromal cells acquire all the typical markers of mature NK cells (CD56, CD94, KIRs, etc), secrete cytokines (IFNγ, TNFα), and kill diverse tumor targets including leukemia cells, ovarian cancer, pancreatic cancer, and myeloma cells. Interestingly, we found that stroma free spin-EB cultures produce their own feeder layer capable of driving NK cell development. We have characterized these feeder layers expressing endothelial markers (CD31) as well as human leukocyte antigen (HLA) -A, -B, -C, and -E, which are important in the education and licensing of human NK cells. The feeder cells also support NK cell development from umbilical cord blood hematopoietic stem cells (HSCs). Additionally, to demonstrate the direct in vivo anti-tumor activity, we have used in vivo bioluminescent imaging using firefly-luciferase hESC-derived NK cells and membrane bound gaussian-luciferase expressing tumor cells to show direct co-localization of the NK cells to the tumor. Based on our calculations, we estimate that one plate of undifferentiated hESCs or iPSCs (typically with 5-10 x 106 undifferentiated cells) would provide enough NK cells to treat a single patient with a dose of 750 x 106 NK cells. Therefore could treat several patients. Together, these studies demonstrate clinical translation of hESC and/or iPSC-derived NK cells for treatment of refractory malignancies is now very feasible.

ANTIBODY-BASED IMMUNOTHERAPY USING REPROGRAMMED ANTIGEN-SPECIFIC EFFECTOR B-CELLS

Kamata, Masakazu[1], **Nagaoka, Yoshiko**[2], **Hsu, Joy-Fon**[3], **Kim, Patrick**[4], **Gudeman, Vee**[3], **Wang, Ying**[2], **Jiang, Meisheng**[2], **Ferbas, Kathie Grovit**[5], **Chen, Irvin SY**[4]
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Introduction: Developing technologies that allow for the identification and maturation of antibodies (Abs) with potent effector function is desperately needed for generating effective prophylactic vaccines against incurable infectious diseases such as acquired immune deficiency syndrome (AIDS). Recent induced pluripotent stem cell (iPSC) technologies allow the reprogramming of antigen (Ag) specific B-cells into iPSC which can then be used to reconstitute and create a chimeric immune system

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with defined Ab specificities. Ig genes of iPSC-derived cells could potentially evolve further in response to vaccination through affinity maturation, so-called somatic hypermutation, to even higher affinity. Moreover, expected results have the potential to represent a new technological advance for the field to identify Abs with novel and/or superior effector function for prophylactic vaccine development. Here we use a mouse model to induce a broad potent neutralizing Ab response using a well-characterized whole virion HIV-1 vaccine. Materials and Methods: We first established a B6-129 hybrid mouse strain with high iPSC reprogramming efficiencies, termed iPSC mouse. The mice were immunized 5 times with a whole virion HIV-1 vaccine. At six days after final immunization, B220-/lowCD138+ B-cell populations in the spleen and bone marrow were isolated with magnetic beads. Reprogramming into iPSCs was induced by culturing in ES medium (15% FCS/1% NEAA/1% GlutaMax/0.1mM 2-mercaptoethanol/KO-DMEM) supplemented with 1000U/ml leukemia inhibitory factor and 4 µg/ml doxcycline. To support mouse B-cell growth, mIL-4, mIL-7, BAFF, and anti-mCD40 Ab were also added in the medium. At 2 weeks post-induction, iPSC-like clones were picked and expanded on irradiated mouse embryonic fibroblasts. For an initial assessment of Ig specificity, these iPSCs were differentiated into embryoid bodies (EBs) and the culture supernatants after 6 days of differentiation were collected and subjected to mouse Ig quantitation by NeoClone Biotechnology (Madison, WI) via the Octet system (ForteBio, CA). Results and Conclusions: We obtained over 300 iPSC-like colonies. Of those, we randomly selected 67 clones derived from bone marrow and induced differentiation upon EB formation. We confirmed 59 out of the 67 clones were secreted mouse Igs into culture supernatant at concentrations ranging from 1060 - 2520ng/ml. We further identified 14 out of the 59 clones secreted Igs against HIV-1 envelope protein, gp120. We then assessed HIV-1 neutralizing activity of those supernatants and found that 4 out of the 14 clones showed >50% inhibition against the RHPA4259 HIV-1 strain. We are now cloning Ig heavy and light chain genes from those clones. Above studies demonstrate that iPSC could be derived from Ag-specific B-cells. Future improved technology should allow for reprogramming of pathogen-specific B-cells obtained from patients, which can be used for Ab-based immunotherapies and/or prevention of AIDS.

COMPARATIVE ANALYSIS OF THE IMMUNOREGULATORY AND REGENERATIVE PROPERTIES OF HUMAN MESENCHYMAL STROMAL CELLS -HYALURONIC ACID HYDROGEL CONSTRUCTS

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Mesenchymal stromal cells (MSCs) together with hyaluronic acid (HA) hydrogel scaffolds have the potential to enhance tissue regeneration by mediating both the inflammatory response and long-term repair of injured tissue. Macrophages play a critical role in the host defense against biomaterial scaffolds as they can differentiate into distinct immunophenotypes that can either drive or resolve inflammation. MSCs hold promise for clinical application because they secrete a spectrum of bioactive molecules that can alter the tissue microenvironment by regulating hematopoiesis, angiogenesis, inflammation and native cell growth. Given the known immunomodulatory effects of MSCs in vitro, combining these cells with HA hydrogel scaffolds may provide an alternative approach for suppressing macrophages foreign body reaction against biomaterials and restoring normal tissue function. The objective of the current investigation was to analyze immunoregulatory and regenerative properties of MSCs derived from human bone marrow (BM) and adipose tissues (AT) embedded in HA hydrogel (Carbylan - GSX) co-cultured with human peripheral blood derived CD14+ cells for 7 days. Control conditions included MSCs in Carbylan-GSX without CD14+ cells, CD14+ cells cultured on Carbylon-GSX without MSCs and CD14+ cells cultured on tissue culture polystyrene. Bio-plex assays were used to measure cytokine, chemokine and growth factors from the cell culture supernatants on days 3 and 7 of all conditions. Extracellular matrix gene expression was analyzed using real-time PCR from MSCs embedded in Carbylon-GSX after 7 days of co-culture with or without CD14+ cells. Overall, supernatants from CD14+ cells co-cultured with BM or AT MSC hydrogel constructs showed significantly higher levels of IL-1β, MIP-1α, IFN-γ, GM-CSF, and IL-12 compared to hydrogel only conditions. When MSC hydrogel co-culture conditions were compared on day 3 significant increases in protein expression were found between BM (VCAM, VEGF) and AT (IL-1β, TNF-α, GM-CSF, IL-12, MCP-1, IL-10). After 7 days co-culture CD14+ cells cultured on hydrogel constructs without MSCs showed significant increases in protein expression of TNF-α, IL-1β, MIP-1α, and MCP-1, while IL- 6, IL-10, IL-12, IFN-γ, HGF and VEGF remained low. Significant increases in protein expression were found after 7 days between BM (VEGF, VCAM) and AT (MCP-1) co-culture conditions. VEGF, elastin, and HAS2 gene transcripts were upregulated in BM and AT MSCs co-cultured with CD14+ cells. Significant increases in gene expression were found between BM (pro-collagen, collagen-I and -III, and MMP-9) and AT (HYAL2) co-culture conditions. In summary, MSCs derived from different tissue sources embedded in a HA hydrogel construct promoted unique immunoregulatory and regenerative properties that may alter their clinical application. Our results lend support to the use of BM or AT MSC hydrogel constructs to regulate macrophage differentiation to an alternative_anti-inflammatory phenotype through paracrine signaling of several bioactive molecules.

MINOR ANTIGEN-MISMATCHED MSC REACT WITH RESIDUAL HOST T CELLS TO TRIGGER THE PROGRESSION OF CHRONIC GVHD

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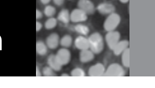
Chronic graft-versus-host disease (cGVHD) is a complication after bone marrow transplantation (BMT) characterized by an autoimmune-type reaction such as systemic sclerosis or Sjögren's syndrome involving exocrine glands. It is believed to be distinct from the potentially lethal acute form of GVHD. Bone marrow transplantation with 8 week-old donor B10.D2 (H-2d) mice and recipient BALB/c mice (H-2d) has been reported as a MHC-compatible, minor antigen (miHA)-incompatible model of cGVHD. Signs of cGVHD appear by 3 weeks after BMT, and progresses to full-blown disease by 8 weeks characterized by low tear volume and excessive fibrosis of the lacrimal gland, conjunctiva, salivary gland, skin, lung, liver and intestine. Accumulation of donor-derived fibroblasts in fibrotic lesions surrounding exocrine ducts was observed, which was similar to human patients as shown in our previous report. These results suggested that donor-derived fibroblasts were part of the pathological process leading to cGVHD.



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Multipotent mesenchymal stem/stromal cells (MSCs) in the bone marrow differentiate into several mesenchymal lineages such as fibroblasts, adipocytes, osteocytes and chondrocytes. However, the in vivo dynamics of MSCs after WBMT are still unknown due to the lack of specific markers. We have succeeded in prospectively isolating murine MSCs based on their expression of PDGF receptor α and Sca-1 (PDGFRα+ Sca-1+ (P αS) cells). Isolated P αS-MSCs without in vitro expansion can differentiate into hematopoietic niche cells, osteoblasts and adipocytes after systemic in vivo transplantation. In this study, we sought to answer the debated role of donor MSCs in the pathogenesis of the autoimmune-like phenotype of cGVHD using prospectively isolated MSCs and HSCs in the cGVHD mouse model. Transplantation of mismatched, but not syngeneic MSCs triggered the onset of cGVHD, and was associated with fibrosis, increased IL-6 secretion, decreased Foxp3+ regulatory T cells and increased Th17 in the peripheral blood. Mismatched MSCs were sufficient to induce cGVHD, while removal of donor MSCs rescued mice from cGVHD. RAG2 knockout recipient mice did not suffer cGVHD, indicating that host T cells were involved. Residual host-derived T cells were significantly higher in cGVHD patients compared to non-cGVHD patients. In conclusion, donor MSCs react with residual host T cells to trigger the progression of cGVHD.

Concurrent Session IVD — Chemical Control of Stem Cell Behavior

FINDING THERAPEUTICS FOR MOTOR NEURON DISEASES

Rubin, Lee
Harvard Stem Cell Inst, Cambridge, MA, USA

ABSTRACT NOT AVAILABLE AT TIME OF PRINTING

COMBINED SMALL MOLECULE INHIBITION ACCELERATES DEVELOPMENTAL TIMING AND CONVERTS HUMAN PLURIPOTENT STEM CELLS INTO NOCICEPTORS

Chambers, Stuart M.[1], Qi, Yuchen[1], Mica, Yvonne[1], Lee, Gabsang[2], Bilsland, James[3], Cao, Lishuang[3], Stevens, Edward[3], Whiting, Paul[3], Studer, Lorenz[1]
[1]Developmental Biology, Sloan Kettering Institute, New York, NY, USA, [2]Institute for Cell Engineering, Johns Hopkins, Baltimore, MD, USA, [3]Neusentis/Pfizer, Cambridge, United Kingdom

Human pluripotent stem cells (hPSCs) have shown promise for modeling human development and disease and for applications in regenerative medicine. Strides have been made in identifying key signaling pathways required for directing differentiation of hPSCs including the derivation of mature neuronal fates. However, The in vitro derivation of postmitotic neurons from hPSCs requires extended culture periods typically lasting 30 days or more mirroring the timing of normal human development. To accelerate this process, we performed a combinatorial small molecule screen for the rapid conversion of hPSCs into postmitotic neurons, and identified a combination of five small molecule pathway inhibitors sufficient to yield neurons at > 75% efficiency within 10 days of differentiation in the absence of any recombinant growth factors. The use of hPSCs allows us to confirm many of the developmental stages observed in rodents can also be demonstrated to occur in human development, though in an accelerated rate using our protocol. For instance, with a SOX10::GFP bacterial artificial chromosome hPSC line we have engineered we observe that > 80% of cells transition through a neural crest intermediate before becoming nociceptors. The resulting human neurons express canonical markers of nociceptive sensory fate including NTRK1, BRN3A, ISL1, NEUROG1, SCN10A, and P2RX3. Electrophysiological and calcium imaging assays confirmed nociceptive identity by demonstrating TTX-resistant, SCN10A-dependent sodium currents and response to nociceptive stimuli including a,b-metATP and capsaicin.

Neuronal fate acquisition occurs in a time frame three-fold faster compared to in vivo development, suggesting that small molecule-mediated inhibition of signaling pathways is sufficient to accelerate developmental timing. In more general terms, our results indicate that it is possible to overcome normal human developmental timing and to accelerate hPSC differentiation via the combinatorial use of small molecules. We anticipate that similar paradigms may become available for the rapid derivation of other hPSC-derived cell types. Work is now underway to better understand how to better modulate the proportions of nociceptor subtypes and ion-channel expression and to identify compounds that inhibit P2RX3 activation. The quick, scalable and high efficiency derivation of functional nociceptors offers unprecedented access to this medically relevant cell type for studies of human pain perception.

HIGH-THROUGHPUT SCREENS USING MOUSE ES CELL-DERIVED NEURONS IDENTIFY DRUGS THAT ENHANCE AXONAL REGENERATION

Henderson, Christopher E.[1], Nikulina, Elena[2], Hou, Jianwei[2], Yang, Wan Seok[3], Lamas, Nuno J.[4], Amoroso, Mackenzie W.[4], Wichterle, Hynek[5], Filbin, Marie T.[2], Stockwell, Brent[3], Li, Hai[5]
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The axons that connect motor neurons to their target muscles are among the longest single structures in the adult body. Even in the embryo, to innervate a muscle in the distal part of the limb a motor neuron needs to generate a process that is >200 cell diameters in length. The quantitative challenge is greater still for an injured adult axon that may need to regenerate over tens of centimeters. Therefore identifying mechanisms that make motor axons longer is critical both for understanding how the neuromuscular system is initially wired and, potentially, for enhancing its regeneration. We are using chemical genetic approaches to study intrinsic mechanisms that stimulate motor axon outgrowth. We believe that these may be particularly important in defining therapeutic targets in amyotrophic lateral sclerosis (ALS), in which the earliest morphological change is the die-back of fast motor axons from their target muscles. If a way could be found to enhance motor axon regrowth, one would expect a delay in the loss of muscle strength that is a debilitating feature of the disease. To define the signaling pathways underlying axonal growth, we screened a 50,000-compound library using an in vitro assay in which mouse ES cell-derived motor neurons are grown on inhibitory substrata. We have identified four distinct compound classes with strong activity on axonal outgrowth. The most active of these were the cholesterol-lowering drugs statins, which were >100-fold more potent than the benchmarking compound and completely overcame growth inhibition. When applied to human ES cell-derived motor neurons they induce a 5-fold increase in axon growth over

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the first 20 hours. In vivo, they enhance optic nerve regeneration 5-fold over controls. Statins stimulate motor axon growth by inhibiting HMG-CoA reductase and thereby downstream protein prenylation. Since HMG-CoA reductase is expressed in motor neurons at high levels throughout embryogenesis and postnatal life, it may potentially play a role in inhibiting axonal growth in multiple contexts. Overall, these studies show that specific post-translational mechanisms govern motor axon length and regeneration and suggest that they may be potential therapeutic targets in situations requiring stimulation of axonal growth. More generally, this study demonstrates the value of differentiated derivatives of stem cells for biological screens involving large numbers of otherwise inaccessible cell types. This work was funded by New York State Spinal Cord Injury Research Board, P2ALS, Project A.L.S., NYSTEM, Helmsley Foundation and NINDS.

2D VASCULAR CONVERSION OF HUMAN PLURIPOTENT STEM CELLS BY A NOVEL GSK3B INHIBITOR - A CELLULAR MODEL TO EVALUATE ENDOTHELIAL DYSFUNCTION IN TYPE 2 DIABETES VASCULAR COMPLICATIONS

Iacone, Roberto[1], Patsch, Christoph[1], Urich, Eduard[1], Thoma, Eva Christina[1], Oi Lan Lui, Kathy[2], Christensen, Klaus[1], Heckel, Tobias[1], Certa, Ulrich[1], Freskard, Per-Ola[1], Huang, Paul[3], Kling, Dorothee[1], Chien, Ken[3], Graf, Martin[1]

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Endothelial dysfunction has been documented in Type 2 Diabetes (T2D) associated with macro-vascular complications such as Coronary Artery Disease (CAD). The ability to generate induced pluripotent stem cells (iPSCs) from T2D patients with accelerated progression to CAD represents a unique opportunity to derive pathophysiological relevant endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) for disease modeling and drug discovery. Herewith, we describe the generation of T2D patient-specific iPSCs and the development of a 2D protocol to directly convert them into vascular cells employing a novel GSK3β inhibitor. The newly identified GSK3β inhibitor shows a superior performance in terms of WNT signaling activation and allows the generation of large numbers of VE-Cadherin+ cells with high efficiency (up to 85%) or SMA+ cells in only 6 days. Conceptually, the protocol can be divided into three steps: (i) single-cell attachment of PSCs; (ii) priming towards an intermediate mesodermal progenitor state and (iii) induction into ECs or VSMCs under chemically defined conditions. Using expression profiling we describe the dynamic gene expression pattern orchestrating the conversion into the mesoderm intermediate and consequentially into the ECs or VSMCs identity. Functional characterization of the stem-cell derived ECs, which have been purified by magnetic activated cell sorting (MACS) confirm an endothelial phenotype: They form tube-like structures in a matrigel angiogenesis assay, respond to anti-angiogenic compounds, take up acetylated-LDL, and upregulate adhesion molecules in response to pathophysiological stressors. Moreover, the stem-cell derived vascular unit has been characterized for in vivo engraftment into immunodeficient mice models.Thus, functional patient-specific ECs in combination with disease modeling assays may represent a powerful tool to identify novel targets controlling vascular biology and therapeutic approaches for diseases with underlying endothelial dysfunction.

A REGENERATIVE MEDICINE APPROACH TO DIABETES AND OBESITY - DIFFERENTIATING ADIPOSE AND SKELETAL MUSCLE PROGENITOR CELLS INTO FUNCTIONAL BROWN ADIPOSE TISSUE

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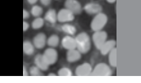
Due to the increasing epidemic of obesity and metabolic diseases, there has been significant interest in targeting adipose tissue. Obesity occurs when there is a long-term disregulation between energy intake and energy expenditure, leading to an increase in white fat deposits where the excess energy is stored. There is another type of adipose tissue, brown fat, which uses energy in a process known as thermogenesis. Brown fat is characterized by expression of uncoupling protein 1 (UCP-1) in the mitochondrial membranes and provides the major means of energy dissipation in the form of heat. The energy consumption properties of brown fat make both pushing the differentiation of adipose derived stem cells to brown adipose as well as increasing activity of the existing brown adipose tissue attractive therapeutic targets. To complicate the issue, there are two distinct developmental origins of brown fat, each with different properties. One subset is derived from an adipogenic precursor in fat depots and is responsive to β-adrenergic stimuli. The other cell type is found in the skeletal muscle and arises from a myf5+ myogenic precursor (myoblasts), appearing to be dependent on the transcriptional regulator PRDM16. Here, we describe the development and characterization of cellular models of brown adipose differentiation from both developmental origins in order to develop a robust assay for compounds that can selectively target the adipose tissue. Adipose derived stem cells (ADSCs) or myoblasts were plated in 384-well plates and maintained in differentiation media for 1-2 weeks after treatment with several small molecule screening sets, including compounds drawn from the literature that have been shown to influence brown adipose differentiation. To fully assess brown adipose differentiation, the cells were stained with UCP-1, a mitochondrial marker, and a neutral lipid marker, with cell nuclei counterstained with Hoescht 33342. In ADSCs, we demonstrated a significant increase in UCP-1 and mitochondria expression in the presence of different thiazolidinedione PPARγ-agonists including pioglitazone and rosiglitazone compared to cells treated with differentiation media alone. In contrast, ADSCs not exposed to differentiation media showed no increase in UCP-1 expression. In skeletal myoblasts, all of the PPARγ-agonists also induced expression of UCP-1, though not to the same levels as in ADSCs. In both cell types, there were cells that were UCP-1/mitochondrial + but did not express neutral lipid, which could be a separate population of brown adipose cells or could be precursors to fully differentiated cells. We demonstrate the application of multiparametric high-content technology to monitor differentiation of cells to brown fat following small molecule intervention. Furthermore, we apply this approach to cells from distinct developmental origins in order that compounds with selective effects are identified. These assays have potential to be used as the basis for regenerative medicine screens looking to identify modulators of adipose and skeletal muscle tissue for indications such as obesity and diabetes.



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IMPROVED GENERATION OF HUMAN HEMATOPOIETIC STEM/PROGENITOR CELLS FROM ES AND PATIENT DERIVED IPS CELLS USING SMALL MOLECULES AND FACTORS

Rönn, Roger E., Moraghebi, Roksana, Guibentif, Carolina, Woods, Niels-Bjarne

Gene Therapy and Molecular Medicine, Lund University, Lund, Sweden

Hematopoietic stem and progenitor cells generated from patient derived induced pluripotent stem (iPS) cells could provide an unlimited supply of HLA matched transplantable cells for the treatment of both hematological disorders and malignancies. The goal of this project is to identify novel pathways involved in hematopoietic stem and progenitor cell generation and expansion from human ES and iPS cells. By using a small molecule compound library with our optimized ES/iPS-2-Blood differentiation protocol, we have identified several novel molecular pathways that enhance the generation of hematopoietic progenitors cells (CD45/43+CD34+) and cells with an adult hematopoietic stem cell phenotype (CD45/43+CD34+CD38-CD90+CD45RA-). By inhibiting the cellular synthesis of retinoic acid (RA) and subsequent retinoid signaling we significantly increased the output of cells generated possessing an adult HSC surface phenotype by 2.7-fold (p-value: 0,024, n=7) compared to DMSO carrier controls, and increased clonogenic progenitors (CFUs) by 2.5-fold (p-value: 0,065, n=7). This improvement is consistent when using both a human ES line (Hues3) and a human iPS-line (RB9-CB1 generated from cord-blood derived endothelial cells). We also increased total blood cell output by increasing the frequency of embryoid bodies (EB) that successfully give rise to blood generating colonies. Conversely, and in support of our findings, directly adding RA was found to severely decrease the blood generation efficiency of our protocol. When comparing the colony forming potential of sorted cells gated on umbilical cord blood (CB), our system enabled the generation of CFU's at efficiencies on par with that of CB (preliminary data). Following plating equal numbers of sorted CD45/43+CD34+ blood progenitors, we saw 79 CFU/500 cells for the CB control, 65 CFU/500 cells from iPS + retinoic acid signaling inhibition, and 23 CFU/500 cells from iPS + DMSO control. The hematopoietic cells generated with our ES/iPS-2-Blood system also possess lymphoid lineage potential following plating of CD45/43+CD34+ cells into an in vitro based lymphoid assay, generating cells expressing markers of T-cells, B-cells, and NK-cells. We are currently performing transplantation experiments to assess the repopulating potential of these cells. We are also investigating additional factors that have demonstrated significant increases in hematopoietic stem/ progenitor cell outputs in preliminary experiments, with one combination of factors showing more than 12-fold increase in CFU-counts. We are further investigating the molecular mechanisms of these different factors for hematopoietic stem and progenitor cell generation with the aim to advance iPS cell technology towards treatments for hematological diseases and malignancies.

Concurrent Session IVE — Stem Cells and Cancer

Saturday, June 16, 2012, 1:30 pm - 3:15 pm

ANTIBODY-BASED TARGETING OF CANCER STEM CELLS IN HEMATOLOGICAL MALIGNANCIES

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Key obstacles to more effective treatment of cancer are a lack of durable responses and a high frequency of recurrent and/ or metastatic disease that is resistant to standard therapies. Evidence suggests that these problems are driven by lack of effective eradication of a subset of cells present in tumors that are referred to as tumor initiating or cancer stem cells (CSCs). These are characterized as having tumor initiating and metastatic potential as well as dividing asymmetrically, with one daughter cell having high proliferative capacity, generating the "bulk" of the tumor burden and metastases in the host and being responsive to antiproliferative agents, while the other daughter cell is quiescent, thereby being resistant to most standard treatments. We have isolated and characterized CSCs from AML patients and identified cell surface targets selectively expressed on AML CSCs and not their normal hematopoietic stem cell counterparts. A series of potent, specific, mononoclonal antibodies (mAbs) have been generated and screened against these AML CSC targets. Select mAb clones were characterized for patient binding properties and also evaluated in in vitro and in vivo in cell based and tumor xenograft models. Promising primary AML patient binding profiles as well as robust antitumor activity have been demonstrated in vitro and in vivo. Specifically, select clones have a high degree of specific binding to AML primary patient samples (>90%), and also specifically kill primary patient AML cells via a complement-dependent cytotoxicity (CDC) mechanism. Additionally, in multiple subcutaneous and orthotopic AML tumor xenograft models, significant antitumor activity has been demonstrated with tumor growth inhibition effects ranging from 80->90% noted for select mAb clones. Because a key driver of CSC resistance is their quiescence, we also evaluated the in vitro antitumor effects of our mAbs on quiescent AML cells in vitro and showed that quiescent AML cells are also susceptible to CDC-based killing. In summary, the patient binding profiles, in vitro and in vivo efficacy against proliferating and quiescent AML cells suggests the mAbs and their targets are promising novel therapeutic approaches for the treatment of AML and have the potential for more effective eradication of AML CSCs and greater efficacy in the clinic.

CD24 MARKS METASTATIC LUNG TUMOR-PROPAGATING CELLS WITH ACTIVE HIPPO SIGNALING

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Tumor-propagating cells (TPCs) contribute to cancer progression and metastasis. Our earlier studies found that the marker Sca-1 enriched for TPCs in murine lung tumors driven by oncogenic Kras and p53 loss. Here, we used this mouse model of lung cancer to probe the connection between tumor-propagating ability and metastatic capacity. To identify cell surface markers and further characterize TPCs, we conducted microarray analysis of Sca-1+ and Sca-1- tumor cells and found the marker CD24 was upregu-

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lated 3-fold (p=0.0057) in Sca-1+ cells. CD24 has been implicated in cancer progression, yet its role in metastatic lung cancer is lacking. Selection of CD24-expressing cells from primary murine lung tumors enriched for tumor-propagating activity and metastatic capacity in orthotopic transplantations. All recipient mice developed at least one lung tumor after transplantation of 10,000 CD24+ (13/13) or CD24- cells (14/14). 50% of recipients of 1,000 CD24+ cells developed tumors (3/6), whereas recipients of 1,000 CD24- cells did not exhibit lesions (0/3). The average number of tumors (14±6 vs 4.8±4.2, p=0.0019) and the average percentage of lung area filled with tumor (58%±23 vs 19%±20%, p=0.0017) were significantly greater in recipients of CD24+ vs. CD24- cells. Strikingly, there was an increase in metastases in recipients of CD24+ (11/12) vs. CD24- cells (6/12, p =0.03). CD24+ recipients developed metastatic lesions in the chest wall (11/11), local lymph nodes (4/11), and distant sites (4/11). CD24- recipients also had small metastases in the chest cavity (6/6) yet had limited development of local lymph node metastases (1/6) and no distant metastases (0/6). This demonstrated that CD24 prospectively marked metastatic tumor cells. In lung cancer cell lines, knockdown of CD24 decreased migration and reduced tumorigenicity post-transplantation. In transwell migration assays, shCD24 lines had reduced migration compared to shGFP cells (p<0.001). While shCD24 cells were capable of subcutaneous tumor formation, they yielded smaller tumors 3 weeks post-transplant. Orthotopic injections demonstrated that shCD24 cells were devoid of TPC activity compared to shGFP controls (tumors in 0/4 vs. 5/6 recipients, p= 0.02). These findings strongly suggest that CD24 plays a functional role in TPC activity and metastasis. In an effort to evaluate other genetic programs that could participate in metastasis, we performed gene set enrichment analysis on the Kras;p53 TPC gene expression data. Two upstream regulators of the Hippo signaling pathway, Nf2 and Lats1, were part of the core enrichment in many of the top gene sets. Knockdown of the Hippo pathway mediators Yap and Taz decreased migration of murine lung cancer cell lines, (3.8-10-fold compared to shGFP, p0.05) supporting a role for this pathway in lung cancer progression. These Hippo knockdown cell lines were injected subcutaneously to assess metastatic capacity in vivo and tumors were removed at equivalent sizes to allow for the development of metastatic lesions in lungs of recipient mice. Metastases were less frequent in recipients of shYap1 or shTaz cells compared to shGFP. Our data suggest a novel role for CD24 and the Hippo pathway in regulating tumor-propagating cells and metastasis. Our characterization of aggressive TPCs within lung tumors has defined a subset of cells involved in metastatic lung cancer and the interruption of CD24 and Hippo signaling pathways may lead to new therapeutics for metastasis.

DISTINCT SELF-RENEWING STEM-LIKE CELL POPULATIONS INITIATE AND MAINTAIN AGGRESSIVE HUMAN PROSTATE CANCER

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The stepwise progression to cancer begins with the acquisition of genetic damage in a normal cell, termed the cell of origin. After the disease is initiated, established tumors are often sustained by self-renewing cancer stem-like cells. However, the relationship between the cells of origin that initiate tumorigenesis and the cancer stem cells that maintain the disease is poorly defined in the majority of human cancers, including prostate cancer. The human prostate is predominantly comprised of two cell types, p63+ CD49f-hi basal stem-like cells that can self-renew and p63- Keratin 8/18+ CD49f-lo luminal secretory cells. The predominant histological subtype of prostate cancer, termed acinar-type adenocarcinoma, is comprised of p63- K8/18+ luminal cells and lacks p63+ basal cells. We have previously established that transformation of naïve benign CD49f-hi basal cells from the human prostate can initiate luminal/acinar-type adenocarcinoma. A fundamental issue is whether the stem-like cells of origin that initiate prostate cancer are continually required to sustain the disease or if luminal-like cancer cells can self-renew and propagate tumors in the absence of basal-like cells. Using a genetically-defined primary human prostate direct transformation assay, we identified the phenotype of cells capable of initiating and propagating the aggressive subtype of human prostate cancer. Freshly-isolated naïve benign human prostate epithelial cells taken from patients undergoing radical prostatectomy were sorted into basal and luminal populations and engineered to express the oncogenes Myc and AKT through lentiviral transduction. Transduced epithelial cells were implanted subcutaneously in immune-deficient mice. Only CD49f-hi basal cells were capable of responding to both oncogenes to initiate aggressive primary human prostate tumors in as little as 6 weeks. Importantly, we found that while CD49f-hi basal cells are required for the initiation of aggressive prostate cancer, they are not required for tumor maintenance. Cancer stem-like cells with the phenotype of CD49f-lo p63- K8/18+ luminal-like cells isolated from primary tumors have the capacity to self-renew and serially propagate adenocarcinoma upon transplantation in vivo. These data indicate that distinct self-renewing stem-like cell populations are driving discrete phases of the disease.

ROLES OF RING1A/B IN STEM CELL POTENTIAL OF MOZ AND OTHER ACUTE MYELOID LEUKEMIAS

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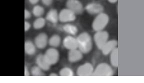
Leukemia and other cancers possess self-renewing stem cells that help maintain cancer. Chromosomal translocations are often involved in the development of human acute myeloid leukemia (AML). The monocytic leukemia zinc finger (MOZ) gene is one of the targets of such translocations. While MOZ is essential for the self-renewal of hematopoietic stem cells, the leukemia associated MOZ-fusion proteins enable the transformation of non-self-renewing myeloid progenitors into leukemia stem cells.
Ring1A and Ring1B are catalytic subunits of the polycomb-group repressive complex 1 (PRC1) containing Bmi1, and PRC1 plays an important role in the maintenance of stem cells. However, the functional role of Ring1A/B in the regulation of leukemia stem cells remains to be unknown. Using Ring1A-null and Ring1B-conditional deficient mice, we performed colony formation assay and transplantation assay. Firstly, c-kit+ progenitors isolated from mice bone marrow were retrovirally transduced with MOZ-TIF2 and were subjected to colony formation assay. Ring1B was conditionally deleted by 4-hydroxytamoxifen treatment in vitro. While Ring1A-deleted cells continuously produced large compact colonies, Ring1A/B-deleted cells formed loose colonies containing high proportion of differentiated cells such as macrophages, and finally lost their ability to self-renew. Ring1A/B were also required for continuous colony forming ability enabled by other AML-associated fusions such as MLL-AF10, AML1-ETO, and PML-RARα.



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Furthermore, we transplanted MOZ-TIF2- and MLL-AF10-induced AML cells into recipient mice, and administered intraperitoneally tamoxifen to ablate Ring1B of transplanted cells. While all the recipients transplanted with Ring1A-deleted cells developed AML, Ring1A/B-deleted cells were incapable of initiating leukemia in recipient mice. To clarify the mechanism of stemness regulation in AML stem cells by PRC1, we compared gene expression profiles of Ring1A-deleted and Ring1A/B-deleted MOZ-TIF2-induced AML cells. As expected, Ink4a/Arf, a known major target of PRC1 involved in stem cell functions, was derepressed by deletion of Ring1A/B. Although deletion of Ink4a/Arf in Ring1A/B-deficient AML cells partially restored colony formation ability, it was not substantial to initiate leukemia in recipient mice. Among several target genes which were derepressed by deletion of Ring1A/B, we focused on Glis2, known to be required for neuronal differentiation and kidney morphogenesis. Enforced expression of Glis2 in MOZ-TIF2-induced AML cells stimulated differentiation of AML progenitors into macrophages. On the other hand, knock-down of Glis2 blocked cell differentiation and restored the colony morphology despite of the absence of Ring1A/B. Collectively, our data demonstrate that Ring 1A/B play crucial roles in the maintenance of AML stem cells through repression of Glis2, which strongly promotes differentiation of leukemia stem cells.

IDENTIFICATION OF RELATIVELY QUIESCENT SOX2-EXPRESSING MOUSE BRAIN TUMOUR STEM CELLS IN MEDULLOBLASTOMA IN VIVO

Vanner, Robert[1], Lee, Lilian[2], Aviv, Tzvi[2], Gallo, Marco[2], Zhu, Xueming[2], Clarke, Ian[2], Dirks, Peter B.[3]
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Brain tumour growth is dependent upon a subpopulation of self-renewing cells termed brain tumour stem cells (BTSCs). These phenotypically primitive cells engraft immunodeficient mice at low numbers and produce tumours comprised of more differentiated cells lacking self-renewal capability. In the Patched1+/- mouse model of the malignant pediatric brain tumour medulloblastoma, BTSCs can be preferentially enriched using the cell surface antigen CD15, express neural stem cell markers Sox2 and Nestin when cultured in vitro, and express multi-lineage markers in differentiation conditions. Quiescence, a transient withdrawal from the cell cycle, is a stem cell property linked to self-renewal in several somatic compartments. It is also a proposed mechanism of BTSC therapy resistance, though there has been no comprehensive study of cell cycle heterogeneity in brain tumours in vivo to determine if quiescent BTSCs exist. Quiescent cells are label-retaining in a heterogeneous population of dividing cells. Prolonged thymidine analogue labeling of Patched1+/- mice irradiated at birth effectively marks over 90% of all cells when started at postnatal day 30. After a three-week chase period, less than 1% of cells retain the initial label, indicating rapid dilution by tumour bulk. To determine if these label-retaining cells continue to divide, mice were administered a second thymidine analogue prior to sacrifice. A fraction of label-retaining cells are slowly cycling, since they acquire the second thymidine analogue. Having incorporated both markers, these relatively quiescent cells are distinct from the majority of tumour cells that have divided sufficiently to dilute out the first label and are positive solely for the second proliferative label. Immunohistochemistry reveals that the relatively quiescent double-labelled cells express neural stem cell and putative cancer stem cell markers Sox2, Nestin, BMI-1 and GFAP. These cells are mutually exclusive from neuroblast and neuron-like cells expressing NeuN, Doublecortin, and Tuj-1. A thymidine analogue pulse-chase time course shows that NeuN+ cells are a rapidly proliferating population comprising 35% of the tumour. NeuN+ cells acquire thymidine analogue label approximately three times faster than Sox2+ cells (under 5% of all tumour cells). Consistent with rapidly and slowly cycling populations of cells, respectively, 80% of NeuN+ cells dilute label by proliferation in 14 days, whereas Sox2+ cells maintain label beyond 3 weeks chase. Label-retaining cells are enriched in the CD15+ tumour-initiating compartment, and experiments are underway to determine the in vivo self-renewal potential of Sox2-expressing label-retaining cells. This is the first characterization of cell cycle heterogeneity in an in vivo brain tumour model. We found that a rare subpopulation of putative cancer stem cells expresses neural stem cell markers and are relatively quiescent compared to the rapidly dividing neuroblast-like cells comprising tumour bulk. Ongoing studies will determine the link between cancer stem cell quiescence, self-renewal, tumourigenicity and therapy resistance in medulloblastoma.

PARALLEL FUNCTIONS OF THE ENDOPLASMIC RETICULUM CHAPERONE PROTEIN GRP78 IN TUMORIGENESIS AND THE INDUCTION OF PLURIPOTENCY

Panopoulos, Athanasia D.[1], Ruiz, Sergio[1], Kelber, Jonathan A.[2], Vale, Wylie W.[3], Gray, Peter C.[3], Izpisua Belmonte, Juan Carlos[1]
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Increasing evidence supports the concept that instances of cancer recurrence may be due to a subpopulation of cells within a tumor that behave as stem cells. It is hypothesized that these cancer cell populations either contain or acquire aberrant mechanisms that were once similar to their normal stem cell counterparts. Work from our laboratory and others have demonstrated that pathways critical in oncogenesis parallel those necessary for the induction of pluripotency, suggesting that similar mechanisms regulate both processes. By therefore understanding the mechanisms that govern reprogramming, we may gain insight into the methods by which cancer cells acquire and exploit stem cell properties to thus enable more strategic targeting of these cell populations and prevent malignant relapse. Members of the TGF-beta superfamily (e.g. activin/Nodal), which regulate many important normal cellular responses including cell growth and differentiation, have also been shown to promote tumorigenesis. Cripto, a regulator of TGF-beta superfamily ligand signaling expressed on human embryonic stem cells (hESCs), is overexpressed in many types of cancer, and has also been shown to promote tumor growth and metastasis. Previous work has demonstrated that Cripto regulates TGF-beta function in tumor cell lines by forming a complex with GRP78, a protein generally restricted to the endoplasmic reticulum in normal tissues, but expressed at the cell surface in many types of tumors. Targeting GRP78 in mouse models suppresses tumor growth, and cell surface GRP78 has been shown to be a molecular target on human tumor samples. Although these studies have suggested an important role for GRP78 in promoting tumorigenesis, the mechanisms are not yet fully understood. We have discovered that GRP78 expression is induced during reprogramming, and becomes localized to the cell surface in pluripotent cells, where it also co-localizes with Cripto. Overexpressing GRP78 induces reprogramming efficiency when

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transduced with the factors Oct4, Sox2, Klf-4 and c-Myc (OSKM). Interestingly, priming cells by overexpressing GRP78 levels before OSKM transduction resulted in a more pronounced increase in reprogramming efficiency (~6-fold). We further demonstrate that a GRP78 antibody, that disrupts cell surface GRP78/Cripto binding and Cripto-mediated TGF-beta superfamily signaling, inhibits reprogramming. In addition to the function of GRP78/Cripto in regulating TGF-beta signaling, previous findings have shown that GRP78/Cripto on the cell surface of hematopoietic stem cells induces a glycolysis-biased metabolism. We demonstrate that somatic cells convert from an oxidative to a glycolytic state when they are reprogrammed, that overexpression of GRP78 shifts somatic cells towards a more glycolytic metabolism, and that manipulating these bioenergetic changes can affect reprogramming. These combined findings suggest that GRP78 may be localized to the cell surface during reprogramming where it functions to inhibit TGF-beta signaling and stimulate glycolysis. These stem cell functions of GRP78 parallel many of the functions ascribed to GRP78 on the surface of cancer cells, and thus provide insight into understanding how cancer cells acquire and exploit stem cell properties.

Plenary VII — Stem Cells and Fate Control

Supported by Salk Institute for Biological Studies

Saturday, June 16, 2012, 4:00 pm - 6:30 pm

LGR5 STEM CELLS IN SELF-RENEWAL AND CANCER

Clevers, Hans
Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences & University Medical Centre Utrecht, Utrecht, Netherlands

The intestinal epithelium is the most rapidly self-renewing tissue in adult mammals. We originally defined Lgr5 as a Wnt target gene, transcribed in colon cancer cells. Two knock-in alleles revealed exclusive expression of Lgr5 in cycling, columnar cells at the crypt base. Using an inducible Cre knock-in allele and the Rosa26-LacZ reporter strain, lineage tracing experiments were performed in adult mice. The Lgr5+ve crypt base columnar cells (CBC) generated all epithelial lineages throughout life, implying that it represents the stem cell of the small intestine and colon. Similar obserations were made in hair follicles and stomach epithelium. Single sorted Lgr5+ve stem cells can initiate ever-expanding crypt-villus organoids in 3D culture. Tracing experiments indicate that the Lgr5+ve stem cell hierarchy is maintained in these organoids. We conclude that intestinal crypt-villus units are self-organizing structures, which can be built from a single stem cell in the absence of a non-epithelial cellular niche. The same technology has now been developed for the Lgr5+ve stomach stem cells. Intestinal cancer is initiated by Wnt pathway-activating mutations in genes such as APC. As in most cancers, the cell of origin has remained elusive. Deletion of APC in stem cells, but not in other crypt cells results in progressively growing neoplasia, identifying the stem cell as the cell-of-origin of adenomas. Moreover, a stem cell/progenitor cell hierarchy is maintained in early stem cell-derived adenomas, lending support to the "cancer stem cell"-concept. Fate mapping of individual crypt stem cells using a multicolor Cre-reporter revealed that, as a population, Lgr5 stem cells persist life-long, yet crypts drift toward clonality within a period of 1-6 months. Lgr5 cell divisions occur symmetrically. The cellular dynamics are consistent with a model in which the resident stem cells double their numbers each day and stochastically adopt stem or TA fates after cell division. Lgr5 stem cells are interspersed between terminally differentiated Paneth cells that are known to produce bactericidal products. We find that Paneth cells are CD24+ and express EGF, TGF-a, Wnt3 and the Notch ligand Dll4, all essential signals for stem-cell maintenance in culture. Co-culturing of sorted stem cells with Paneth cells dramatically improves organoid formation. This Paneth cell requirement can be substituted by a pulse of exogenous Wnt. Genetic removal of Paneth cells in vivo results in the concomitantloss of Lgr5 stem cells. In colon crypts, CD24+ cells residing between Lgr5 stem cells may represent the Paneth cell equivalents. We conclude that Lgr5 stem cells compete for essential niche signals provided by a specialized daughter cell, the Paneth cell.

ANALYSIS OF NEURAL STEM CELLS IN THE ADULT BRAIN, ONE AT A TIME

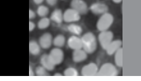
Song, Hongjun
Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Neurogenesis and gliogenesis continue in discrete regions of the adult mammalian brain. A fundamental question in the neural stem cell field is whether cell genesis occurs from distinct lineage-restricted progenitors or from self-renewing and multipotent neural stem cells in the adult brain. We have developed genetic marking strategies for lineage-tracing of individual neural precursors in the adult mouse brain. Using this clonal approach, we have shown that individual nestin-expressing radial glia-like precursors in the adult dentate gyrus are normally quiescent and, once activated, they can continue to self-renew, give rise to both neurons and glia, and are maintained for an extended period of time. They also exhibit different modes of self-renewal, including both symmetric self-renewal to expand the stem cell pool and asymmetric self-renewal to generate neurons or astrocytes. We have been exploring the underlying molecular mechanisms regulating precursor maintenance, activation, self-renewal mode and fate specification in vivo, and have identified several intrinsic regulators. We are also interested in the identification of niche mechanisms regulating adult neural stem cells. Using optogenetic approaches to manipulate specific neuronal subtypes, we have uncovered specific neuronal circuitry mechanisms regulating quiescent neural stem cell behaviors in vivo. Finally, we are developing new approaches using different Cre-drivers and reporter lines to address the question about neural precursor heterogeneity in the adult brain.

REPROGRAMMING GERM CELLS INTO NEURONS

Hobert, Oliver
Columbia University, HHMI, New York, NY, USA

The ability of transcription factors to directly reprogram the identity of cell types is usually restricted and defined by cellular context. We show here that through ectopic expression of single C.elegans transcription factors, the identity of mitotic germ cells can be directly converted into that of specific neuron types (glutamatergic, cholinergic or GABAergic). This reprogramming event requires the removal of the histone chaperone LIN-53/RbAp48, a component of several histone remodeling and modifying complexes, and can be mimicked by chemical inhibition of histone deacetylases. Our findings illustrate the susceptibility specifically of germ cells to be directly converted into individual, terminally differentiated neuron types, demonstrate that a specific chromatin factor provides a barrier for cellular reprogramming and suggest drug-mediated strategies to generate cell types for in vitro studies and therapeutic replacement approaches.



Detailed Program and Abstracts — Saturday, June 16

Anne McLaren Memorial Lecture

INTEGRATING INTRINSIC AND EXTRINSIC SIGNALS TO REGULATE EPIDERMAL STEM CELL FATE

Watt, Fiona M.
King's College London, Cambridge, United Kingdom

Markers that are co-expressed by stem cells on a population basis can exhibit heterogeneous expression at single-cell resolution, reflecting transcriptional noise or covert functional differences. We previously reported that human interfollicular epidermis is maintained by stem cells that express high levels of β1 integrin, Delta-like 1 (DLL1; Notch ligand) and the EGFR antagonist, LRIG1. However, we observe cell-to-cell variation in the relative abundance of DLL1 and LRIG1 transcripts and single-cell global gene expression profiling shows that stem cells fall into two clusters delineated by DLL1 expression. There is a hierarchical relationship between the two stem cell subtypes, with DLL1+ cells giving rise to DLL1- cells. DLL1+ cells exhibit elevated expression of genes associated with endocytosis, integrin-mediated adhesion and receptor tyrosine kinase, but not Notch, signalling. By examining the effects of overexpressing DLL1 and LRIG1 we have obtained evidence that the epidermal stem cell heterogeneity revealed by gene expression profiling results in altered niche responsiveness.

Innovation Showcases

Thursday, June 14 11:45 a.m - 12:15 p.m.

CELL SURFACE MARKER DISCOVERY USING ANTIBODY LIBRARIES AND HIGH-THROUGHPUT FLOW CYTOMETRY

Christian Carson, Ph.D.
BD Biosciences
Rooms 501-502

This tutorial will demonstrate how surface marker screening can be used to identify unique differentially expressed markers of stem cells and their derivatives. An analysis tool and a representative set of applications will be discussed:

- Identification of unique surface signatures for the isolation of hESC/hiPSC-derived neural cell types
- Immunophenotyping of brain tumor cells from multiple patients simultaneously using fluorescent cell barcoding
- Analysis of markers expressed on adipose-derived stem and regenerative cells (ADRCs)

Thursday, June 14 11:45 a.m - 12:15 p.m.

PREDICTIVE ASSAYS FOR HIGH THROUGHPUT ASSESSMENT OF DRUG TOXICITY USING IPSC DERIVED CELL MODELS

Oksana Sirenko, Ph.D. and Evan F Cromwell, Ph.D.
Molecular Devices LLC
Rooms 411-412

Stem-cell derived models are becoming increasingly important for modeling diseases and predictive toxicology. Next-generation high content, high throughput tools for imaging and electrophysiology offer contemporary and automated techniques for evaluating this complex biology. We will present examples of novel multi-parametric assays for testing the effects of compounds on the rate of beating cardiomyocytes, hepatotoxicity, and neuronal development. We also will highlight innovative software that provides step-by-step assistance for designing customized, reusable, and distributable analysis algorithms.

Thursday, June 14 11:45 a.m - 12:15 p.m.

LARGE SCALE ANALYSES OF MOLECULAR SIGNATURES AND BEHAVIORS OF HUMAN PLURIPOTENT STEM CELLS

Michiyo Koyanagi-Aoi PhD, Center for iPS Cell Research and Application (CiRA),
Kyoto University
Qiagen
Rooms 301-304

There are some variations in molecular signatures and differentiation propensity among clones of human embryonic stem (hES) cells and induced pluripotent stem (hiPS) cells. We examined the global gene/miRNA expression and DNA methylation status of 10 hES and 49 hiPS cells and evaluated the correlation between these molecular signatures and cellular propensities. Of them, we report the results of pyrosequencing, a very useful method for checking DNA methylation status of many clones quickly and easily.

Thursday, June 14 11:45 a.m - 12:15 p.m.

LARGE PARTICLE FLOW CYTOMETRY FOR CELL CLUSTERS (EBS, SPHEROIDS) AND MICROCARRIES OF 3D CELL CULTURES

Rock Pulak, PhD.
Union Biometrica Inc.
Room 503

Cells growing in clusters communicate with each other and behave differently than cells grown as monolayers or in suspension. These interactions are likely to be important for proper function. Union Biometrica Large Particle Flow Cytometers automate the analysis and dispensing of objects too large (10-1500µm) or too fragile for traditional cytometers, including those studied by stem cell researchers such as embryoid bodies, neurospheres, and microcarriers of 3D cell cultures.

Thursday, June 14 12:30 p.m. - 1 p.m.

ISOLATION AND ANALYSIS OF HESCS, HIPSCS AND THEIR ECTODERM AND ENDODERM DERIVATIVES BY FLOW CYTOMETRY

Nil Emre, Ph.D.
BD Biosciences
Rooms 501-502

This tutorial will demonstrate how flow cytometry enables the stem cell research workflow--from Isolation to Analysis. Applications discussed will include:

- Efficient cell sorting of hESCs
- Enrichment of hiPSCs during reprogramming processes
- Analysis of neural differentiation of hESCs using intracellular flow cytometry
- Analysis of endoderm differentiation of hESCs using intracellular flow cytometry

Innovation Showcases

Thursday, June 14 12:30 p.m. - 1 p.m.

LAMININS- EXTRACELLULAR MODULATORS OF STEM CELLS AND CELL LINEAGES

Karl Tryggvason
BioLamina
Rooms 301-304

Pluripotent human ES and iPS cells have *in vitro* been difficult to maintain, as has more differentiated cellular phenotypes. A possible solution is usage of tissue-specific laminin isoforms that have major roles of *in vivo* cell niches. Laminin-521, expressed by hESCs in the ICM, enables hESC line derivation and self-renewal completely defined, feeder-free and xeno-free. Laminin-521 allows single-cell hESCs survival without ROCK. Other laminins support survival and maintained functionality of more mature cell types.

Thursday, June 14 12:30 p.m. - 1 p.m.

ADVANCEMENT IN IN VITRO MODELING USING IPSC-DERIVED CELLS

Chris Parker
Cellular Dynamics International
Rooms 411-412

iPSC technology offers unprecedented access to human biology and opportunities to significantly advance the fields of drug development, disease research, and regenerative medicine. CDI enables these opportunities by providing unlimited access to consistent, industrial quantities of highly pure (>95%) human cells that recapitulate normal human biology. This tutorial will highlight recent studies demonstrating the benefits of using CDI's iCell® products (cardiomyocytes, neurons, hepatocytes) and MyCell™ Custom Services for disease modeling, drug screening, and safety testing.

Thursday, June 14 12:30 p.m. - 1 p.m.

ADVANCES IN REPROGRAMMING AND NEURAL INDUCTION OF HUMAN iPS CELLS

Vi Chu, PhD
Merck Millipore
Room 503

Induced pluripotent stem cell (iPSC) derived neural cells provide powerful tools to help model human neurodegenerative diseases. This presentation will focus on practical topics including (1) how to efficiently generate, identify & characterize iPSCs, (2) how to excise the transgene using a recombinant TAT-CRE protein, (3) how to direct the differentiation of iPSCs to neural progenitor cells, and (4) how to preferentially differentiate iPS-derived neural progenitor cells to enriched populations of neurons, astrocytes and oligodendrocytes.

Friday, June 15 11:45 a.m. - 12:15 p.m.

A NOVEL ECM MIMETIC SURFACE FOR STEM CELL EXPANSION: ANALYSIS OF HUMAN MESENCHYMAL STEM CELLS UNDER XENO-FREE CONDITIONS

Marshall Kosovsky, Ph.D.
BD Biosciences
Rooms 501-502

Stem cell research is progressing towards the development of new cell-based therapies. To address requirements for defined environments, we developed a novel synthetic surface for expansion and differentiation of human mesenchymal stem cells (hMSCs). BD PureCoat™ ECM Mimetic Fibronectin Peptide (FN mimetic surface) is manufactured with an RGD-based peptide that supports attachment of α5-integrin positive cells. We demonstrate that hMSCs exhibit comparable properties when cultured on FN mimetic or ECM protein-coated surfaces in xeno-free media.

Friday, June 15 11:45 a.m. - 12:15 p.m.

LEAVING NOTHING TO CHANCE: INCREASING PRECISION IN MAKING AND MODIFYING IPSCS

Dr. Mark Tomoshima, *SKI Stem Cell Research Facility, Center for Stem Cell Biology and Center for Cell Engineering, Developmental Biology Program, Sloan-Kettering Institute*
Life Technologies
Rooms 411-412

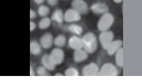
Induced pluripotent stem cells (iPSCs) offer a new cellular window into disease and therapy. Traditional reprogramming and transgenic methods can disrupt gene function through random DNA insertion. My lab has evolved our methods to reprogram cells using Sendai viral vectors that do not alter the genome. We now also target transgene insertion through the use of zinc finger nucleases. These two technologies allow the production of engineered iPSC lines that lack randomly inserted DNA.

Friday, June 15 11:45 a.m. - 12:15 p.m.

STREAMLINING THE IPS CELL WORKFLOW FROM REPROGRAMMING TO DIFFERENTIATION

Sebastian Knöbel
Miltenyi Biotec GmbH
Room 503

Scarless reprogramming of human fibroblasts into iPSCs by repeated transfection with mRNA paves the way for translational use of iPS cells. Here, we introduce tools for stem cell separation, culture, and analysis that streamline the whole iPSC workflow from fibroblast isolation to final differentiation. Straightforward magnetic cell sorting improves the generation, maintenance and differentiation of iPSC lines, guarantees predictable and reproducible results and makes the whole workflow amenable to automation.



Innovation Showcases

Friday, June 15 11:45 a.m. - 12:15 p.m.

EFFICIENT DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO DEFINITIVE ENDODERM USING THE FULLY DEFINED, XENO-FREE STEMDIFF™ DEFINITIVE ENDODERM KIT

Dr. Michael Riedel
STEMCELL Technologies Inc.
Rooms 301-304

This tutorial takes you through the efficient generation of multipotent definitive endoderm from human pluripotent stem cells using the STEMdiff™ Definitive Endoderm Kit. Details include the kit's method of use and experimental results obtained from multiple hESC and hiPSC lines. This tutorial may be useful to researchers studying the differentiation of hPSCs to endoderm lineages or users interested in developing serum-free protocols for endoderm lineage differentiation.

Friday, June 15 12:30 p.m. - 1 p.m.

ISOLATION AND EXPANSION OF HUMAN BONE MARROW-DERIVED MESENCHYMAL STEM CELLS AND HUMAN ADIPOSE-DERIVED STEM CELLS IN A SERUM-FREE MEDIUM

Bob E. Newman, Ph.D.
BD Biosciences
Rooms 501-502

Researchers are recognizing the need to switch to serum-free culture conditions due to safety and efficacy concerns, and issues with lot-to-lot variability with serum-containing media. While serum-free media has been shown to expand BM-MSCs and AD-SCs, isolating them under serum-free conditions has traditionally been problematic. BD has developed a serum-free media formulation, BD Mosaic™ hMSC SF, that can be used to isolate and expand MSCs from bone marrow, and isolate and expand ADSCs from fat.

Friday, June 15 12:30 p.m. - 1 p.m.

TALENs®: THE NEXT GENERATION GENE CUSTOMIZATION TOOLS FOR AFFORDABLE AND PRECISE REPROGRAMMING OF PLURIPOTENT STEM CELLS

Dr Inga Gerard
Cellectis bioresearch
Rooms 301-304

TALENs® from Cellectis bioresearch open new fields of use as powerful tools for gene knock-out and targeted manipulation of genomes. We present TAL Effector Nucleases and show how they can be used as genome customization tools; in particular their use in pluripotent stem cells and the key advantages they have over First Generation Nuclease technologies, such as Zinc Finger Nucleases. Finally, to demonstrate their affordability, we present our award-winning Custom TALEN® offer.

Friday, June 15 12:30 p.m. - 1 p.m.

EVOLUTION OF IPSC CULTURE MEDIUM

Dr. Nirupama (Rupa) Shevde
Life Technologies
Rooms 411-412

Human induced pluripotent stem cells (iPSCs) provide the potential for generating large numbers of wild-type and disease iPSC lines to advance discoveries in developmental biology and disease research. Two main challenges in expanding iPSCs are reproducibility and cost of the culture system, particularly for larger-scale studies. Here we present a defined feeder-free culture system that provides only the essential components necessary for optimal iPSC culture and expansion, improving reproducibility and differentiation potential while reducing cost.

Friday, June 15 12:30 p.m. - 1 p.m.

CELLULAR THERAPY: MANY TALK ABOUT IT — WE ENABLE IT

Stefan Miltenyi
Miltenyi Biotec GmbH
Room 503

The FDA advisory panel recommended that the CliniMACS CD34 Reagent System application be approved as a Humanitarian Use Device in the United States. The voting results were overwhelmingly positive that the CliniMACS CD34 Reagent System is "Safe" and also has "Probable Benefit" in treating AML patients undergoing allogeneic PBSC transplantation from a matched related donor. Data concerning this breakthrough and how this can impinge on other areas of cell-based therapy will be discussed.

Saturday, June 16 11:45 a.m. - 12:15 p.m.

NOVEL DISCOVERIES IN STEM CELL BIOLOGY THROUGH SINGLE-CELL GENOMIC APPLICATIONS: PART I

Brittnee Jones, Fluidigm Corporation
Reef Hardy, Ph.D. *IUPUI: Indiana University-Purdue University Indianapolis*
Fluidigm Corporation
Rooms 411-412

Single-cell gene expression profiling is unlocking unique properties in individual cells to analyze underlying heterogeneity in cell populations. Fluidigm is creating innovative microfluidic technologies to enable highly parallel gene expression analysis from samples containing only a few hundred cells. Herein we will discuss integrated workflows to isolate, prepare and detect mRNA using Dynamic Array™ IFCs on the Biomark HD™ system. Listen to our scientific collaborators about novel discoveries made with the Fluidigm technology for single-cell genomics.

Innovation Showcases

Saturday, June 16 11:45 a.m. - 12:15 p.m.

ADVANCED SERUM-FREE, XENO-FREE CULTURE SYSTEM FOR HUMAN MESENCHYMAL STEM CELLS

David Fiorentini
Biological Industries Israel Beit Haemek Ltd.
Rooms 501-502

Human mesenchymal stem cells (hMSC) are multipotent cells with the ability to differentiate into cells of connective tissue lineages, including mainly adipocytes, osteoblasts and chondrocytes. hMSC have advantages over human embryonic stem cells, due to the broad variety of their tissue sources and for being immuno-privileged. Application of hMSC in regenerative medicine and cell therapy needs the elaboration of appropriate serum-free, xeno-free culture media and auxiliary solutions.

Saturday, June 16 11:45 a.m. - 12:15 p.m.

LONG-TERM CELL CULTURE OBSERVATION SYSTEM AND ADVANCED IMAGE ANALYSIS TECHNOLOGY

Lee Rubin, *Harvard University*
Nikon Corporation
Rooms 301-304

Nikon has been provided the microscopic system over 85 years. Since 2007, the cell culture observation system (BioStation CT) has been released and well accepted in the stem cell research field. This advanced system enables to handle multiple samples automatically and maintain the stable environmental condition during the microscopic image acquisition of all the moments of reprogramming and differentiation process with image analysis technology.

Saturday, June 16 11:45 a.m. - 12:15 p.m.

STEMDIFF™ CARDIOMYOCYTE KIT FOR DIFFERENTIATION OF HUMAN IPSCS TO THE CARDIOMYOCYTE LINEAGE

Dr. Jennifer Antonchuk
STEMCELL Technologies Inc.
Room 503

Summary: This tutorial covers the differentiation of human induced pluripotent stem cells to cardiomyocytes using the STEMdiff™ Cardiomyocyte kit. We've used the kit to generate a population of synchronously beating EBs that express high levels of cardiac Troponin T. In this tutorial, we describe the kit, its method of use, and results from differentiation of multiple hiPSC lines.

Saturday, June 16 12:30 p.m. - 1 p.m.

NOVEL DISCOVERIES IN STEM CELL BIOLOGY THROUGH SINGLE-CELL GENOMIC APPLICATIONS: PART II

Brittnee Jones, *Fluidigm Corporation*
Reef Hardy, Ph.D. *IUPUI: Indiana University-Purdue University Indianapolis*
Fluidigm Corporation
Rooms 411-412

Single-cell gene expression profiling is unlocking unique properties in individual cells to analyze underlying heterogeneity in cell populations. Fluidigm is creating innovative microfluidic technologies to enable highly parallel gene expression analysis from samples containing only a few hundred cells. Herein we will discuss integrated workflows to isolate, prepare and detect mRNA using Dynamic Array™ IFCs on the Biomark HD™ system. Listen to our scientific collaborators about novel discoveries made with the Fluidigm technology for single-cell genomics.

Saturday, June 16 12:30 p.m. - 1 p.m.

DEFINING STEM CELL POPULATIONS FOR EXPERIMENTAL SUCCESS: CHARACTERIZATION AND SELECTION *IN VITRO*

Joy Aho, PhD
R&D Systems Inc.
Rooms 501-502

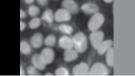
To maintain optimum quality of stem cell populations grown in vitro, it is essential to properly characterize your cells. Stem cells are defined by their marker expression and ability to renew and differentiate into multiple lineages. Here we will describe R&D Systems reagents and kits available for the characterization of stem cells through marker expression and differentiation potential. These reagents and methods will aid researchers in ensuring that they consistently use defined stem cell populations.

Saturday, June 16 12:30 p.m. - 1 p.m.

MRNA FOR INTEGRATION-FREE CELL FATE MANIPULATION

Brad Hamilton, *R&D Segment Manager - Reprogramming*
Stemgent
Room 503

Messenger RNA (mRNA) mediated cellular reprogramming has proven to be a fast and efficient method for the derivation integration-free human iPS cell lines from patient fibroblasts. This presentation will highlight significant advancements made to mRNA reprogramming as well as the more recent application of mRNA to directing cell fate and differentiation. Data presented will demonstrate the potential of this technology for high-throughput scalability and GMP compatibility.



Innovation Showcases

Saturday, June 16 12:30 – 1:00

CORNING® SYNTHEMAX® SURFACE: A NOVEL SYNTHETIC, XENO-FREE REAGENT FOR STEM CELL CULTURE IN FEEDER-FREE, SERUM-FREE ENVIRONMENT

Dr. Julien Maruotti, *Johns Hopkins University*
Corning Inc.

Rooms 301-304

Recent advancements have enabled feeder-free culture of human stem cells on vessels pre-coated with animal or human-derived biologicals, such as ECM proteins or serum. These materials are undefined, inherently variable and need time-consuming and costly testing to ensure they are pathogen-free. Corning® Life Sciences has developed Synthemax®, a synthetic, xeno-free, ready-to-use cell culture surface to address these concerns.

Poster Floorplan

NO PHOTOGRAPHY OR RECORDINGS PERMITTED IN THE POSTER AREAS

Mesenchymal Cell Lineage Analysis

STAGE

Chromatin in Stem Cells

Germline Cells

Cardiac Cells

Cancer Cells

Hematopoietic Cells

Epidermal Cells

Lung Cells

Mesenchymal Stem Cell Differentiation

Endothelial Cells/Hemangioblasts

Muscle Cells

Intestinal/Gut Cells

Pancreatic Cells

Liver Cells

Epithelial Cells (Not Skin)

Internet Cafe

Neural Cells

Eye or Retinal Cells

AV

ISSCR SALES

MAIN ENTRANCE

ABSTRACTS

EXHIBITOR HELP DESK

Poster Session Schedule

Wednesday, June 13

Exhibit Hall Open, Posters Open for Viewing: . . 3 p.m. – 8 p.m.

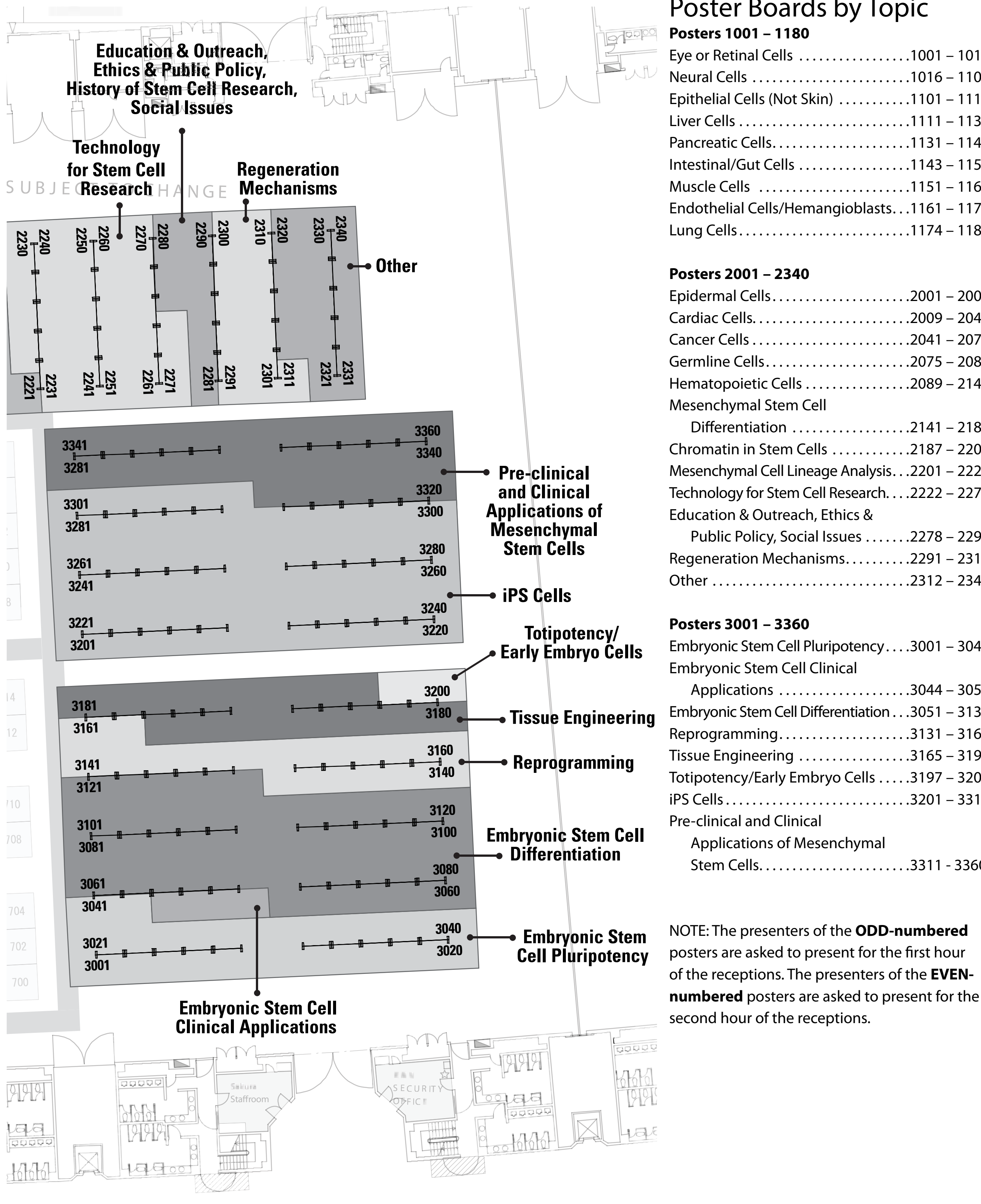
ISSCR 10th Anniversary Reception: . . . . 7 - 9 p.m.

Thursday, June 14

Exhibit Hall Open, Posters Open for Viewing: . . 11 a.m. – 8 p.m.

Poster Presentation 1/Exhibit Reception: . . . . . . 6 p.m. – 8 p.m.

Poster Floorplan



Poster Boards by Topic

Posters 1001 – 1180

Eye or Retinal Cells .... 1001 – 1015
Neural Cells .... 1016 – 1100
Epithelial Cells (Not Skin) .... 1101 – 1110
Liver Cells .... 1111 – 1130
Pancreatic Cells..................... 1131 – 1142
Intestinal/Gut Cells .... 1143 – 1150
Muscle Cells .... 1151 – 1160
Endothelial Cells/Hemangioblasts. . . 1161 – 1173
Lung Cells.......................... 1174 – 1180

Posters 2001 – 2340

Epidermal Cells..................... 2001 – 2008
Cardiac Cells........................ 2009 – 2040
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Mesenchymal Stem Cell
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Technology for Stem Cell Research. . . . 2222 – 2277
Education & Outreach, Ethics &
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NOTE: The presenters of the **ODD-numbered** posters are asked to present for the first hour of the receptions. The presenters of the **EVEN-numbered** posters are asked to present for the second hour of the receptions.

Friday, June 15

Exhibit Hall Open, Posters Open for Viewing: . . 11:00 a.m. – 8:00 p.m.
Poster Presentation 2/Exhibit Reception: . . . . . . 6:00 p.m. – 8:00 p.m.

Saturday, June 16

Exhibit Hall Open, Posters Open for Viewing:
. . . . 11:00 a.m. – 4:00 p.m.

INTRODUCING

Get connected!

The ISSCR's new online educational and interactive portal will debut at the ISSCR 10th Annual Meeting this June in Yokohama, Japan.

At its launch, ***ISSCR Connect*** will allow annual meeting registrants online access to:

- View talks they may have missed from select concurrent and plenary sessions for a limited time after the meeting
- Visit "virtual" exhibit booths and resources
- Interact with your colleagues

Following the annual meeting, the ISSCR will broaden the access to ***ISSCR Connect*** and provide additional learning opportunities, including webinars with live Q&A sessions, continued viewing of the "virtual" exhibit booths, peer-to-peer interaction and more!

Watch your inbox for more information.

The ISSCR would like to thank the Federation of Pharmaceutical Manufacturers' Associations of JAPAN for their support of the ISSCR 10th Annual Meeting

The Federation of Pharmaceutical Manufacturers' Associations of JAPAN

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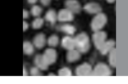

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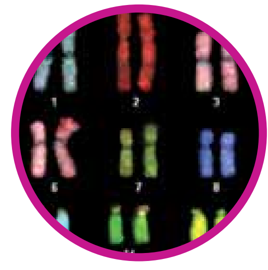

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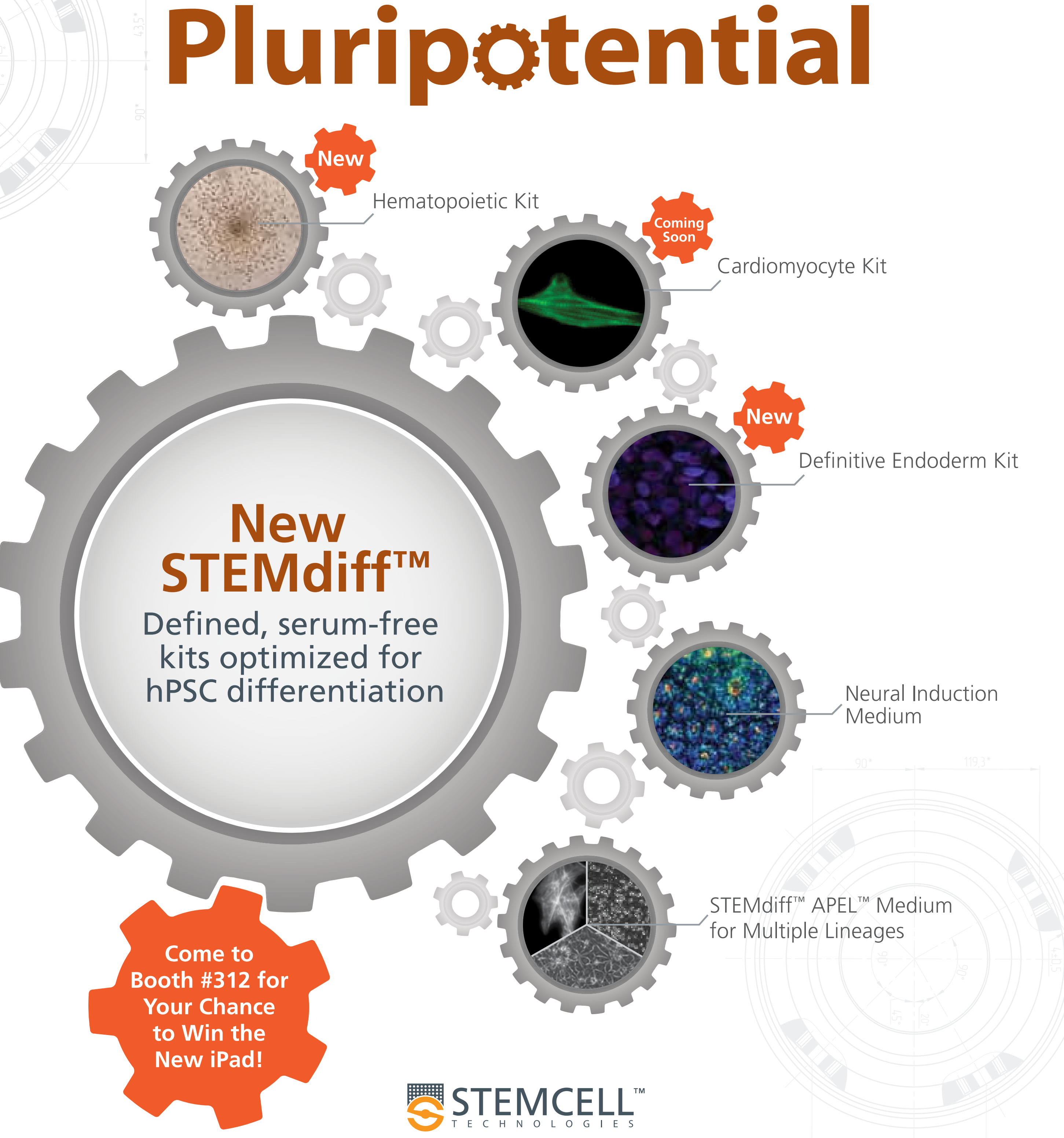

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