ISSCR

INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

FINAL PROGRAM

11TH ANNUAL MEETING

June 12-15, BOSTON, MA USA

2013

Co-Sponsored by

HSCI
HARVARD STEM CELL INSTITUTE®

ISSCR

Dear Colleagues:

On behalf of the International Society for Stem Cell Research, we are delighted to welcome you to our 11th Annual Meeting, the largest forum for stem cell and regenerative medicine professionals from around the world. It is also a pleasure to be back in Boston, a historic city that played a key role in the ISSCR's development, and which features a vibrant stem cell research, biotech, and life science research community.

The primary goal of our annual meeting is to provide you with an unparalleled array of opportunities to learn from and interact with your peers, and this year you'll have more ways to do that than ever before.

Additional Poster Session
We enjoyed a record number of submitted poster abstracts for 2013. In response to past attendees' requests for additional time to view and discuss poster presentations, we've added a third poster session, giving you a quick and efficient way to keep abreast of the latest scientific advances.

"Poster Teasers" and New "Poster Briefs"
In 2011, we introduced "poster teasers," which gave delegates time to share their findings in plenary sessions via 1-minute discussions. They've proved so popular with attendees that this year, we're adding "poster briefs," 3-minute mini-presentations that give authors of the most highly-rated poster abstracts an opportunity to discuss their work during the concurrent sessions.

The burgeoning interest in stem cell research is reflected in our record number of exhibitors this year, 26% of whom are new. They make our meeting possible, and we encourage you to visit them and investigate all the tools and services they offer.

ISSCR's New Mobile Application and Journal
With so much to see, it's critical to manage your time here wisely. To that end, we are introducing the ISSCR2013 mobile application. Use it to access meeting information, and to search abstracts to find the scientific content of most interest to you.

And finally, we are proud to launch the new ISSCR journal, *Stem Cell Reports*, which is intended to further the exchange of information related to stem cell research and heighten the visibility of researchers. During the meeting, you'll have a chance to speak with the editors. We hope you'll consider submitting a manuscript and, if you're presenting at our meeting, that you'll keep *Stem Cell Reports* in mind when it's time to publish your data.

As always, we are grateful for your support. Thank you for coming -- we believe you'll agree that this year's meeting offers you an unprecedented opportunity to gain a comprehensive understanding of our important and dynamic field.

George Q. Daley MD, PhD
PROGRAM CHAIR

Shinya Yamanaka MD, PhD
PRESIDENT

HSCI
HARVARD STEM CELL INSTITUTE®

Dear Friends,

On behalf of the Harvard Stem Cell Institute and our many colleagues in the Boston area, we are pleased to welcome you to the 11th Annual Meeting of the International Society for Stem Cell Research. It is only fitting that ten years after the ISSCR's first annual meeting in Washington, DC that the meeting is in Boston where the idea for ISSCR was first launched.

Since its inception, ISSCR has recognized that stem cell research is inherently a multi-disciplinary effort that transcends geographic, organizational and domain boundaries. Over those years, progress in that science has sometimes been frustratingly slow; other times, unforeseen break-throughs have advanced the pace of discovery amazingly quickly. The award of this year's Nobel Prize in Medicine to Drs. Gurdon and Yamanaka demonstrates that our field is no longer just a potentially interesting idea, but is fundamental to the future of science and medicine. As we collectively share our recent results and new ideas with one another over the next several days, we can only be even more excited about what the next ten years will bring.

HSCI is delighted to welcome you to the vibrant eco-system of the Boston area stem cell and life sciences community that will be home for the next few days to the larger, global eco-system of stem cell science that is ISSCR.

We hope you have a productive meeting and enjoyable time.

Sincerely,

Douglas Melton CO-DIRECTOR
David Scadden CO-DIRECTOR
Leonard Zon CHAIR, EXECUTIVE COMMITTEE
Brock Reeve EXECUTIVE DIRECTOR

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We are grateful to the organizations supporting the ISSCR 11th Annual Meeting. Their generous support sustains us in our mission to serve as the responsible voice for stem cell science worldwide.

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THE NEW YORK STEM CELL FOUNDATION

ONTARIO STEM CELL INITIATIVE (OSCI)

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SPECIAL EVENTS

JUNIOR INVESTIGATOR EVENTS

Trainees and early-career investigators: take advantage of these JI-focused networking and career-building sessions

MEET THE EXPERTS NETWORKING LUNCHES

THURSDAY & FRIDAY
11:30 AM -1:00 PM

The ISSCR is pleased to host the popular "Meet the Experts", which offers the opportunity to meet with one of 40 stem cell research experts over lunch. Pre-registration for this event is required, but check with the registration desk to see if there are slots still available.

ISSCR JUNIOR INVESTIGATOR SOCIAL NIGHT

THURSDAY
9:00 PM – Midnight

The ISSCR Junior Investigators committee invites Junior Investigators to join us for an exclusive evening of networking and fun supported by iPS Academia Japan, Inc.

Meet, mingle, dance and socialize with fellow young investigators from around the world at Boston's famous Ned Devine's Irish Pub.

CAREER PANEL "STRATEGIES FOR SUCCESS: MAKING THE MOST OF YOUR PHD DEGREE"

SATURDAY
11:30 AM – 12:45 PM

Excitement!! Opportunities! Jobs? Are you finding yourself wondering what comes next? You don't need commentaries in Nature to remind you that jobs and funding in academia are as elusive as ever. In this career panel, our PhD panelists will discuss how they chose their varied career paths. Come and hear strategies for success in finding a rewarding job that leverages your skill set and, as always, come with your questions!

Pre-registration for this event is required, but check with the registration desk to see if there are slots still available.

EXHIBITION HALL

Find business solutions when you meet with exhibiting companies. The ISSCR 11th Annual Meeting features nearly 200 leading suppliers and vendors in the Exhibition Hall. Please support ISSCR Exhibitors who help make this meeting possible.

REFRESHMENT BREAKS IN THE EXHIBIT HALL

WEDNESDAY, JUNE 12
PM Refreshment break 3:30-4:30PM

THURSDAY, JUNE 13
PM Refreshment break 3:00-4:00PM

FRIDAY, JUNE 14
PM Refreshment break 3:00-4:00PM

MEET UP HUBS

Collaborate with your colleagues from around the world at the Meet Up hubs located in the Exhibit Hall. Supported by BD Biosciences

NETWORKING LOUNGE

The ISSCR Networking Lounge is the perfect place to check your emails, take a rest and plan your day, or collaborate with colleagues. Located in the center of the Exhibition Hall this lounge offers:

- Information on future ISSCR Meetings and Forums
- Tourism Vancouver promoting the ISSCR 2014 Annual Meeting
- Internet terminals

BOSTON COMMON

Located at the front of the Exhibit Hall, the Boston Common features charging stations for your phone and seating to relax and recharge your batteries.

POSTER PRESENTATIONS/ EXHIBIT RECEPTIONS

Join us for three poster presentations, your opportunity to view and discuss the exceptional work on display. Enjoy light refreshments and snacks while viewing the posters and visiting with exhibitors.

WEDNESDAY, JUNE 12

EXHIBIT HALL OPEN AND POSTERS OPEN FOR VIEWING
3:30 PM – 8:30 PM

POSTER PRESENTATION I
Supported by Harvard Stem Cell Institute
6:30 PM – 8:30 PM

Presentations for Odd-Numbered Posters
6:30 PM – 7:30 PM

Presentations for Even-Numbered Posters
7:30 PM – 8:30 PM

THURSDAY, JUNE 13

EXHIBIT HALL AND POSTERS OPEN FOR VIEWING
11:00 AM – 4:00 PM and 6:00 PM – 8:00 PM

POSTER PRESENTATION II
Supported by Massachusetts General Hospital Center for Regenerative Medicine and Boston Children's Hospital/Stem Cell Program.
6:00 PM – 8:00 PM

Presentations for Odd-Numbered Posters
6:00 PM – 7:00 PM

Presentations for Even-Numbered Posters
7:00 PM – 8:00 PM

FRIDAY, JUNE 14

EXHIBIT HALL AND POSTERS OPEN FOR VIEWING
11:00 AM – 4:00 PM and 6:00 PM – 8:00 PM

POSTER PRESENTATION III
6:00 PM – 8:00 PM

Presentations for Odd-Numbered Posters
6:00 PM – 7:00 PM

Presentations for Even-Numbered Posters
7:00 PM – 8:00 PM

SATURDAY, JUNE 15

Exhibit Hall Open

11:00 AM – 1:00 PM

Closing Reception
Supported by GlaxoSmithKline

6:00 PM– 7:00 PM
• Northeast Lobby - Level Zero

GENERAL INFORMATION

ISSCR REGISTRATION DESK

The ISSCR Registration Desk is located at the Boston Convention and Exhibition Center (BCEC), North Lobby and will be open during the following times:

TUESDAY JUNE 11 2:00 PM – 6:00 PM
WEDNESDAY JUNE 12 7:30 AM – 8:00 PM
THURSDAY JUNE 13 7:30 AM – 6:00 PM
FRIDAY JUNE 14 7:30 AM – 5:00 PM
SATURDAY JUNE 15 8:00 AM – 5:00 PM

HOUSING ASSISTANCE

For hotel related matters please visit the Housing Assistance desk in the registration area.

BCEC INFORMATION DESK

Need help getting around the Boston Convention & Exhibition Center? For directions inside the BCEC, stop by the Public Safety / Information desk located on the east side of the North Lobby and on Level Zero. Also, watch for the "Red Coat" guest service ambassadors at each entrance.

Pick up your copy of The BCEC Navigator, a helpful guide to the BCEC at the Information desk located on the east side of the North Lobby and on Level Zero.

MESSAGE CENTER

For your convenience, a message board is located in the North Lobby of the Boston Convention and Exhibition Center (BCEC). We are unable to page meeting delegates.

JOB OPPORTUNITIES

Need a job? Need to hire? Post resumes and employment opportunities on the designated boards located in the North Lobby of the BCEC.

BOSTON HOSPITALITY BOOTH

The Boston Convention and Visitors Bureau has a permanent hospitality booth in the North Lobby of the BCEC Lobby to help you plan dining options, activities, and tours ideas. The booth will be open during the following hours:

WEDNESDAY JUNE 12 Noon – 5:00 PM
THURSDAY JUNE 13 10:00 AM – 5:00 PM
FRIDAY JUNE 15 10:00 AM – 5:00 PM
SATURDAY JUNE 15 10:00 AM – 4:00 PM

RECORDINGS PROHIBITED

Still photography, video and/or audio taping of the sessions, presentations and posters at the ISSCR 11th Annual Meeting is strictly prohibited. Additionally, blogging, tweeting and other intent to communicate or disseminate results or discussion presented at the meeting is prohibited until the start of each individual presentation. Thank you for your cooperation.

SMOKING

Smoking is prohibited in the BCEC. For your convenience, there is a designated smoker's patio outside the level Zero Entrance.

TWITTER

Please share your Annual Meeting experience at #ISSCR2013

WIFI CONNECTIVITY

Enjoy complimentary Wi-Fi connectivity throughout the BCEC. Just open the wireless network connection on any device and click on the "BCEC Wireless Network".

MY BCEC APP

Download the complimentary My BCEC App for iPhone, iPad, Android or Blackberry to navigate the Boston Convention and Exhibition Center and the surrounding neighborhood, and scan real time travel imformation from Logan International Airport and the MBTA.

INTERNET ACCESS

A limited number of internet terminals are available in the Networking Lounge located in the center of the exhibition hall.

ATM / BANKING

There are (3) Citizen's Bank ATM machines, located in: North Lobby (Level 1), near the Food Court, NE Lobby (Level 1), and at SE Lobby (Level 0).

COAT CHECK

For your convenience, coat check is available on the East side of the North Lobby, Level One.

LOST AND FOUND

Please bring found items to the ISSCR Registration Desk. If you lost something, stop by during registration hours for assistance.

BUSINESS CENTER

A Fed Ex Office business center is located just off the North Lobby, Level One. The normal business hours are Mon-Fri 9:00 AM - 5:00 PM. The center does not accept exhibitor freight.

MEETING ROOMS AVAILABLE

Rooms are available for ISSCR ad hoc meetings on a first come, first-served basis. Sign-up sheets are posted outside each meeting room. These meeting rooms are located in the Westin Boston Waterfront, Concourse Level:

EXECUTIVE BOARD ROOM, FANEUIL, DOUGLAS, ALCOTT:

WEDNESDAY JUNE 12 7:00 AM – 7:00 PM
THURSDAY JUNE 13 7:00 AM – 7:00 PM
FRIDAY JUNE 14 7:00 AM – 7:00 PM
SATURDAY JUNE 15 7:00 AM – 7:00 PM

There are also informal seating areas available in the BCEC Exhibition Hall, Level Zero.

WICKED GOOD MARKETPLACE

LEVEL TWO – NORTHWEST LOBBY, LEVEL TWO
Easy access from the exhibition hall or North Lobby. The Wicked Good Marketplace is a perfect place for lunch, offering a variety of local Boston fare and healthy options.

The food court will be available each day for lunch:

WEDNESDAY JUNE 12 11:00 AM – 5:00 PM
THURSDAY JUNE 13 11:00 AM – 5:00 PM
FRIDAY JUNE 14 11:00 AM – 5:00 PM
SATURDAY JUNE 15 11:00 AM – 4:00 PM

MOTHER'S ROOM

A Mother's Room is provided for conference attendees. Room 201, Level 2, West side of the convention center.

This semi-private room will be available Wednesday through Saturday between 8:00 AM and 5:00 PM. If you require access outside of these hours please visit the Registration Desk.

ATTENTION SPEAKERS

If you have not loaded your presentation remotely in advance of the meeting, you must bring your CD or USB flash drive to the AV technician in the Speaker Ready Room the day before your session begins. Your program will be loaded to the network in the Speaker Ready Room so you can access it from the podium.

The Speaker Ready Room technician will review your presentation with you to ensure that everything runs as you expect. We have many speakers scheduled each day, so you MUST visit the Speaker Ready Room well in advance of your presentation to ensure you will be able to access your files during your presentation.

SPEAKER READY ROOM

Location: BCEC Room #251

TUESDAY JUNE 11 2:00 PM – 6:00 PM
WEDNESDAY JUNE 12 7:30 AM – 5:00 PM
THURSDAY JUNE 13 7:30 AM – 5:00 PM
FRIDAY JUNE 14 7:30 AM – 5:00 PM
SATURDAY JUNE 15 8:00 AM – 4:00 PM

Wednesday Speakers **MUST** visit the Speaker Ready Room Tuesday afternoon or Wednesday morning.

Thursday Speakers **MUST** visit the Speaker Ready Room on Tuesday or Wednesday.

Friday Speakers **MUST** visit the Speaker Ready Room on Wednesday or Thursday.

Saturday Speakers **MUST** visit the Speaker Ready Room no later than Friday afternoon.

2013 FEATURED SPEAKERS

PRESIDENTIAL SYMPOSIUM

Wednesday, 1:00 PM

James A. Thomson derived the first human embryonic stem (ES) cell line in 1998 and derived human induced pluripotent stem (iPS) cells in 2007. He serves as Director of Regenerative Biology at the Morgridge Institute for Research in Madison, Wisconsin, is a professor in the Department of Cell and Regenerative Biology at the University of Wisconsin's School of Medicine and Public Health and a professor in the Molecular, Cellular, and Developmental Biology Department at the University of California, Santa Barbara. He is also a founder and Chief Scientific Officer for Cellular Dynamics International, a Madison, WI-based company producing derivatives of human induced pluripotent stem cells for drug discovery and toxicity testing.

Douglas A. Melton is the Xander University Professor and an Investigator of the Howard Hughes Medical Institute. He is also a Co-Director of Harvard's Stem Cell Institute and Co-Chair of the Department of Stem Cell and Regenerative Biology in the Harvard Medical School and the Faculty of Arts and Sciences. Dr. Melton is on the Scientific Advisory Boards of several pharmaceutical companies and is the scientific co-founder of Gilead Sciences and Curis (Ontogeny), Inc. Research in the Melton laboratory focuses on the developmental biology of the pancreas and Type 1 diabetes. One aim is to understand how the pancreas normally develops and use that information to develop pancreatic cells (islets of Langerhans) from stem cells or other precursors. The longer-term goal is to apply the findings to human cells and provide a source of insulin-producing beta-cells for diabetics.

Edith Heard is the leader of the Mammalian Developmental Epigenetics team and Director of the Unit of Genetics and Developmental Biology Department at the Institut Curie and Professor of the Collège de France. Professor Heard has made many important contributions to the emerging field of epigenetics through her work on one of its most classic examples, X-chromosome inactivation. Her pioneering work, using X inactivation as a model system, has shed light on epigenetic mechanisms at multiple timescales: over the cell cycle, during development, across generations and in evolution. She and her colleagues were the first to discover the evolutionary diversity of events underlying X inactivation; the importance of the spatial organization and dynamics of the two X chromosomes in the nucleus during the initiation of X inactivation; and some of the first changes in the chromatin status of the X chromosome that can be considered as potential epigenetic marks ensuring the cellular memory of the inactive state.

Richard A. Young is a Professor of Biology at MIT and a Member of the Whitehead Institute. Dr. Young studies gene regulatory circuitry in health and disease. His research accomplishments range from the development of genome-wide analysis technologies to identification of the core regulatory circuitry of human embryonic stem cells. Dr. Young has served as an advisor to Science magazine, the National Institutes of Health and the World Health Organization. Dr. Young's honors include Membership in the National Academy of Sciences, the Chiron Corporation Biotechnology Research Award, Yale's Wilbur Cross Medal, and in 2006 Scientific American recognized him as one of the top 50 leaders in science, technology and business.

ANNE McLAREN MEMORIAL LECTURE

PLENARY I

Wednesday, 2:50 PM

Elaine Fuchs is the Rebecca C. Lancefield Professor in Mammalian Cell Biology and Development at The Rockefeller University. She is also an Investigator, Howard Hughes Medical Institute. Dr. Fuchs's research centers on skin biology, its stem cells and its associated human genetic disorders, which include skin cancers. Dr. Fuchs's current research focuses on the molecular mechanisms that underlie how multipotent stem cells of the skin are able to both self-renew long-term and to maintain and regenerate the epidermis, sebaceous glands and hair follicles. She is interested in how stem cells respond to signals from their neighbors, adjust their program of gene expression and adopt specific fates. In addition to elucidating how these pathways are regulated in normal homeostasis, Dr. Fuchs's team also investigates the mobilization of stem cells in wound repair and the abnormalities in the process that lead to human skin cancers.

ERNEST McCULLOCH MEMORIAL LECTURE

PLENARY II: *REGENERATION, ENGRAFTMENT MIGRATION*

Wednesday, 5:50 PM

George Q. Daley is the Samuel E. Lux, IV Professor of Hematology and the Director of the Stem Cell Transplantation Program at Boston Children's Hospital. He is also a past-President of the ISSCR and currently serves as Clerk on the ISSCR Board of Directors. Dr. Daley seeks to translate insights in stem cell biology into improved therapies for genetic and malignant diseases. Important research contributions from his laboratory include the creation of customized stem cells to treat genetic immune deficiency in a mouse model, the differentiation of germ cells from embryonic stem cells, and the generation of disease-specific pluripotent stem cells by direct reprogramming of human fibroblasts. Dr. Daley's recent studies have clarified mechanisms of Gleevec resistance and informed novel combination chemotherapeutic regimens.

KEYNOTE ADDRESS

PLENARY VII

Saturday, 5:10 PM

Eric Lander is a Professor of Biology at MIT and Founding Director of the Broad Institute. One of the principal leaders of the Human Genome Project, he and his colleagues have a long-standing interest in applying genomics to understand the molecular basis of human physiology and disease. The work of Dr. Lander and his colleagues includes mapping and sequencing of the human, mouse, and other genomes; understanding the functional elements encoded in genomes through comparative analysis; understanding the genetic variation in the human population and its relationship to disease susceptibility; understanding the distinctive cellular signatures of diseases and of response to drugs; understanding gene regulation; and understanding the mutations underlying cancer.

JOIN US IN HONORING THE RECIPIENTS OF THE **2013 ISSCR AWARDS**

McEWEN AWARD FOR INNOVATION
PRESIDENTIAL SYMPOSIUM

Wednesday, 1:00 PM

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.

James Thomson, PhD, is the recipient of the 2013 McEwen Award for Innovation. He is recognized for his work that reproducibly isolated pluripotent cell lines from human blastocysts, which opened the door for the study of human embryonic stem cells and revealed new possibilities for developing cell-based therapies, disease models and reagents for toxicity testing. Dr. Thomson will be presented with the award during the Presidential Symposium on Wednesday, June 12, 2013.

Dr. Thomson has been a member of the faculty of the University of Wisconsin-Madison since 1992 and serves as Director of Regenerative Biology at the Morgridge Institute for Research in Madison, Wisconsin. Dr. Thomson is also a founder and the Chief Scientific Officer of Cellular Dynamics International, a Madison-based company producing derivatives of human induced pluripotent stem cells for drug discovery and toxicity testing.

Join us for the 2013 award presentation which will take place during the Presidential Symposium on the afternoon of Wednesday, June 12.

Supported by McEwen Centre for Regenerative Medicine.

ISSCR PUBLIC SERVICE AWARD
PLENARY II
REGENERATION, EGRAFTMENT AND MIGRATION

Wednesday, 4:15 PM

The ISSCR Public Service Award is given to an individual(s) 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.

Hiromitsu and Betty Jean Crouch Ogawa are the recipients of the 2013 ISSCR Public Service Award. The Ogawas are recognized for their extraordinary support of stem cell research in Japan, through the work of Nobel Prize winner Shinya Yamanaka, and in the US, with their support of The Gladstone Institute. Their work with the ISSCR Global Advisory Council fortifies the growing global effort to engage contributors involved in stem cell research.

Join us for this special award presentation which will take place during Plenary Session II on the afternoon of Wednesday, June 12, 2013.

ISSCR-UNIVERSITY OF PITTSBURGH OUTSTANDING YOUNG INVESTIGATOR AWARD
PLENARY VI
GENOMICS AND EPIGENOMICS

Saturday, 1:00 PM

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.

Marius Wernig, MD, PhD, is the recipient of the 2013 ISSCR-University of Pittsburgh Outstanding Young Investigator Award. Dr. Wernig is recognized for his research demonstrating the ability of previously specified cells to be reprogrammed directly to other, distantly related cell types, which has transformed the field of cellular reprogramming.

Dr. Wernig is an independent investigator and holds a tenure-track Assistant Professor position at Stanford University School of Medicine.

Join us for the 2013 award presentation which will take place during Plenary Session VI on the afternoon of Saturday, June 15.

Supported by the University of Pittsburgh – McGowan Institute for Regenerative Medicine.

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2013 ANNUAL MEETING

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Joslin Diabetes Center

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Ex Officio Member, Center for iPS Cell Research & Application (CiRA)

CITY OF BOSTON · MASSACHUSETTS

OFFICE OF THE MAYOR
THOMAS M. MENINO

June 12, 2013

Dear Friends:

On behalf of the people of Boston, it gives me great pleasure to welcome the attendees, sponsors, and exhibitors to The International Society for Stem Cell Research Annual Meeting.

As home to some of the finest medical institutions and facilities in the world, Boston is proud to have been selected as host city for ISSCR.

I would like to wish you a successful and productive meeting and invite you to take advantage of all that Boston has to offer. As you will discover, Boston is one of the great meeting centers of the world. Our Old World charm and wealth of historical, cultural, artistic, and sports attractions will make your visit a memorable one.

Enjoy your stay. We are pleased that you could be with us.

Sincerely,

Thomas M. Menino
Mayor of Boston

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LIEUTENANT GOVERNOR

June 2013

Dear Friends:

On behalf of the Commonwealth of Massachusetts, I send warm greetings as you gather for the 11th Annual Meeting of the International Society for Stem Cell Research. Welcome to Boston!

Massachusetts is home to world-renowned universities, research hospitals and labs that are the center of medical advancement. We are proud to host the International Society for Stem Cell Research forum this year where leading stem cell and regenerative medicine professionals collaborate and advance research involving stem cells.

Through stem cell research, we have gained a greater understanding of our genetic code and uncovered tremendous possibilities for the treatment of diseases such as cancer. This weekend, you have the opportunity to attend informational sessions, listen to keynote speakers and share research and experiences with your esteemed colleagues.

During your stay in Boston, I encourage you to visit and enjoy our many museums, historic sites and fantastic restaurants. Please accept my best wishes for a successful and informative meeting.

Sincerely,

NEW FOR 2013! ISSCR2013 MOBILE APP

Capitalize on your time at the ISSCR 11th Annual Meeting

Download the ISSCR2013 mobile app today! Your all inclusive guide for this year's scientific program, oral and poster abstracts, maps as well as exhibitor and supporter listings. The app is supported on Apple devices (iPhone, iPad, and iPod Touch) and Android devices.

USE THE ISSCR2013 MOBILE APP TO:

- Build your personal itinerary on your mobile device or synchronize with the itinerary you built on the web-based Program Planner
- Browse or search for the scientific content, presenters, exhibitors or events of most interest to you
- Transfer your itinerary to your device calendar or Outlook
- Take notes directly to sessions, speakers and exhibitors. At the end of the meeting, export all notes via e-mail
- Receive up-to-the minute updates and location information
- Join the conversation via twitter with #ISSCR2013

FOLLOW THE INSTRUCTIONS BELOW TO DOWNLOAD THE ISSCR2013 MOBILE APP TO YOUR DEVICE OF CHOICE

1. Go to your Apple or Google Play store depending on your device
2. Search for and Install the Oasis Mobile Meeting Planner
3. After opening the App, you will automatically be directed to the Home screen. You will need to go to the Events page to download the ISSCR2013 event. Choose the Events icon on the bottom of the Home page
4. Select the ISSCR2013 App from the list of Upcoming Events and download the ISSCR2013 event
5. After installing the ISSCR2013 event, select the Activate button
6. The ISSCR2013 App will automatically open the next time you select the Oasis App on your smart device

PROGRAM SCHEDULE

Visit the Exhibit Hall. Say thank you to the exhibitors who make this meeting possible.

TUESDAY, JUNE 11, 2013

2:00 PM - 6:00 PM	REGISTRATION OPEN	Boston Convention and Exhibition Center North Lobby

WEDNESDAY, JUNE 12, 2013

7:30 AM - 8:00 PM	REGISTRATION OPEN	Boston Convention and Exhibition Center North Lobby
8:30 AM - 12:30 PM	INDUSTRY WEDNESDAY SYMPOSIUM Thermo Fisher Scientific NEW CONCEPTS AND TOOLS FOR ADVANCING STEM CELL RESEARCH AND THERAPEUTIC APPROACH	Room 253
8:30 AM - 12:30 PM	INDUSTRY WEDNESDAY SYMPOSIUM PerkinElmer PRECLINICAL ADVANCES IN STEM CELL RESEARCH- TECHNOLOGIES ACCELERATING CLINICAL TRANSLATION	Room 257
8:30 AM - 12:30 PM	FOCUS SESSION Stem Cell COREdinates with the Allen Institute of Brain Science GENERATING COLLECTIONS OF HUMAN IPSC AND ES LINES: ESTABLISHING BEST PRACTICES FOR SHARING PROTOCOLS AND CELL LINES	Room 254
9:30 AM - 12:30 PM	FOCUS SESSION ISSCR Ethics and Public Policy Committee SOMATIC CELL DONATION FOR STEM CELL RESEARCH: CURRENT CHALLENGES -- FUTURE DIRECTIONS	Room 255
1:00 PM - 3:30 PM	PRESIDENTIAL SYMPOSIUM *Supported By Janssen Research & Development* **Chair: Shinya Yamanaka** *Kyoto University, Center for IPS Cell Research and Application (CiRA)*	Plenary Hall B1
1:00 PM - 1:10 PM	**Welcome Remarks:** David Scadden, Co-founder and Co-director, Harvard Stem Cell Institute	
1:10 PM - 1:20 PM	**Presidential Address:** Shinya Yamanaka, ISSCR President	
1:20 PM - 1:30 PM	**McEwen Award for Innovation presented to James A. Thomson**	
1:30 PM - 1:50 PM	**James A. Thomson,** *Morgridge Institute for Research, USA* INNER CELL MASS HYSTERIA	
1:50 PM - 2:10 PM	**Douglas A. Melton,** *Harvard Stem Cell Institute, Harvard University, USA* MAKING PANCREATIC BETA CELLS	
2:10 PM - 2:30 PM	**Edith Heard,** *INSERM, Institut Curie, France* DEVELOPMENTAL DYNAMICS AND EPIGENETIC PLASTICITY OF X- CHROMOSOME INACTIVATION	
2:30 PM - 2:50 PM	**Richard A. Young,** *Whitehead Institute and Massachusetts Institute of Technology, USA* TRANSCRIPTIONAL CONTROL OF CELL IDENTITY	
2:50 PM - 3:25 PM	ANNE McLAREN MEMORIAL LECTURE **Elaine Fuchs,** *Rockefeller University, USA* SKIN STEM CELLS: IN SILENCE AND IN ACTION	
3:30 PM - 8:30 PM	EXHIBITION HALL OPEN	Exhibit Hall A
3:30 PM - 8:30 PM	NETWORKING CAFE OPEN	Exhibit Hall A
3:30 PM - 4:15 PM	REFRESHMENT BREAK IN EXHIBIT HALL	Exhibit Hall A

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PROGRAM SCHEDULE

WEDNESDAY, JUNE 12, 2013 *(continued)*

4:15 PM - 6:30 PM	**PLENARY II: REGENERATION, ENGRAFTMENT AND MIGRATION** *Supported by The New York Stem Cell Foundation* **Chair: Austin Smith** *Wellcome Trust Medical Research Council, Cambridge Stem Cell Institute, UK*	Plenary Hall B1
4:15 PM - 4:25 PM	**ISSCR Public Service Award presented to Hiromitsu and Betty Jean Ogawa**	
4:25 PM - 4:50 PM	**Paul S. Frenette,** *Albert Einstein College of Medicine, USA* DISSECTING HEMATOPOIETIC STEM CELL MICROENVIRONMENT	
4:50 PM - 5:15 PM	**Charles Lin,** *Massachusetts General Hospital, USA* VISUALIZING STEM CELL BIOLOGY IN VIVO	
5:15 PM - 5:40 PM	**Timm T. Schröder,** *Swiss Federal Institute of Technology, Switzerland* LONG-TERM SINGLE CELL QUANTIFICATION: NEW TOOLS FOR OLD QUESTIONS IN STEM CELL RESEARCH	
5:40 PM - 5:50 PM	**Poster Teasers**	
5:50 PM - 6:25 PM	**ERNEST McCULLOCH MEMORIAL LECTURE** **George Q. Daley,** *Boston Children's Hospital, USA* MILESTONES AND BARRIERS IN HEMATOPOIETIC STEM CELL DERIVATION FROM PLURIPOTENT STEM CELLS	
6:30 PM - 8:30 PM	**POSTER RECEPTION** *Supported by Harvard Stem Cell Institute*	Exhibit Hall A
6:30 PM - 8:30 PM	**POSTER PRESENTATION I** *Supported by Harvard Stem Cell Institute* 6:30 to 7:30: Odd numbered posters presented 7:30 to 8:30: Even numbered posters presented	Exhibit Hall A

THURSDAY, JUNE 13, 2013

7:30 AM - 6:00 PM	**REGISTRATION OPEN**	Boston Convention and Exhibition Center North Lobby
8:15 AM - 9:00 AM	**MORNING COFFEE**	NorthEast (NE)Lobby
9:00 AM - 11:20 AM	**PLENARY III: DISEASE MODELING** *Supported by California Institute for Regenerative Medicine (CIRM)* **Chair: Rudolph Jaenisch** *Whitehead Institute for Biomedical Research, USA*	Plenary Hall B1
9:00 AM - 9:15 AM	**ISSCR Business Meeting**	
9:15 AM - 9:40 AM	**Ludovic Vallier,** *Cambridge Stem Cell Institute and Wellcome Trust Sanger Institute, UK* MODELING GENETIC VARIABILITY USING HUMAN INDUCED PLURIPOTENT STEM CELLS	
9:40 AM - 10:05 AM	**Lawrence S.B. Goldstein,** *University of California, San Diego, USA* USING INDUCED PLURIPOTENT STEM CELLS TO UNDERSTAND ALZHEIMER'S DISEASE	
10:05 AM - 10:30 AM	**Haruhisa Inoue,** *Center for iPS Cell Research & Application, Japan* UNRAVELING NEURODEGENERATIVE-DISEASE MECHANISMS USING PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS	
10:30 AM - 10:55 AM	**Joseph C. Wu,** *Stanford University School of Medicine, USA* HUMAN INDUCED PLURIPOTENT STEM CELLS FOR CARDIOVASCULAR DISEASE MODELING	
10:55 AM - 11:05 AM	**PATIENT ADVOCATE ADDRESS** **Andres Trevino,** *Boston Children's Hospital Trust, USA* ANDY AND SOFIA	

PROGRAM SCHEDULE

THURSDAY, JUNE 13, 2013 *(Plenary III continued)*

11:05 AM - 11:15 AM	Meet the Editors of *Stem Cell Reports*	
11:00 AM - 4:00 PM	EXHIBITION HALL OPEN	Exhibit Hall A
11:00 AM - 4:00 PM	NETWORKING CAFE OPEN	Exhibit Hall A
11:20 AM - 1:15 PM	LUNCH BREAK ON YOUR OWN	
11:30 AM - 1:00 PM	MEET THE EXPERTS: NETWORKING LUNCH *Junior Investigator event, reservations required*	Ballroom Lobby Level 3
11:45 AM - 12:15 PM	INNOVATION SHOWCASES	
	Irvine Scientific Jessie H.-T. Ni MESENCHYMAL STROMAL/STEM CELLS EXPANSION	Room 255
	R & D Systems, Inc. Joy Aho DEFINING STEM CELL POPULATIONS FOR EXPERIMENTAL SUCCESS	Room 254 A
	Stemgent Brad Hamilton ADVANCEMENTS IN HUMAN IPS CELL DERIVATION TECHNIQUES FROM BLOOD DERIVED CELL TYPES	Room 257
	EMD Millipore Corporation Vi Chu PLURISTEM MEDIUM: WEEKEND & HOLIDAY FREE CULTURE OF PLURIPOTENT HUMAN ES/IPS CELLS	Room 253
	STEMCELL Technologies Michael Riedel EFFICIENT DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO SPECIFIC CELL TYPES USING FULLY DEFINED STEMDIFF™ REAGENTS	Room 258
12:30 PM - 1:00 PM	INNOVATION SHOWCASES	
	Molecular Devices, LLC. Oksana Sirenko PREDICTIVE HIGH-THROUGHPUT ASSAYS FOR TOXICITY ASSESSMENT USING IPSC-DERIVED CELL MODELS	Room 255
	Union Biometrica, Inc. Rock Pulak LARGE PARTICLE FLOW CYTOMETRY PROVIDES HIGH THROUGHPUT ANALYSIS AND AUTOMATION FOR CELL CLUSTERS (EBS, SPHEROIDS) AND ENCAPSULATED 3D CELL CULTURES	Room 254 A
	Lonza Thomas Fellner BUILDING BRIDGES FROM RESEARCH TO THERAPY: DEVELOPMENT OF A NOVEL REPROGRAMMING AND CULTURE SYSTEM FOR THE GENERATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS (HIPSCS) UNDER DEFINED CONDITIONS	Room 257
	BD Biosciences Christian Carson FLOW CYTOMETRY APPLICATIONS FOR ISOLATING AND ANALYZING COMPLEX HETEROGENOUS STEM CELL CULTURES	Room 253
	STEMCELL Technologies Erik Hadley COMPLETE AND FLEXIBLE CULTURE SYSTEMS TO SUPPORT HUMAN PLURIPOTENT STEM CELL RESEARCH	Room 258

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PROGRAM SCHEDULE

THURSDAY, JUNE 13, 2013 *(continued)*

1:15 PM - 3:05 PM — **CONCURRENT 1A: PLURIPOTENT STEM CELLS I** — Ballroom East
Supported by Ontario Stem Cell Initiative

Chair: Janet Rossant
Hospital For Sick Children, Canada

1:20 PM - 1:45 PM — **Jacob Hanna,** *Weizmann Institute, Israel*
THE EPIGENETIC STABILITY OF PLURIPOTENT AND SOMATIC CELL STATES

1:45 PM - 2:00 PM — **Ron Parchem,** *University of California, San Francisco, USA*
MIR-290 AND MIR-302 CLUSTERS DEFINE SEQUENTIAL STATES OF PLURIPOTENCY DURING DEVELOPMENT THAT ARE NOT RECAPITULATED IN REVERSE DURING REPROGRAMMING

2:00 PM - 2:15 PM — **Raffaella Di Micco,** *New York University School of Medicine, USA*
BET PROTEINS REGULATE EMBRYONIC STEM CELL IDENTITY BY CONTROLLING TRANSCRIPTIONAL ELONGATION OF CORE PLURIPOTENCY GENES

2:15 PM - 2:30 PM — **Kathleen Worringer,** *Gladstone Institutes, USA*
THE LET-7 TARGET LIN-41 PROMOTES INDUCED PLURIPOTENT STEM CELL REPROGRAMMING

2:30 PM - 2:45 PM — **Poster Briefs**

2:45 PM - 3:00 PM — **Dalit Ben Yosef,** *Tel Aviv Sourasky Medical Center, Israel*
GENOMIC ANALYSIS OF HESC PEDIGREES ENABLES IDENTIFICATION OF DE NOVO GENETIC ALTERATIONS AND DETERMINATION OF THE TIMING AND ORIGIN OF MUTATIONAL EVENTS

1:15 PM - 3:05 PM — **CONCURRENT 1B: NEURAL STEM CELLS** — Room 253

Chair: Elena Cattaneo
University of Milan, Italy

1:20 PM - 1:45 PM — **Fiona Doetsch,** *Columbia University, USA*
STEM CELLS AND THEIR NICHE IN THE ADULT MAMMALIAN BRAIN

1:45 PM - 2:00 PM — **Luis C. Fuentealba,** *University of California, San Francisco, USA*
ADULT V-SVZ NEURAL STEM CELLS HAVE A DISTINCT EMBRYONIC ORIGIN

2:00 PM - 2:15 PM — **Benedikt Berninger,** *University Medical Center Johannes Gutenberg University Mainz, Germany*
OLIGODENDROGLIOGENIC AND NEUROGENIC ADULT SUBEPENDYMAL ZONE NEURAL STEM CELLS CONSTITUTE DISTINCT LINEAGES AND EXHIBIT DIFFERENTIAL RESPONSIVENESS TO WNT SIGNALING

2:15 PM - 2:30 PM — **Xiao-Ling Hu,** *Tsinghua University, China*
VCAM1 IS A NICHE FACTOR IN THE DEVELOPING MOUSE TELENCEPHALON ACTING THROUGH BOTH CELL AUTONOMOUS AND NON-AUTONOMOUS EFFECTS

2:30 PM - 2:45 PM — **Poster Briefs**

2:45 PM - 3:00 PM — **Magdalena Goetz,** *Institute of Stem Cell Research, Helmholtz Center Munich, Germany*
A NOVEL NEURAL STEM CELL FACTOR WITH POTENT ROLES IN REGULATING BRAIN SIZE

1:15 PM - 3:05 PM — **CONCURRENT 1C: STEM CELLS AND TISSUE ENGINEERING** — Room 258

Chair: John McNeish
GlaxoSmithKline, USA

1:20 PM - 1:45 PM — **Molly S. Shoichet,** *University of Toronto, Canada*
BIOENGINEERED STRATEGIES TO PROMOTE TISSUE AND FUNCTIONAL REPAIR IN THE CENTRAL NERVOUS SYSTEM

1:45 PM - 2:00 PM — **Yi-Dong Lin,** *Academia Sinica, Taiwan*
INJECTION OF PEPTIDE NANOGELS AUGMENTS AND PROLONGS THE THERAPEUTIC EFFICACY OF AUTOLOGOUS BONE MARROW CELLS IN CARDIAC REPAIR

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THURSDAY, JUNE 13, 2013 *(Concurrent IC continued)*

2:00 PM - 2:15 PM — **Jaime Rivera,** *Massachusetts Institute of Technology, USA*
TETHERED EPIDERMAL GROWTH FACTOR ENHANCES BONE MARROW-DERIVED HUMAN CONNECTIVE TISSUE PROGENITOR COLONY FORMATION IN VITRO AND PROGENY SURVIVAL POST-TRANSPLANTATION

2:15 PM - 2:30 PM — **Andrea Meinhardt,** *Technische Universität, Germany*
IN VITRO RECONSTITUTION OF THE EMBRYONIC NEURAL TUBE FROM MOUSE EMBRYONIC AND MOUSE INDUCED PLURIPOTENT STEM CELLS

2:30 PM - 2:45 PM — **Poster Briefs**

2:45 PM - 3:00 PM — **Todd C. McDevitt,** *Georgia Tech / Emory University, USA*
ENGINEERING OF 3D PLURIPOTENT STEM CELL MICROENVIRONMENTS VIA BIOMATERIALS INCORPORATION

1:15 PM - 3:05 PM — CONCURRENT ID: STEM CELL SIGNALING AND NICHES — Room 205

Chair: Christine Mummery
Leiden University Medical Center, Netherlands

1:20 PM - 1:45 PM — **Tannishtha Reya,** *University of California, San Diego School of Medicine, USA*
BLOODLESS: THE CRITICAL ROLE OF ASYMMETRIC DIVISION IN BLOOD DEVELOPMENT AND CANCER

1:45 PM - 2:00 PM — **Chiung-Ying Chang,** *Rockefeller University, USA*
NFIB: A GOVERNOR OF EPITHELIAL-MELANOCYTE STEM CELL BEHAVIOUR IN A SHARED NICHE

2:00 PM - 2:15 PM — **Joo-Hyeon Lee,** *Boston Children's Hospital/Harvard Stem Cell Institute, USA*
LUNG ENDOTHELIAL CELLS DRIVE LINEAGE SPECIFIC DIFFERENTIATION OF LUNG EPITHELIAL STEM CELLS VIA BMP4-TSP1 REGULATION

2:15 PM - 2:30 PM — **Valentina Annese,** *Instituto de Biomedicina de Sevilla (IBiS), Hospital Virgen del Rocio/CSIC/Universidad de Sevilla, Spain*
NEURAL CREST-DERIVED STEM CELLS CONTRIBUTE TO HYPOXIA-INDUCED PHYSIOLOGICAL ANGIOGENESIS IN THE ADULT MAMMALIAN CAROTID BODY.

2:30 PM - 2:45 PM — **Poster Briefs**

2:45 PM - 3:00 PM — **Helen M. Blau,** *Stanford University School of Medicine Baxter Lab for Stem Cell Biology, USA*
BIOENGINEERED NICHES IDENTIFY REGULATORS THAT REJUVENATE THE FUNCTION OF AGED MUSCLE STEM CELL POPULATIONS

1:15 PM - 3:05 PM — CONCURRENT IE: STEM CELLS, INJURY AND REGENERATION — Ballroom West
Supported by StemCells, Inc

Chair: Elly Tanaka
Technical University Dresden, Center for Regenerative Therapies Dresden, Germany

1:20 PM - 1:45 PM — **András Simon,** *Karolinska Institute, Sweden*
SKELETAL MUSCLE DEDIFFERENTIATION DURING ADULT LIMB REGENERATION

1:45 PM - 2:00 PM — **Eugeniu Nacu,** *Center for Regenerative Therapies Dresden / Max Planck Institute of Molecular Cell Biology and Genetics, Germany*
ACTIVATION OF PROXIMO-DISTAL POSITIONAL INFORMATION MOLECULES, MEIS AND HOX, DURING AXOLOTL LIMB REGENERATION

2:00 PM - 2:15 PM — **Evan Barry,** *Boston Children's Hospital, USA*
YAP REPRESSES WNT-INDUCED INTESTINAL STEM CELL EXPANSION AND ORGAN GROWTH DURING REGENERATIVE STRESS

2:15 PM - 2:30 PM — **Denise Gay,** *University of Pennsylvania, USA*
FGF9 FROM DERMAL GAMMA DELTA T CELLS INDUCES HAIR FOLLICLE NEOGENESIS AFTER WOUNDING

2:30 PM - 2:45 PM — **Poster Briefs**

2:45 PM - 3:00 PM — **Albert Edge,** *Harvard Medical School, USA*
REGENERATION OF HAIR CELLS FROM COCHLEAR STEM CELLS

PROGRAM SCHEDULE

THURSDAY, JUNE 13, 2013 *(continued)*

3:05 PM - 4:00 PM — REFRESHMENT BREAK — Exhibit Hall A

4:00 PM - 5:50 PM — CONCURRENT IIA: PLURIPOTENT STEM CELLS II — Ballroom East
Supported by Fluidigm Corporation

Chair: Nissim Benvenisty
Hebrew University, Israel

4:05 PM - 4:30 PM — **Jeanne F. Loring,** *The Scripps Research Institute, USA*
EPIGENETIC MAPPING OF NORMAL AND DISEASE-SPECIFIC IPSC DIFFERENTIATION

4:30 PM - 4:45 PM — **Jian Long Wang,** *Mount Sinai School of Medicine, USA*
NANOG-DEPENDENT FUNCTION OF TET1 AND TET2 IN ESTABLISHMENT OF PLURIPOTENCY

4:45 PM - 5:00 PM — **Shannon M. Buckley,** *New York University School of Medicine, USA*
PROTEIN DEGRADATION BY THE UBIQUITIN PROTEASOME SYSTEM PLAYS A KEY ROLE IN MAINTAINING AND INDUCING PLURIPOTENCY

5:00 PM - 5:15 PM — **Shinji Masui,** *Center for iPS Cell Research and Application (CiRA), Kyoto University, Japan*
TRANSCRIPTION FACTORS INTERFERING WITH DEDIFFERENTIATION INDUCE CELL TYPE-SPECIFIC TRANSCRIPTIONAL PROFILES

5:15 PM - 5:30 PM — **Poster Briefs**

5:30 PM -5:45 PM — **Roshan M. Kumar,** *Harvard University, USA*
DECONSTRUCTION OF THE DYNAMIC PLURIPOTENT STEM CELL TRANSCRIPTION PROGRAM

4:00 PM - 5:50 PM — CONCURRENT IIB: NEW TECHNOLOGIES FOR CONTROLLING AND OBSERVING STEM CELL BEHAVIOR — Room 258

Chair: Sally Temple
Neural Stem Cell Institute, USA

4:05 PM - 4:30 PM — **Karl Deisseroth,** *Stanford University, USA*
OPTICAL DECONSTRUCTION OF FULLY-ASSEMBLED BIOLOGICAL SYSTEMS

4:30 PM - 4:45 PM — **Magali Soumillon,** *Harvard University, USA*
HIGH-THROUGHPUT SINGLE CELL RNA-SEQ TO DEFINE STEM CELL POPULATION HETEROGENEITY

4:45 PM - 5:00 PM — **Hideki Masaki,** *Institute for Medical Science, Japan*
FUNCTIONAL SCREENING OF PLURIPOTENT STEM CELLS FOR CHIMERA FORMING ABILITY

5:00 PM - 5:15 PM — **Marta Roccio,** *University of Bern, Switzerland*
PREDICTING STEM CELL FATE CHANGES BY DIFFERENTIAL CELL CYCLE PROGRESSION PATTERNS

5:15 PM - 5:30 PM — **Poster Briefs**

5:30 PM - 5:45 PM — **Janet Zoldan,** *Massachusetts Institute of Technology, USA*
A MICROFLUIDIC APPROACH TO IPS GENERATION

4:00 PM - 5:50 PM — CONCURRENT IIC: CELL FATE CONVERSION — Room 253

Chair: Fiona Watt
Center for Stem Cells and Regenerative Medicine, UK

4:05 PM - 4:30 PM — **Malin Parmar,** *Lund University, Sweden*
GENERATION OF INDUCED NEURONS VIA DIRECT CONVERSION IN VIVO AND IN VITRO

4:30 PM - 4:45 PM — **Nan Yang,** *Stanford University, USA*
GENERATION OF OLIGODENDROGLIAL CELLS BY DIRECT LINEAGE CONVERSION

PROGRAM SCHEDULE

THURSDAY, JUNE 13, 2013 *(Concurrent IIC continued)*

4:45 PM - 5:00 PM	**Jared J. Ganis,** *Harvard Medical School; Boston Children's Hospital and Dana Farber Cancer Institute; HHMI, USA* REGULATORS OF GLOBIN SWITCHING IN THE ZEBRAFISH EMBRYO
5:00 PM - 5:15 PM	**Pentao Liu,** *Wellcome Trust Sanger Institute, UK* REPROGRAMMING TO PLURIPOTENCY USING DESIGNER TALE TRANSCRIPTION FACTORS
5:15 PM - 5:30 PM	**Poster Briefs**
5:30 PM - 5:45 PM	**Vania Broccoli,** *San Raffaele Scientific Institute, Italy* EFFICIENT GENERATION OF SAFE INDUCED DOPAMINERGIC NEURONAL (IDAN) CELLS FROM ADULT HUMAN FIBROBLASTS BY DIRECT REPROGRAMMING

4:00 PM - 5:50 PM — **CONCURRENT IID: MODELING HUMAN DISEASE** — **Ballroom West**

Chair: Hideyuki Okano
Keio University School of Medicine, Japan

4:05 PM - 4:30 PM	**Lisa Ellerby,** *Buck Institute for Age Research, USA* MODELING HUNTINGTON'S DISEASE WITH INDUCED PLURIPOTENT STEM CELLS
4:30 PM - 4:45 PM	**Toshiyuki Araki,** *Ontario Cancer Institute, Canada* NOONAN SYNDROME ASSOCIATED-RAF1 MUTANT EVOKES HYPERTROPHIC CARDIOMYOPATHY FEATURES IN HUMAN CARDIOMYOCYTES IN VITRO.
4:45 PM - 5:00 PM	**Jeremy Sugarman,** *Berman Institute of Bioethics, Johns Hopkins University, USA* ATTITUDES OF PATIENTS TOWARD THE DONATION OF BIOLOGICAL MATERIALS FOR THE DERIVATION OF INDUCED PLURIPOTENT STEM CELLS
5:00 PM - 5:15 PM	**Ying Jin,** *Institute of Health Sciences, China* DISCOVERING AN EARLY NEURAL PHENOTYPE OF FAMILIAL ALZHEIMER'S DISEASES USING INDUCED PLURIPOTENT STEM CELLS
5:15 PM - 5:30 PM	**Poster Briefs**
5:30 PM - 5:45 PM	**ChengZhong Wang,** *Gladstone Institute of Neurological Disease, University of California, San Francisco, USA* HUMAN NEURONS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS REVEAL DETRIMENTAL EFFECTS OF APOLIPOPROTEIN E4: IMPLICATIONS FOR ALZHEIMER'S DISEASE

4:00 PM - 5:50 PM — **CONCURRENT IIE: STEM CELL AGING AND METABOLISM** — **Room 205**

Chair: Sean Morrison
University of Texas Southwestern Medical Center, USA

4:05 PM - 4:30 PM	**David Sabatini,** *Whitehead Institute, USA* REGULATION OF GROWTH BY THE MTOR PATHWAY
4:30 PM - 4:45 PM	**Floris Foijer,** *European Institute for the Biology of Ageing - University Medical Center Groningen, Netherlands* WHOLE CHROMOSOME INSTABILITY IS TOLERATED BY INTERFOLLICULAR EPIDERMAL CELLS, BUT NOT HAIR FOLLICLE STEM CELLS IN MOUSE SKIN
4:45 PM - 5:00 PM	**Keiyo Takubo,** *Keio University School of Medicine, Japan* PROLYL AND ASPARAGINYL HYDROXYLATION IN HYPOXIA-INDUCIBLE FACTOR PLAYS A CRITICAL ROLE IN HEMATOPOIETIC STEM CELL MAINTENANCE
5:00 PM - 5:15 PM	**Alessandro Prigione,** *Max Delbrueck Center for Molecular Medicine, Germany* HUMAN INDUCED PLURIPOTENT STEM CELLS EXHIBIT HIGH PKM2 LEVELS AND HIF-1 ALPHA DRIVEN EARLY RECONFIGURATION OF ENERGY METABOLISM
5:15 PM - 5:30 PM	**Poster Briefs**
5:30 PM - 5:45 PM	**Justine D. Miller,** *Memorial Sloan-Kettering Cancer Center, USA* MODELING NEURONAL AGING TO STUDY LATE-ONSET NEURODEGENERATIVE DISEASES WITH IPSCS

PROGRAM SCHEDULE

THURSDAY, JUNE 13, 2013 *(continued)*

6:00 PM - 8:00 PM	EXHIBITION HALL OPEN	Exhibit Hall A
6:00 PM - 8:00 PM	NETWORKING CAFE OPEN	Exhibit Hall A
6:00 PM - 8:00 PM	POSTER RECEPTION *Supported by Massachusetts General Hospital Center for Regenerative Medicine and Boston Children's Hospital Stem Cell Program*	Exhibit Hall A
6:00 PM - 8:00 PM	POSTER PRESENTATION II 6:00 to 7:00-Odd numbered posters presented 7:00 to 8:00-Even numbered posters presented	Exhibit Hall A
9:00 PM - 12:00 AM	JUNIOR INVESTIGATOR SOCIAL EVENT *Supported by iPS Academia Japan, Inc.* Reservations required	

FRIDAY, JUNE 14, 2013

7:30 AM - 5:00 PM	REGISTRATION OPEN	Boston Convention and Exhibition Center North Lobby
8:15 AM - 9:00 AM	MORNING COFFEE	NorthEast (NE) Lobby
9:00 AM - 11:20 AM	PLENARY IV: CELL AND GENE THERAPY *Joint Session with the American Society of Gene & Cell Therapy and the European Society of Gene & Cell Therapy* **Chair: David Scadden** *Harvard Stem Cell Institute and Massachusetts General Hospital Center for Regenerative Medicine, USA*	Plenary Hall B1
9:00 AM - 9:25 AM	**David Williams,** *Boston Children's Hospital, USA* INTEGRATING VIRAL VECTORS, GENE TRANSFER INTO HEMATOPOIETIC STEM CELLS AND THERAPIES IN HUMAN MONOGENIC DISEASES	
9:25 AM - 9:50 AM	**Alessandra Biffi,** *Ospedale San Raffaele, Italy* PHASE I/II CLINICAL TRIAL OF HEMATOPOIETIC STEM CELL GENE THERAPY FOR THE TREATMENT OF METACHROMATIC LEUKODYSTROPHY	
9:50 AM - 10:15 AM	**Nancy King,** *Wake Forest University Health Sciences, USA* ETHICAL ISSUES IN CELL- AND GENE-BASED RESEARCH AND THERAPY	
10:15 AM - 10:40 AM	**Sarah Ferber,** *Sheba Medical Center and Tel-Aviv University, Israel* TRANSDIFFERENTIATION AND ITS IMPLEMENTATION IN AUTOLOGOUS CELL REPLACEMENT THERAPY FOR DIABETES	
10:40 AM - 10:50 AM	**5 Poster Teasers**	
10:50 AM - 11:15 AM	**Charles Murry,** *University of Washington - Center for Cardiovascular Biology, USA* REGENERATING THE HEART OF A NON-HUMAN PRIMATE	
11:00 AM - 4:00 PM	EXHIBITION HALL OPEN	Exhibit Hall A
11:00 AM - 4:00 PM	NETWORKING CAFE OPEN	Exhibit Hall A
11:20 AM - 1:15 PM	LUNCH BREAK ON YOUR OWN	
11:30 AM - 1:00 PM	MEET THE EXPERTS: NETWORKING LUNCH	Ballroom Lobby Level 3

FRIDAY, JUNE 14, 2013 *(continued)*

11:45 AM - 12:15 PM	INNOVATION SHOWCASES	
	Biological Industries Israel Beit Haemek Ltd. Mark Weiss and David Fiorentini TOWARD A CLINICAL GRADE EXPANSION OF HUMAN MESENCHYMAL STEM CELLS: A COMPLETE SERUM-FREE, XENO-FREE CULTURE SYSTEM	Room 255
	Miltenyi Biotec GmbH Sebastian Knoebel CLEANING UP THE MESS: ISOLATION OF PURE GERM LAYER DERIVATIVES	Room 254 A
	Life Technologies Birgitt Schuele GENERATING PARKINSON'S DISEASE MODELS USING FOOTPRINT-FREE REPROGRAMMING AND A NOVEL METHOD FOR NSC DIFFERENTIATION	Room 257
	BD Biosciences Justin D. Lathia GAINING INSIGHTS INTO TUMOR HETEROGENEITY AND CANCER STEM CELL PATHWAYS BY USING HIGH-THROUGHPUT FLOW CYTOMETRY	Room 253
	Nikon Instruments Lee Rubin LONG-TERM CELL CULTURE OBSERVATION SYSTEM AND ADVANCED IMAGE ANALYSIS TECHNOLOGY	Room 258
12:30 PM - 1:00 PM	INNOVATION SHOWCASES	
	Corning Incorporated Deepa Saxena NOVEL, ANIMAL-FREE FIBRONECTIN AND COLLAGEN-I ECM MIMETIC SURFACES, ENABLING THE DEFINED EXPANSION OF HMSCS AND OTHER PRIMARY AND ADULT STEM CELL TYPES	Room 255
	Miltenyi Biotec GmbH Stefan Miltenyi INTEGRATED SOLUTIONS FOR CELL MANUFACTURING AND SCREENING	Room 254 A
	Life Technologies Alex Meissner TRANSCRIPTIONAL AND EPIGENETIC DYNAMICS DURING SPECIFICATION OF HUMAN PLURIPOTENT STEM CELLS	Room 257
	BD Biosciences Paulina Ordonez FLOW CYTOMETRY ANALYSIS OF CELLULAR AND FUNCTIONAL PHENOTYPES OF DISEASE IN PATIENT SPECIFIC HUMAN IPSC DERIVED CELLS	Room 253
	Corning Incorporated Mark Rothenberg TWO UNIQUE TECHNOLOGIES FOR NEURAL STEM AND INDUCED PLURIPOTENT CELL LINES: THE SYNTHEMAX® SELF-COATING SYNTHETIC SUBSTRATE AND FLOWELL™ A PERFUSION-BASED TECHNOLOGY FOR CONTINUOUS CULTURING	Room 258
1:15 PM - 3:05 PM	CONCURRENT IIIA: HEMATOPOIETIC STEM CELLS **Chair: Amy Wagers** *Joslin Diabetes Center, USA*	Ballroom East
1:20 PM - 1:45 PM	**Corey Cutler,** *Dana-Farber Cancer Institute, USA* PROSTAGLANDIN-MODULATED UMBILICAL CORD BLOOD HEMATOPOIETIC STEM CELL TRANSPLANTATION	

PROGRAM SCHEDULE

FRIDAY, JUNE 14, 2013 *(Concurrent IIIA continued)*

1:45 PM - 2:00 PM
Michel Cohen-Tannoudji, *Institut Pasteur, France*
NOTCHLESS AT THE CROSSROAD BETWEEN RIBOSOME BIOGENESIS AND ADULT HEMATOPOIETIC STEM CELL MAINTENANCE

2:00 PM - 2:15 PM
Catherine Frelin, *University Health Network, Ontario Cancer Institute, Canada*
NUCLEAR TRANSLOCATION OF GATA3 NEGATIVELY REGULATES SELF-RENEWAL IN ACTIVATED LONG-TERM HEMATOPOIETIC STEM CELLS

2:15 PM - 2:30 PM
Michael J. White, *The Walter and Eliza Hall Institute of Medical Research, Australia*
THE INTRINSIC APOPTOSIS CASPASE CASCADE REGULATES HEMATOPOIETIC STEM CELL HOMEOSTASIS AND FUNCTION

2:30 PM - 2:45 PM
Poster Briefs

2:45 PM - 3:00 PM
Christopher M. Sturgeon, *McEwen Centre for Regenerative Medicine, Canada*
HUMAN DEFINITIVE AND PRIMITIVE HEMATOPOIESIS ARE SPECIFIED SIMULTANEOUSLY FROM HUMAN PLURIPOTENT STEM CELLS

1:15 PM - 3:05 PM CONCURRENT IIIB: EPIGENETICS OF STEM CELLS — Room 258

Chair: Kathrin Plath
University of California, Los Angeles School of Medicine, USA

1:20 PM - 1:45 PM
Michaela Frye, *Wellcome Trust-Centre for Stem Cell Research, UK*
CYTOSINE-5 RNA METHYLATION IN NORMAL TISSUES AND DISEASES

1:45 PM - 2:00 PM
Christian Elabd, *University of California, Berkeley, USA*
DNA METHYLTRANSFERASE 3 DEPENDENT NON RANDOM SEGREGATION OF DNA STRANDS IN DIFFERENTIATING EMBRYONIC STEM CELLS

2:00 PM - 2:15 PM
Ritu Kumar, *Weill Cornell Medical College, USA*
DURING REPROGRAMMING, AID STABILIZES A STEM CELL PHENOTYPE BY REMOVING EPIGENETIC MEMORY OF SECONDARY PLURIPOTENCY NETWORK GENES

2:15 PM - 2:30 PM
Shu Xiao, *University of California San Diego, USA*
COMPARATIVE AND SPATIOTEMPORAL EPIGENOMIC ANNOTATION OF REGULATORY DNA

2:30 PM - 2:45 PM
Poster Briefs

2:45 PM - 3:00 PM
Peter Lansdorp, *European Institute for the Biology of Ageing, Netherlands*
IDENTIFICATION OF SISTER CHROMATIDS IN CELLS USING DNA TEMPLATE STRANDS

1:15 PM - 3:05 PM CONCURRENT IIIC: STEM CELL THERAPIES — Ballroom West

Chair: Deepak Srivastava
Gladstone Institute of Cardiovascular Disease, USA

1:20 PM - 1:45 PM
Nicholas Boulis, *The Emory Clinic, USA*
SURGICAL APPROACHES TO HUMAN SPINAL CORD STEM CELL TRANSPLANTATION IN AMYOTROPHIC LATERAL SCLEROSIS

1:45 PM - 2:00 PM
Martha S. Windrem, *University of Rochester Medical Center, USA*
HUMAN IPSC-DERIVED OLIGODENDROCYTE PROGENITOR CELLS CAN EFFICIENTLY AND COMPLETELY RESTORE CENTRAL MYELIN IN ANIMAL MODELS OF BOTH CONGENITAL AND ACQUIRED DEMYELINATION

2:00 PM - 2:15 PM
Matthew Pouliot, *The Orthopedic Stem Cell Institute, USA*
AUTOGENOUS POINT OF CARE BONE MARROW CONCENTRATE (BMC) FOR THE TREATMENT OF LUMBAR DEGENERATIVE DISC DISEASE: IRB CONTROLLED PROSPECTIVE STUDY

2:15 PM - 2:30 PM
Jason S. Robert, *Arizona State University, USA*
OUGHT PATIENTS TO PAY TO PARTICIPATE IN CELL TRANSFER CLINICAL TRIALS?

2:30 PM - 2:45 PM
Poster Briefs

FRIDAY, JUNE 14, 2013 *(Concurrent IIIC continued)*

2:45 PM - 3:00 PM
Angelo Lombardo, *San Raffaele Telethon Institute for Gene Therapy, San Raffaele Scientific Institute, Italy*
EXPLOITING ARTIFICIAL NUCLEASES FOR TARGETED TRANSGENE INTEGRATION IN HUMAN HEMATOPOIETIC STEM CELLS AND INDUCED PLURIPOTENT STEM CELLS DERIVED FORM NORMAL DONORS AND SCID-X1 PATIENTS

1:15 PM - 3:05 PM CONCURRENT IIID: CHEMICAL CONTROL OF STEM CELL BEHAVIOR — Room 253

Chair: Hongkui Deng
Peking University, China

1:20 PM - 1:45 PM
Stephen Minger, *GE Healthcare, UK*
INNOVATING PRECLINICAL DRUG DEVELOPMENT & HUMAN CELL THERAPY

1:45 PM - 2:00 PM
Uri Ben-David, *Hebrew University Stem Cell Unit, Israel*
HIGH-THROUGHPUT SCREENING IDENTIFIES A NOVEL OLEATE SYNTHESIS INHIBITOR THAT SELECTIVELY ELIMINATES HUMAN PLURIPOTENT STEM CELLS AND PREVENTS TERATOMA FORMATION

2:00 PM - 2:15 PM
Jing Shan, *Massachusetts Institute of Technology, USA*
IDENTIFICATION OF SMALL MOLECULES FOR MATURATION OF IPS-DERIVED HEPATOCYTE-LIKE CELLS

2:15 PM - 2:30 PM
Lauren Drowley, *AstraZeneca, Sweden*
DEVELOPMENT OF HIGH-CONTENT IMAGING ASSAY TO EVALUATE DIFFERENTIATION TOWARDS FUNCTIONAL BROWN ADIPOSE TISSUE IN ADIPOSE AND SKELETAL MUSCLE PROGENITORS

2:30 PM - 2:45 PM
Poster Briefs

2:45 PM - 3:00 PM
Quinn P. Peterson, *Harvard University, USA*
COMBINATORIAL SMALL MOLECULE MODULATION OF HESC-DERIVED PANCREATIC MULTIPOTENT PROGENITOR CELLS

1:15 PM - 3:05 PM CONCURRENT IIIE: GERM CELL BIOLOGY AND ARTIFICIAL GAMETES — Room 205

Chair: Thomas Graf
Center for Genomic Regulation, Spain

1:20 PM - 1:45 PM
Jinsong Li, *Institute of Biochemistry & Cell Biology SIBS CAS, China*
GENERATION OF MAMMALIAN HAPLOID EMBRYONIC STEM CELLS

1:45 PM - 2:00 PM
Harry G. Leitch, *University of Cambridge, UK*
REBUILDING PLURIPOTENCY FROM MOUSE PRIMORDIAL GERM CELLS

2:00 PM - 2:15 PM
Robin M. Hobbs, *Monash University, Australia*
THE TSC2-MTORC1 SIGNALING AXIS CONTROLS GERMLINE STEM CELL FATE

2:15 PM - 2:30 PM
Hossein Azizi, *Institute for Anatomy and Cell Biology, University of Heidelberg, Germany*
IDENTIFICATION AND PLURIPOTENCY OF MOUSE SPERMATOGONIAL STEM CELLS

2:30 PM - 2:35 PM
Poster Brief

2:35 PM - 3:00 PM
Josef Penninger, *Institute of Molecular Biology (IMBA), Austria*
YEAST GENETICS IN MAMMALIAN STEM CELLS

3:05 PM - 4:00 PM REFRESHMENT BREAK IN EXHIBIT HALL — Exhibit Hall A

4:00 PM - 5:50 PM CONCURRENT IVA: STEM CELLS IN ORGAN DEVELOPMENT — Ballroom West

Chair: Leonard I. Zon
Boston Children's Hospital, USA

4:05 PM - 4:30 PM
Henrik Semb, *University of Copenhagen, Denmark*
SHAPE IS FATE

PROGRAM SCHEDULE

FRIDAY, JUNE 14, 2013 *(Concurrent IVA continued)*

4:30 PM - 4:45 PM — **Akio Kobayashi,** *Harvard Medical School/Brigham and Women's Hospital, USA*
PAX2 MAINTAINS THE NEPHRON LINEAGE BY REPRESSING STROMAL CELL FATES IN NEPHRON PROGENITOR CELLS DURING MAMMALIAN KIDNEY DEVELOPMENT

4:45 PM - 5:00 PM — **Rachael Nimmo,** *UCL Cancer Institute, UK*
MIR-142-3P UNLOCKS THE HEMANGIOBLAST PROGRAM IN THE MESODERMAL PRECURSORS OF HEMATOPOIETIC STEM CELLS AND THE MAJOR VESSELS

5:00 PM - 5:15 PM — **Joan Isern,** *Spanish National Cardiovascular Center (CNIC), Spain*
DEVELOPMENTAL SEGREGATION OF BONE MARROW HSC NICHE FORMATION FROM ENDOCHONDRALOSSIFICATION BY SPECIALIZED NEURAL CREST- AND MESODERM-DERIVED MSCS, RESPECTIVELY

5:15 PM - 5:30 PM — **Poster Briefs**

5:30 PM - 5:45 PM — **Valerie Gouon-Evans,** *Mount Sinai School of Medicine, USA*
VEGFR2/KDR/FLK-1 MARKS A NOVEL, FUNCTIONAL, AND CONSERVED LIVER PROGENITOR CELL

4:00 PM - 5:50 PM — CONCURRENT IVB: SELF RENEWAL MECHANISMS — Room 258

Chair: Arturo Alvarez-Buylla
University of California, San Francisco, USA

4:05 PM - 4:30 PM — **Rongwen Xi,** *National Institute of Biological Sciences, China*
DYNAMIC INTERACTIONS BETWEEN STEM CELLS AND THEIR MICROENVIRONMENT DURING INTESTINAL HOMEOSTASIS AND REGENERATION IN DROSOPHILA

4:30 PM - 4:45 PM — **Claudia Cattoglio,** *HHMI, University of California, Berkeley, USA*
TRANSCRIPTIONAL REGULATION MEDIATED BY THE DNA REPAIR COMPLEX XPC-RAD23B-CETN2 IN EMBRYONIC STEM CELLS

4:45 PM - 5:00 PM — **Guang Hu,** *National Institute of Environmental Health Sciences/National Institute of Health, USA*
THE THO COMPLEX REGULATES PLURIPOTENCY GENE MRNA EXPORT AND MAINTAINS ESC SELF-RENEWAL

5:00 PM - 5:15 PM — **Devendra S. Mistry,** *University of California, San Diego, USA*
THE MECHANISM UNDERLYING EPIDERMAL TISSUE SELF-RENEWAL BY THE RNA DEGRADING EXOSOME COMPLEX

5:15 PM - 5:30 PM — **Poster Briefs**

5:30 PM - 5:45 PM — **Robert A.J. Signer,** *University of Texas Southwestern Medical Center, USA*
THE RATE OF PROTEIN TRANSLATION REGULATES HEMATOPOIETIC STEM CELL SELF-RENEWAL

4:00 PM - 5:50 PM — CONCURRENT IVC: IMMUNOLOGY AND STEM CELLS — Room 253

Chair: Irving Weissman
Stanford University School of Medicine, USA

4:05 PM - 4:30 PM — **Ashleigh S. Boyd,** *Roger Williams Medical Center, Boston University School of Medicine, USA*
ASSESSING THE IMMUNE RESPONSE TO TRANSPLANTATION OF PLURIPOTENT STEM CELL DERIVATIVES

4:30 PM - 4:45 PM — **Audrey Parent,** *University of California, San Francisco, USA*
DIRECTED DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS INTO THYMIC EPITHELIAL PROGENITORS

4:45 PM - 5:00 PM — **Yuqiong Pan,** *Stanford University, USA*
INDUCED TOLERANCE TO EMBRYONIC STEM CELL TRANSPLANTATION BY HOST CONDITIONING WITH TOTAL LYMPHOID IRRADIATION, ANTITHYMOCYTE SERUM, AND REGULATORY T CELL

FRIDAY, JUNE 14, 2013 *(Concurrent IVC continued)*

5:00 PM - 5:15 PM — **Julia D. Ransohoff,** *Stanford School of Medicine, USA*
BLOCKADE OF COSTIMULATORY MOLECULE SIGNALING PROMOTES SURVIVAL OF HUMAN EMBRYONIC STEM CELL-DERIVED ENDOTHELIAL CELLS BY INDUCING T CELL IMMUNOGLOBULIN 3 UPREGULATION AND IMPROVES CARDIAC FUNCTION.

5:15 PM - 5:30 PM — **Poster Briefs**

5:30 PM - 5:45 PM — **Maria Themeli,** *Memorial Sloan-Kettering Cancer Center, USA*
HUMAN IPS CELL-DERIVED ANTIGEN-TARGETED T LYMPHOCYTES ERADICATE CD19-POSITIVE TUMOR CELLS

4:00 PM - 5:50 PM — CONCURRENT IVD: STEM CELLS AND CANCER — Ballroom East

Chair: Connie Eaves
BC Cancer Research Centre, Canada

4:05 PM - 4:30 PM — **Mina J. Bissell,** *Lawrence Berkeley National Lab, USA*
EXAMINING THE 'CANCER STEM CELL' HYPOTHESIS AS IT RELATES TO BREAST CANCER

4:30 PM - 4:45 PM — **Ellen van Rooijen,** *Boston Children's Hospital, Harvard Medical School, USA*
A CHROMATIN FACTOR SCREEN IN ZEBRAFISH IDENTIFIES NEURAL CREST REGULATOR SATB2 AS A NOVEL DRIVER OF MELANOMA

4:45 PM - 5:00 PM — **Marion Chapellier,** *INSERM; Centre Recherche en Cancerologie de Lyon, France*
BMP2 DETERMINES LUMINAL SWITCH AND PROMOTES RELATED BREAST TUMORS THROUGH THE DISRUPTION OF THE FOXC1/FOXA1 BALANCE

5:00 PM - 5:15 PM — **Tzvi Aviv,** *Hospital for Sick Children, Canada*
TARGETING CANCER STEM CELLS IN MEDULLOBLASTOMA IS NECESSARY BUT NOT SUFFICIENT FOR TUMOUR CONTROL

5:15 PM - 5:30 PM — **Poster Briefs**

5:30 PM - 5:45 PM — **Jody Haigh,** *VIB/ Ghent University, Belgium*
OVEREXPRESSION OF THE EMT REGULATOR ZEB2/SIP1 RESULTS IN A BLOCK IN T CELL DEVELOPMENT AND IDENTIFIES THIS GENE AS A NEW DRIVER FOR T CELL LYMPHOBLASTIC LEUKEMIA

4:00 PM - 5:50 PM — CONCURRENT IVE: CHROMATIN REGULATION IN STEM CELLS — Room 205

Chair: Derek van der Kooy
University of Toronto, Canada

4:05 PM - 4:30 PM — **Jay Bradner,** *Dana-Farber Cancer Institute, USA*
CHEMICAL CONTROL OF GENE EXPRESSION

4:30 PM - 4:45 PM — **Andre Catic,** *Massachusetts General Hospital, USA*
A FUNCTIONAL MAP OF NUCLEAR PROTEOLYSIS

4:45 PM - 5:00 PM — **Eran Meshorer,** *The Hebrew University of Jerusalem, Israel*
A LIBRARY OF ENDOGENOUSLY TAGGED FLUORESCENT PROTEINS IN EMBRYONIC STEM CELLS REVEALS A LINKER HISTONE CHAPERONE INVOLVED IN PLURIPOTENCY AND DIFFERENTIATION

5:00 PM - 5:15 PM — **Andrew Xiao,** *Yale Stem Cell Center, USA*
HISTONE VARIANT H2A.X-MEDIATED EPIGENETIC MECHANISMS ARE CRITICAL FOR MAINTAINING GENOME STABILITY AND PLURIPOTENCY IN ES AND IPS CELLS

5:15 PM - 5:30 PM — **Poster Briefs**

5:30 PM - 5:45 PM — **Lawrence W. Stanton,** *Genome Institute of Singapore, Singapore*
OCT4 SWITCHES PARTNERING FROM SOX2 TO SOX17 TO REINTERPRET THE ENHANCER CODE AND SPECIFY ENDODERM

6:00 PM - 8:00 PM — EXHIBITION HALL OPEN — Exhibit Hall A

PROGRAM SCHEDULE

FRIDAY, JUNE 14, 2013 *(continued)*

6:00 PM - 8:00 PM	NETWORKING CAFE OPEN	Exhibit Hall A
6:00 PM - 8:00 PM	POSTER RECEPTION	Exhibit Hall A
6:00 PM - 8:00 PM	POSTER PRESENTATION III 6:00 to 7:00 -Odd numbered posters presented 7:00 to 8:00 -Even numbered posters presented	Exhibit Hall A

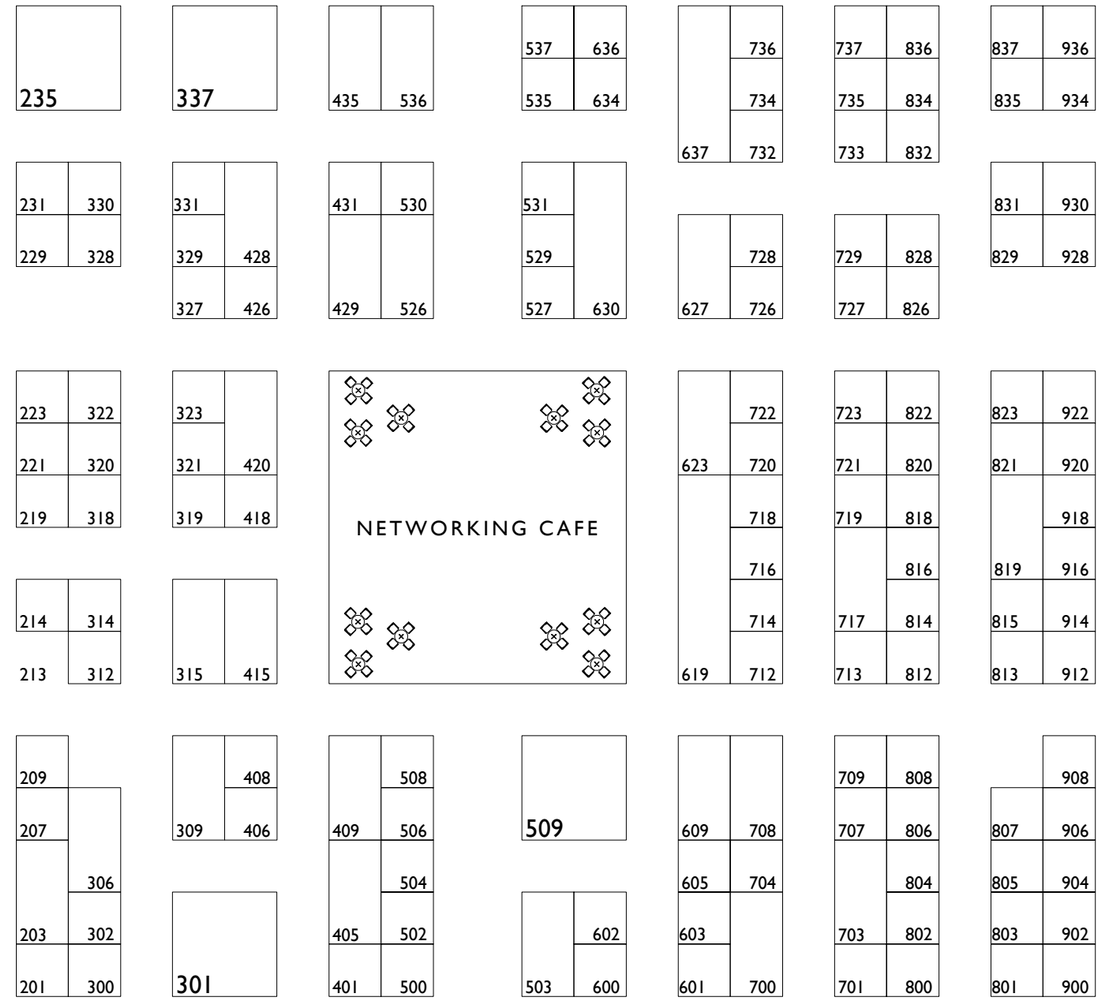
SATURDAY, JUNE 15, 2013

8:00 AM - 5:00 PM	REGISTRATION OPEN	Boston Convention and Exhibition Center North Lobby
8:15 AM - 9:00 AM	MORNING COFFEE BREAK	NorthEast (NE) Lobby
9:00 AM - 11:10 AM	PLENARY V: STEM CELLS AND FATE CONTROL *Supported by Development* **Chair: Fred Gage** *Salk Institute for Biological Studies, USA*	Plenary Hall B1
9:00 AM - 9:25 AM	**Ian Chambers,** *University of Edinburgh, UK* TRANSCRIPTION FACTOR CONTROL OF TRANSITIONS IN PLURIPOTENT CELL STATES	
9:25 AM - 9:50 AM	**Oliver Brüstle,** *University of Bonn Medical Center, Germany* PLURIPOTENT STEM CELLS AND CELL FATE PROGRAMMING FOR MODELING NEUROLOGICAL DISEASE	
9:50 AM - 10:15 AM	**Mitinori Saitou,** *Kyoto University, Graduate School of Medicine, Japan* MECHANISM AND RECONSTITUTION IN VITRO OF GERM CELL DEVELOPMENT IN MICE	
10:15 AM - 10:40 AM	**Elena Cattaneo,** *University of Milano, Italy* TRANSLATING THE NATURAL HISTORY OF HUMAN STRIATAL DEVELOPMENT INTO PLURIPOTENT STEM CELL DIFFERENTIATION	
10:40 AM - 11:05 AM	**Benoit G. Bruneau,** *Gladstone Institute of Cardiovascular Disease, UCSF, USA* EPIGENOMIC REGULATION OF CARDIAC LINEAGE DEVELOPMENT	
11:00 AM - 1:00 PM	EXHIBITION HALL OPEN	Exhibit Hall A
11:00 PM - 1:00 PM	NETWORKING CAFE OPEN	Exhibit Hall A
11:10 AM - 1:00 PM	LUNCH BREAK ON YOUR OWN	
11:30 AM - 12:00 PM	INNOVATION SHOWCASES	
	Fluidigm Corporation HARNESSING THE POWER OF SINGLE CELL GENOMICS TO ACCELERATE STEM CELL DISCOVERY, PART I	Room 255
	PeproTech Rick. I. Cohen STRETCHING EVERY CELL COUNTS: OPTIMIZING THE CULTURE OF PPSC USING NOVEL INSULIN FREE MEDIA	Room 254 A
	Cellectis bioresearch Catharina Ellerström ENGINEERING OF STEM CELLS: TALEN™-MEDIATED APPROACH	Room 257
	ES Cell International, a subsidiary of BioTime, Inc. Tom Zarembinski and Jeffrey Janus NOVEL CLONALLY-PURIFIED PURESTEM EMBRYONIC PROGENITORS AND CUSTOMIZABLE HYSTEM® HYDROGEL SUBSTRATES FOR CELL CULTURE AND DELIVERY	Room 253

SATURDAY, JUNE 15, 2013 *(continued)*

11:30 AM - 12:45 PM	JUNIOR INVESTIGATOR EVENTS: CAREER PANEL	Ballroom Lobby Level 3
12:15 PM - 12:45 PM	INNOVATION SHOWCASES	
	Fluidigm Corporation HARNESSING THE POWER OF SINGLE CELL GENOMICS TO ACCELERATE STEM CELL DISCOVERY, PART II	Room 255
	LifeMap Sciences, Inc. a subsidiary of BioTime, Inc. Ariel Rinon LIFEMAP DISCOVERY, A POWERFUL DATABASE LINKING EMBRYONIC DEVELOPMENT WITH STEM CELL RESEARCH	Room 253 A-C
1:00 PM - 2:55 PM	PLENARY VI: GENOMICS AND EPIGENOMICS OF STEM CELLS *Supported By Lieber Institute for Brain Development* **Chair: Haifan Lin** *Yale University School of Medicine*	Plenary Hall B1
1:00 PM - 1:25 PM	**Kenneth S. Zaret,** *Perelman School of Medicine, University of Pennsylvania, USA* PROGRAMMING AND REPROGRAMMING CELL FATE	
1:25 PM - 1:50 PM	**Kristin Baldwin,** *Scripps Research Institute, USA* PROBING THE SOMATIC MUTATIONAL SPECTRA AND EPIGENETIC PLASTICITY OF NEURONS AND FIBROBLASTS USING REPROGRAMMING AND WHOLE GENOME SEQUENCING	
1:50 PM - 2:15 PM	**Shoukhrat Mitalipov,** *Oregon Health and Science University, USA* REPROGRAMMING OF HUMAN SOMATIC CELLS TO PLURIPOTENCY BY SOMATIC CELL NUCLEAR TRANSFER	
2:15 PM - 2:20 PM	**Outstanding Young Investigator Award presented to Marius Wernig**	
2:20 PM - 2:55 PM	**Marius Wernig,** *Stanford University, USA* DIRECT LINEAGE REPROGRAMMING TOWARDS THE NEURAL LINEAGE	
2:55 PM - 3:40 PM	REFRESHMENT BREAK	Exhibit Hall A
3:40 PM - 6:00 PM	PLENARY VII: MAKING TISSUES AND ORGANS *Supported By GlaxoSmithKline* **Chair: Daniel Marshak** *PerkinElmer, Inc., USA*	Plenary Hall B1
3:40 PM - 3:55 PM	**President-Elect Address:** Janet Rossant	
3:55 PM - 4:20 PM	**Hans Snoeck,** *Columbia University Medical Center, USA* GENERATION OF ANTERIOR FOREGUT ENDODERM DERIVATIVES FROM HUMAN PLURIPOTENT STEM CELLS	
4:20 PM - 4:45 PM	**Graziella Pellegrini,** *University of Modena E Reggio Emilia, Italy* REGENERATION OF SQUAMOUS EPITHELIA FROM STEM CELLS OF CULTURED DRAFTS	
4:45 PM - 5:10 PM	**Timothy Bertram,** *Tengion Inc., USA* REGENERATING KIDNEY AND HOLLOW ORGANS	
5:10 PM - 5:45 PM	**Eric Lander,** *Broad Institute of MIT and Harvard University, USA* Closing Keynote Address: SECRETS OF THE HUMAN GENOME	
5:45 PM - 5:55 PM	**Closing Remarks: Shinya Yamanaka**	
6:00 PM - 7:00 PM	CLOSING RECEPTION *Supported By GlaxoSmithKline*	NorthEast (NE) Lobby

EXHIBIT BOOTH FLOOR PLAN



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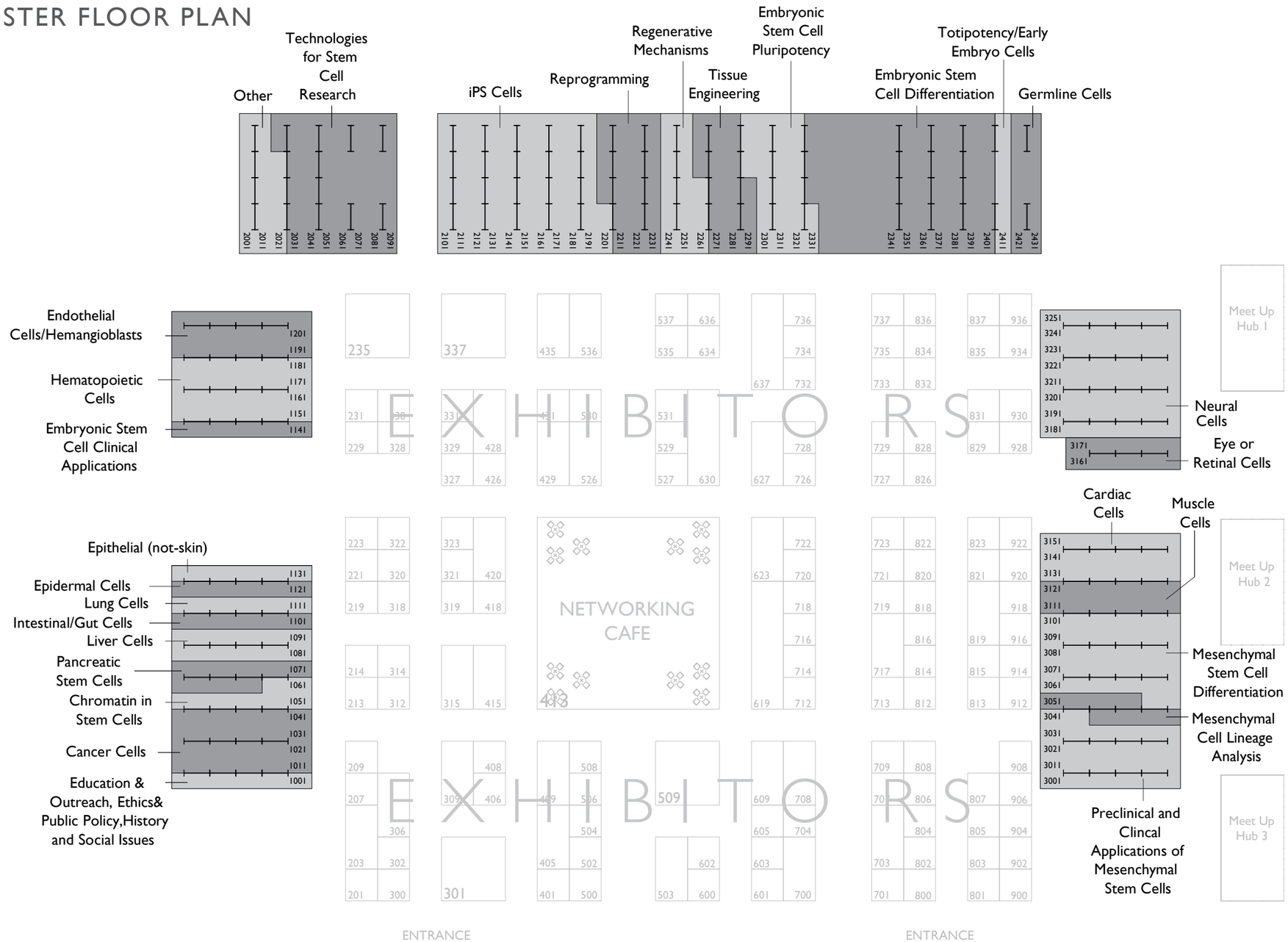

SCHEDULE

WEDNESDAY, Poster Presentation I
6:30 PM - 8:30 PM

6:30 to 7:30- Odd numbered posters presented
7:30 to 8:30- Even numbered posters presented

Poster Reception Supported by Harvard Stem Cell Institute

THURSDAY, Poster Presentation II
6:00 PM - 8:00 PM

6:00 to 7:00 -Odd numbered posters presented
7:00 to 8:00 -Even numbered posters presented

Poster Reception Supported by Massachusetts General Hospital and Boston Children's Hospital

FRIDAY, Poster Presentation III
6:00 PM - 8:00 PM

6:00 to 7:00 -Odd numbered posters presented
7:00 to 8:00 -Even numbered posters presented

NO PHOTOGRAPHY OR VIDEO RECORDINGS IN THE POSTER AREA

POSTER BOARDS BY TOPIC

POSTERS 1000-1210

POSTERS 2001-2440

POSTERS 3001-3260

PLEASE NOTE

ODD numbered poster boards are presented in the ***first*** hour

EVEN numbered poster boards are presented in the ***second*** hour

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Wednesday, June 12, 2013, 1:00 PM - 3:30 PM

Plenary Hall B1

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Thomson, James A.

Morgridge Institute for Research, USA

Dr. Thomson will present a personal historical perspective on the derivation of human pluripotent stem cells.

MAKING PANCREATIC BETA CELLS

Melton, Douglas A.

Harvard Stem Cell Institute, Harvard University, USA

Despite the fact that the primary causes for Type 1 and Type 2 diabetes differ, all diabetics would benefit from an increase in the number and quality of their insulin-producing beta cells. We are actively pursuing to parallel approaches to this end: (1) the directed differentiation of human stem cells into beta cells, with an eye toward transplantation and (2) the manipulation of endogenous beta cell replication. Progress on both projects will be presented, including new results on a hormone that specifically and robustly causes beta cell replication.

DEVELOPMENTAL DYNAMICS AND EPIGENETIC PLASTICITY OF X- CHROMOSOME INACTIVATION

Heard, Edith

INSERM; Institut Curie, France

In female mammals, one of the two X chromosomes is converted from the active euchromatic state into inactive heterochromatin during early embryonic development. This process, known as X-chromosome inactivation, results in the transcriptional silencing of over a thousand genes and ensures dosage compensation between the sexes. The regulation of X inactivation is tightly integrated with the pluripotency network. We are studying the mechanisms underlying the establishment and maintenance of the X-inactivation process, during early embryogenesis and in embryonic stem cells differentiated into epiblast cells or neuronal progenitor cells. We have performed detailed transcriptome analyses of male and female differentiating ES cells, which has revealed that X inactivation represents a developmental checkpoint in cells with two X chromosomes. Our findings on the developmental dynamics of this process, and on the changes in chromatin structure and nuclear organization that accompany X inactivation, will be presented.

TRANSCRIPTIONAL CONTROL OF CELL IDENTITY

Young, Richard A.

Whitehead Institute and Massachusetts Institute of Technology, USA

The gene expression program of any particular cell largely defines its identity and its functions. Learning how gene expression programs are controlled is important for understanding the control of cell identity and development. I will describe new insights into the control of gene expression programs in embryonic stem cells and iPS cells, as well as other cells that may be used for therapeutic purposes. These insights provide a new approach to assess the quality of reprogramming and promise to accelerate progress toward mapping the transcriptional control circuitry of all human cells.

ANNE McLAREN MEMORIAL LECTURE

SKIN STEM CELLS: IN SILENCE AND IN ACTION

Fuchs, Elaine

Rockefeller University, USA

How stem cells balance self-renewal and differentiation is of fundamental importance to our understanding of normal tissue maintenance and wound repair. Moreover, increasing evidence suggests that the regulatory circuitry governing this balancing act is at the root of some types of tumors both in mice and in humans. The hair follicle is an ideal model system for exploring how stem cell behavior is controlled and how this process goes awry in tumor progression. In the adult, hair follicles undergo cyclical bouts of tissue regeneration, destruction and rest. The hair cycle is fueled by stem cells located within a niche referred to as the bulge. In the mouse, hair cycles are synchronous, making them an especially attractive model to explore how quiescent stem cells become mobilized to actively regenerate tissue, how they self-renew to maintain a pool of stem cells, and how they return to quiescence following tissue production. This process is related to a challenge faced by many adult stem cells, namely to be able to respond quickly to injury, repair tissue and then return again to a quiescent state. It is also a process that goes awry in cancer.

Using the hair follicle as our paradigm, we've been dissecting the crosstalk that takes place between a stem cell and its microenvironment (stem cell niche). We're learning that these communication networks govern when stem cells will be activated to make tissue, and then determine the downstream transcriptional changes involved. Our findings provide us with an understanding of how the hair cycle works and why the resting phase gets longer as we age. Our studies also provide new insights into the process of stem cell activation and fate commitment. Finally, we've discovered that the mechanisms we've unearthed become deregulated in squamous cell carcinomas, among the most prevalent and life-threatening cancers world-wide.

Wednesday, June 12, 2013, 4:15 PM - 6:30 PM

Plenary Hall B1

Plenary II: Regeneration, Engraftment and Migration

Supported by The New York Stem Cell Foundation

DISSECTING HEMATOPOIETIC STEM CELL MICROENVIRONMENT

Frenette, Paul S.

Albert Einstein College of Medicine, USA

The identification of niche cells in the bone marrow has proved to be a challenging undertaking due to the complexity of its cellular constituents, the paucity of specific markers to accurately separate stromal cells, and its poorly accessible location in calcified bone. Prior studies have suggested that the endosteal region, populated by osteoblasts, was a niche maintaining quiescent hematopoietic stem cells (HSCs), whereas other studies have suggested that most HSCs are found near blood vessels. Through investigations to identify the mechanisms involved in HSC migration, we have previously described a key role of sympathetic adrenergic nerves in regulating CXCL12 synthesis and HSC retention in the bone marrow. Adrenergic impulses in the bone marrow microenvironment regulate circadian oscillations of HSC egress from the bone marrow via the β3 adrenoreceptor. We have hypothesized that stromal cells targeted adrenergic nerves would provide insight on the HSC niche. These analyses have led to the identification of Nestin+ mesenchymal stem cells (MSC), rare stromal population marked by transgenic expression of GFP under the Nestin promoter, as a putative niche cell. Evidence that Nestin+ MSC are niche cells include the significant histo-

logical association of HSC and MSC near blood vessels of the marrow, the enrichment in "niche factor" expression, including CXCL12, SCF, Angiopoietin-1, and VCAM-1 in Nestin+ cells, and the selective regulation of these factors upon mobilization. While adrenergic signals downregulate the expression of genes that retain HSC in the bone marrow, CD169+ macrophages have the opposite effect; they secrete one or more soluble factors that promote CXCL12 expression in Nestin+ cells. Thus, adrenergic nerves and CD169+ macrophages have antagonizing functions on HSC retention in the niche.
In addition to regulating HSC migration, adrenergic nerves maintain the quiescence of Nestin+ niche cells. Chemotherapy-induced neuropathy disrupts niche cell quiescence, rendering the niche vulnerable to genotoxic insult. Bone marrow neuropathy leads to defects in hematopoietic regeneration following sublethal irradiation or 5-fluorouracil challenge. Neuroprotection of neurotoxic chemotherapy or chemical sympathectomy rescues HSC proliferation and hematopoietic regeneration. Further recent data using whole-mount bone marrow imaging suggest that subsets of Nestin+ cells (high GFP vs low GFP) mark different vascular niches that regulate HSC behavior. These recent studies will be discussed in the context recent advances in our understanding of stem cell microenvironment in the bone marrow.

VISUALIZING STEM CELL BIOLOGY IN VIVO

Lin, Charles
Massachusetts General Hospital, USA

Stem cell migration, engraftment, and regeneration are activities that are dynamic in nature, but the slow pace of these events means lengthy observations are often necessary to reveal the underlying dynamics. Intravital microscopy (IVM) is a technique that enables real-time visualization of cell migration, proliferation, and cell-cell interaction with high spatial resolution in live animals, but matching the spatial scale and especially the temporal scale of observation to the biological problems being studied poses a significant challenge. In this talk I will highlight recent innovations in IVM that aim to overcome these challenges, as well as technical advances that will extend the capability of IVM beyond cell tracking to in vivo sensing and micromanipulation of the stem cell microenvironment.

LONG-TERM SINGLE CELL QUANTIFICATION: NEW TOOLS FOR OLD QUESTIONS IN STEM CELL RESEARCH

Schröder, Timm T.
Swiss Federal Institute of Technology (ETH) Switzerland

Stem cell systems are highly complex and dynamic, and consist of large numbers of different cells expressing many molecules controlling their fates. Despite intensive research, many long-standing questions in stem cell research remain unsolved. One major reason is that usually fates of populations of cells - rather than individual cells - are analyzed at very few time points of an experiment, and without knowing individual cell identities. Real-time tracking of individual cells in culture, tissues or whole organisms would be an extremely powerful approach to fully understand the developmental complexity of stem cell driven regeneration. We are therefore developing culture and imaging systems to follow the fate of individual cells over long periods of time. New software is programmed, helping to record and display the divisional history, position, properties, interaction etc. of all individual cells in a culture over many generations. Our approaches also allow the continuous long term quantification of protein expression levels or activity in living cells. This novel kind of quantitative data of single cell behavior and molecule expression is used as the basis for the improved generation and falsification of models describing the molecular control of stem cell fates. I will discuss how we use these approaches to try to find answers for long standing questions in hematopoiesis and pluripotency research.

ERNEST McCULLOCH MEMORIAL LECTURE

MILESTONES AND BARRIERS IN HEMATOPOIETIC STEM CELL DERIVATION FROM PLURIPOTENT STEM CELLS

Daley, George Q.
Boston Children's Hospital, USA

Pluripotent stem cells can be cultured from mammalian blastocysts as embryonic stem cells (ESCs) or derived via reprogramming of somatic cells (iPSC). Through their differentiation in vitro, ESC/iPSC represent tractable resources for the study of embryonic hematopoietic development and hold promise for modeling genetic diseases of the blood like immune deficiency, bone marrow failure, and hemoglobinopathy. While current protocols for directing hematopoietic differentiation faithfully recapitulate myeloid lineages, and there have been encouraging reports of NK, B and T cell development, recapitulating the various stages of hematopoietic ontogeny and producing bona fide hematopoietic stem cells (HSC) has proven elusive. Novel strategies to produce specific hematopoietic lineages, and to achieve the ultimate goal of HSC derivation will be discussed, alongside illustrations of the utility of ESC/iPSC in disease modeling.

Thursday, June 13, 2013 9:00 AM - 11:20 AM

Plenary Hall B1

Plenary III: Disease Modeling

Supported by California Institute for Regenerative Medicine (CIRM)

MODELING GENETIC VARIABILITY USING HUMAN INDUCED PLURIPOTENT STEM CELLS

Vallier, Ludovic[1], Brimpari, Mina[2], Rouhani, Foad[3], Cho, Candy[2], Rodríguez-Seguí, Santiago[4], Kumasaka, Natsuhiko[3], Bradley, Allan[5], Hanley, Neil[6], Ferrer, Jorge[7], Gaffney, Daniel[3]
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Human induced pluripotent stem cells are generated from somatic cells reprogrammed by overexressing transcription factors. Their pluripotency status confers upon them the capacity to proliferate indefinitely in vitro while maintaining their capacity to differentiate into a broad number of cell types. hIPSCs represent an unique opportunity for regenerative medicine since they could enable the production of patient specific cell types which are fully immuno-compatible with the original donor thereby avoiding the use of immune suppressive treatment during personal cell based therapy. In addition, hIPSCs can be generated from somatic cells isolated from patients suffering from diverse diseases. Then, the resulting "diseased" hIPSCs can be differentiated into the cell type targeted by the disease and thus provide in vitro models to perform large scale studies impossible with primary cell culture or with biopsy material. However, hIPSC lines appear to strongly vary in their capacity of differentiation and the origin of this variability remains unclear.
Here, we derived a panel of 50 hIPSCs lines from 15 patients using different tissues of origin and reprogramming methods. We then analysed the capacity of the resulting lines to differentiate into the three germ layers endoderm, mesoderm and neuroectoderm using fully chemical defined culture conditions. We showed that hIPSC lines can be grouped into two distinct categories: hIPSCs lines with a high capacity to produce mesoendoderm cells and hIPSC lines with a high capacity to produce neuroectoderm cells. These observations were further validated in vivo by showing that hIPSC lines with extreme phenotype could generate teratomas highly enriched with endodermal or neuroectodermal derivatives. Importantly, hIPSC lines derived from one individual systematically belong to the same category independently of the tissue of origin, passage number, method of reprogramming or reprogramming experiments. To further uncover the mechanisms dictating variability between hIPSC lines, we also performed RNA-seq on a panel of 53 human iPSC lines and corresponding somatic cells. We show that, relative to variation between tissue of origin has a minor effect on the transcriptome variability of iPSCs. However, we also identified allele-specific expression at many non-imprinted genes illustrating the potential of these cells for studying the function of naturally occurring genetic variation during development and differentiation.
Considered together, these results suggest that variability between hIPSCs can be an inherent consequence of their genetic background. They also show that hIPSC could represent a unique tool to model the genetic mechanisms imposing variability between individuals and also involved in individual onset of diseases.

USING INDUCED PLURIPOTENT STEM CELLS TO UNDERSTAND ALZHEIMER'S DISEASE

Goldstein, Lawrence S.B.
University of California, San Diego, USA

Stem cell technologies bring new and powerful opportunities to understand and treat previously untreatable brain disorders. In my talk, I will discuss how we are trying to use human stem cells to develop new methods to understand, and eventually treat Alzheimer's Disease and other related diseases of the brain by generating new approaches for understanding disease mechanisms and drug discovery. For example, Alzheimer's Disease is a severe, progressive, and incurable disease characterized by memory loss and dementia. Although common pathological features of the disorder are known, their relationship to the development of the disease remains problematic. Rare hereditary forms of Alzheimer's Disease identify genetic changes that can cause disease. In one approach, we are working to generate human neurons from human stem cells that carry the genetic changes that can cause hereditary Alzheimer's Disease to try and test several ideas about what causes this terrible disease. However, the relationship of hereditary forms of Alzheimer's Disease to the common "sporadic" form of the disease remains unclear. Human stem cell technology is letting us begin to probe the relationship of the two forms of the disease and to assess mechanisms of risk factor action at the cellular level.

UNRAVELING NEURODEGENERATIVE-DISEASE MECHANISMS USING PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS

Inoue, Haruhisa
Center for IPS Cell Research & Application, (CiRA), Japan

Neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS) and Alzheimer's disease (AD), were discovered more than 100 years ago. Many approaches and modelings of the diseases have been used to unravel the disease mechanisms. However, we still do not understand them completely, since it has been difficult to analyze patient neural cells directly, at least before the appearance of induced pluripotent stem cell (iPSC) technology. We have been analyzing ALS and AD neural cells from patient-specific iPSCs to reveal the disease mechanisms that may trigger sporadic, as well as familial, forms of the diseases. In this session, I would like to present our data and discuss disease mechanisms of neurodegenerative diseases.
We generated motor neurons from iPSCs from familial ALS patients, who carry mutations in Tar DNA binding protein-43 (TDP-43). ALS patient-specific iPSC-derived motor neurons recapitulated ALS-associated phenotypes, and they were used for further analysis. We found that motor neurons generated from ALS patient-derived iPSCs may provide a useful tool for clarifying ALS disease pathogenesis as well as for screening drug candidates.

HUMAN INDUCED PLURIPOTENT STEM CELLS FOR CARDIOVASCULAR DISEASE MODELING

Wu, Joseph C.
Stanford University School of Medicine, USA

The successful derivation of human iPS cells from somatic cells offers significant potential to overcome obstacles in the field of cardiovascular disease. hiPSC-derived cardiomyocytes can now provide incredible potential for disease modeling in vitro and regenerative medicine therapies in vivo. This talk will focus on the usage of iPSC-CMs for (i) cardiac disease modeling, (ii) drug screening, and (iii) cell therapy.

Thursday, June 13, 2013 1:15 PM - 3:05 PM

Ballroom East

Concurrent IA: Pluripotent Stem Cells I

Supported by Ontario Stem Cell Initiative

THE EPIGENETIC STABILITY OF PLURIPOTENT AND SOMATIC CELL STATES

Hanna, Jacob
Weizmann Institute, Israel

The identity of somatic and pluripotent cells can be epigenetically reprogrammed and forced to adapt a new functional cell state by different methods and distinct combinations of exogenous factors. The aspiration to utilize such ex vivo reprogrammed pluripotent and somatic cells for therapeutic purposes necessitates understanding of the mechanisms of reprogramming and elucidating the extent of equivalence of the in vitro derived cells to their in vivo counterparts. I will present and analyze our recent advances toward understanding these fundamental questions.
I will further highlight future possibilities for utilizing epigenetic reprogramming for experimental and theoretical modeling of gene expression regulation, cell fate decisions and early mammalian development.

MIR-290 AND MIR-302 CLUSTERS DEFINE SEQUENTIAL STATES OF PLURIPOTENCY DURING DEVELOPMENT THAT ARE NOT RECAPITULATED IN REVERSE DURING REPROGRAMMING

Parchem, Ron, Judson, Robert, La Russa, Marie, Blelloch, Robert
University of California, San Francisco, USA

The transcription factors Oct4, Sox2, and Klf4 (OSK) are sufficient to induce somatic cells such as fibroblasts to reprogram into induced pluripotent stem cells (iPSCs), which functionally resemble embryonic stem cells (ESCs) derived from early blastocyst stage embryos. It remains unclear whether reprogramming of an individual fibroblast cell is accomplished by an actual reversal of the stages of normal development or by direct conversion to a pluripotent state. Here, we use novel markers of pluripotency to follow reprogramming of individual cells in real-time. Red (mCherry) and green fluorescent protein (GFP) reporters were targeted to the microRNA (miRNA) clusters, miR-290 and miR-302, respectively. In the developing mouse, pluripotent cells expressed only miR-290-mCherry at the blastocyst stage (embryonic day 3.5, or E3.5), both miR-290-mCherry and miR-302-GFP at E5.5, and only miR-302-GFP at E7.5, thus defining three distinct and transient pluripotent states. Similarly, naïve ESCs derived from blastocysts expressed miR-290-mCherry exclusively and primed epiblast stem cells (EpiSCs) derived from epiblast expressed miR-302-GFP exclusively. Differentiation of ESCs in vitro revealed identical transitions in

reporter expression to those seen in vivo, demonstrating the presence of all three pluripotent states during in vivo development and in vitro differentiation. During reprogramming to iPSCs, however, cells rarely passed through the intermediate pluripotent states marked by miR-302-GFP. Moreover, the two miRNA loci were activated stochastically rather than in reverse order. Similarly, Oct4-GFP and miR-290-mCherry were activated in random order. Efficiency of individual cells to form iPSC colonies was correlated with the number of activated pluripotency loci rather than the order with which the miR-290, miR-302 and Oct4 loci were activated. Together, these results identify a defined order of marker expression during normal differentiation, both in vivo and in vitro, that is absent during de-differentiation. We conclude that reprogramming bypasses intermediate pluripotent states and instead transitions directly to a naïve-like identity.

BET PROTEINS REGULATE EMBRYONIC STEM CELL IDENTITY BY CONTROLLING TRANSCRIPTIONAL ELONGATION OF CORE PLURIPOTENCY GENES

Di Micco, Raffaella[1], Fontanals-Cirera, Barbara[1], Tsirigos, Aristotelis[2], Ntziachristos, Panagiotis[1], Aifantis, Iannis[1], Zhou, Ming-Ming[3], Hernando, Eva[1]
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Limited understanding of the molecular mechanisms that govern self-renewal and pluripotency of embryonic stem cells (ESC) represents a major barrier towards stem cells therapeutic applications. Transcription factors and chromatin remodeling complexes are key determinants of ESC identity. The bromodomain and extra-terminal domain (BET) family of proteins regulates chromatin dynamics through modulating acetylation-mediated protein-chromatin interactions critical for gene transcription. Here, we sought to investigate the mechanistic role of BET proteins in governing ESC properties, self-renewal and pluripotency. Small-molecule chemical inhibition of the BET bromodomain acetyl-lysine binding activity significantly reduced the number of undifferentiated human and mouse ESC colonies, without affecting their viability. Moreover, BET inhibition compromised the ability of ESC to self-renew and to form embryoid bodies (EB). Global gene expression profile of compound-treated human ESC revealed a marked suppression of the pluripotency gene program, including OCT4 and PRDM14. Chromatin immunoprecipitation followed by next generation sequencing (ChIP-seq) of compound-treated human ESC revealed preferential displacement of BRD3 and BRD4 proteins from the transcription start sites (TSS) and gene bodies of stem cell genes. ChIP-based assessment of RNA polymerase II (Pol II) density along gene sequences and of the transcriptional elongation marks H3 lysine 36 trimethylation (H3K36me3) and Pol II serine-2 phosphorylation (Pol II pS2) revealed a specific defect in the elongation of pluripotency genes, following BET displacement. Moreover, consistent with the reported association of BRD4 with the super elongation transcriptional complex (SEC), Pol II was preferentially co-displaced with BRD4 from the gene bodies of down-regulated stem cell genes. Conversely, the expression of epithelial to mesenchymal transition (EMT) markers and neuroectodermal lineage genes increased following compound treatment in a BET-independent manner. Individual RNAi knockdown of BRD4, but not BRD2 or BRD3, phenocopied the effects of BET chemical inhibition on ESC, and teratomas derived from Brd4-suppressed ESC exhibited a marked defect in multidifferentiation potential. In addition, both BET inhibition and Brd4 suppression impaired the somatic reprogramming of fibroblasts to induced pluripotent stem cells (iPSC). All together, these results unravel a key role for BET proteins, and particularly for BRD4, in regulating the transcriptional elongation of the pluripotency network essential to maintain the ESC state. Our findings add a new level to the complex ESC regulatory system and could be exploited to improve the efficiency of ESC differentiation and somatic reprogramming.

THE LET-7 TARGET LIN-41 PROMOTES INDUCED PLURIPOTENT STEM CELL REPROGRAMMING

Worringer, Kathleen, Hayashi, Yohei, Sami, Salma, Srivastava, Deepak, Yamanaka, Shinya
Gladstone Institutes, San Francisco, CA, USA

Fibroblasts can be directly reprogrammed into induced pluripotent stem cells (iPSCs) by expressing the pluripotency genes OCT4, SOX2, KLF4, and c-MYC (OSKM). Without c-MYC, reprogramming efficiency is greatly reduced. We found that inhibiting the let-7 family of microRNAs in human cells promoted reprogramming with OSK alone to a level of efficiency comparable to reprogramming with OSKM, and that persistence of let-7 inhibited reprogramming. The let-7 target LIN-41/TRIM71, a TRIM-NHL protein, was upregulated during reprogramming, and LIN-41 activity was important for normal reprogramming efficiency. Furthermore, LIN-41 expression partially rescued the decrease in reprogramming efficiency observed upon let-7 overexpression. These data indicate that LIN-41 is one component of the mechanism by which let-7 inhibition promotes reprogramming.

Poster Briefs

SUPER-ENHANCERS AT KEY CELL IDENTITY GENES

Hnisz, Denes, Whyte, Warren A., Orlando, David A., Abraham, Brian J., Lin, Charles Y., Rahl, Peter B., Lee, Tong Ihn, Young, Richard A.

VASCULARIZED AND FUNCTIONAL HUMAN LIVER TISSUE FROM AN INDUCED PLURIPOTENT STEM CELL-DERIVED ORGAN BUD TRANSPLANT

Takebe, Takanori, Sekine, Keisuke, Enomura, Masahiro, Koike, Hiroyuki, Zhang, Ran-Ran, Kimura, Masaki, Ogaeri, Takunori, Koike, Naoto, Ueno, Yasuharu, Zheng, Yun-Wen, Taniguchi, Hideki, Yokohama City University, Yokohama, Japan

UNDERSTANDING EARLY FATE DECISIONS BY STUDYING HETEROGENEITY IN HUMAN PLURIPOTENT STEM CELLS

Bhatia, Sonam, Pilquil, Carlos, Draper, Jonathan
Department of Biochemistry and Biomedical Sciences, McMaster Unviersity, Hamilton, ON, Canada

GENOMIC ANALYSIS OF HESC PEDIGREES ENABLES IDENTIFICATION OF DE NOVO GENETIC ALTERATIONS AND DETERMINATION OF THE TIMING AND ORIGIN OF MUTATIONAL EVENTS

Ben Yosef, Dalit[1], Boscolo, Francesca S.[2], Amir, Hadar[1], Malcov, Mira[1], Lynch, Candace[3], Zdravkovic, Tamara[4], Genbacev, Olga[4], Amit, Ami[5], Loring, Jeanne F.[3], Fisher, Susan[6], Laurent, Louise C.[7]
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Human pluripotent stem cells, including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), have tremendous potential as sources of material for cell transplantation therapy. However, given the association between a high mutational load and cancer, and the observation that genetic aberrations are frequently found in human pluripotent stem cell cultures, the potential genetic instability of human pluripotent stem cells is of significant concern. Prior studies in hiPSCs have shown that deletions and regions of loss-of-heterozygosity (LOH) tend to arise during reprogramming and early culture, while duplications more frequently occur during long-term culture. The procedures involved in reprogramming to generate hiPSCs and derivation of hESCs are quite different. Most significantly, all current reprogramming methods involve increasing the expression or concentration of pluripotency-associated factors, often through integration of exogenous transgenes; these manipulations may increase the incidence or accumulation of genetic aberrations. Therefore, it is reasonable to hypothesize that early in their derivation, hiPSC and hESC lines are subjected to different mutagenic and selective pressures.
The relevant experiments in hESCs have not been previously conducted, due to the lack of availability of DNA from parents or siblings related to the blastocysts from which the hESC lines were derived. We have overcome this obstacle by using two strategies: for one set of hESC lines, we obtained DNA from the parents who donated the blastocysts; and a second set of hESC lines were generated from single blastomeres from four sibling embryos. Using a combination of cytogenetic and molecular analysis approaches, including high-resolution SNP genotyping, karyotyping, and STR analysis, we have identified de novo copy number variants in hESCs, defined the stages at which these events occurred, and traced the parent of origin of the affected alleles.
Our results show that early stages in the generation of hESCs (including preimplantation embryo development, hESC derivation and early passage) are prone to deletions and LOH, while duplications arise more commonly during long-term culture of hESCs. These results highlight the importance of close monitoring of genomic integrity and the development of improved methods and reagents for derivation and culture of human pluripotent stem cell cultures to minimize selection for genetically abnormal cells and ensure the safety of pluripotent stem cell-derived cells for clinical use.

1:15 PM - 3:05 PM

Room 253

Concurrent IB: Neural Stem Cells

STEM CELLS AND THEIR NICHE IN THE ADULT MAMMALIAN BRAIN

Doetsch, Fiona
Columbia University, USA

Stem cells persist in specialized niches in the adult mammalian brain where they continuously generate large numbers of neurons that become functionally integrated into neural circuits. The subventricular zone (SVZ) is an extensive germinal layer adjacent to the lateral ventricles. A subset of astrocytes are stem cells in this region and generate rapidly dividing transit-amplifying cells, which in turn produce neuroblasts that migrate to the olfactory bulb. A major limitation in the neural stem cell field has been the ability to prospectively purify stem cell astrocytes from other brain astrocytes. We have recently developed a simple method to simultaneously purify each SVZ cell type in the lineage by means of fluorescence activated cell sorting, including quiescent stem cells for the first time. I will present our recent findings about the functional and molecular properties of quiescent and activated adult neural stem cells, and about two compartments of the specialized niche that support adult neurogenesis, the vasculature and the cerebrospinal fluid.

ADULT V-SVZ NEURAL STEM CELLS HAVE A DISTINCT EMBRYONIC ORIGIN

Fuentealba, Luis C.[1], Rompani, Santiago[2], Romero, Ricardo[1], Cepko, Constance L.[3], Alvarez-Buylla, Arturo[1]
[1]Neurological Surgery and Institute for Regeneration Medicine, University of California, San Francisco, CA, USA, [2]Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland, [3]Departments of Genetics and Ophthalmology, Howard Hughes Medical Institute, Harvard Medical School, Boston, MA, USA

Neural stem cells (B1 cells) are retained in the ventricular-subventricular zone (V-SVZ) next to the striatum, cortex and septum of the adult rodent brain. These cells represent an exception to the general rule that neural stem cells are present only in development. They continue to produce, throughout life, large numbers of young neurons that migrate into the olfactory bulb to differentiate into granular and periglomerular cells. B1 cells have marker expression and morphological characteristics of astrocytes, but also retain neuroepithelial characteristics, including basal contacts with blood vessels and an apical compartment that contacts the ventricle. These adult B1 cells are derived from radial glia (RG), the neural stem cells in the developing embryonic brain. It has been suggested that adult neural stem cells are modified RG and are derived from neural stem cells that, earlier in development, produce striatal, cortical and septal neurons. While this hypothesis is widely accepted, there is no direct proof that the neural stem cells in the adult are derived from these embryonic cells. We first asked when in embryonic development there were mitotic cells that generated these adult stem cells. Using BrdU and in utero delivery of GFP-expressing retroviruses, we found that the majority of adult neural stem cells were generated from embryonic cells that divided during mid-late stages of development. These results indicate that this subpopulation of embryonic cells generates precursors of B1 cells that remain relatively quiescent until they are reactivated later to produce adult olfactory neurons. This observation raised the alternative hypothesis that adult neural stem cells may be uniquely specified, i.e. they may be generated in a separate lineage that is distinct from the lineage that generates the neurons destined for other forebrain locations. To test this hypothesis, we performed clonal lineage analysis of forebrain progenitor cells. We injected embryonically a library of GFP-expressing retroviruses, each containing a unique sequence or "barcode". Nestin-CreER;R26tdTomato transgenic mice were used to label adult stem cells and their neuronal progeny. The barcode sequence from individual GFP/tdTomato-labeled interneurons in the olfactory bulb was amplified and sequenced. We also identified the barcode of GFP-labeled neurons in the striatum, cortex and septum (i.e. cells born in the embryo). If the adult-generated OB neurons shared their origin with embryonically-generated forebrain neurons, they should have the same barcode. Out of 700 clones analyzed, we have not found any sibling relationships between forebrain neurons born in the embryo and OB interneurons generated by adult stem cells. These data provide strong support for the second hypothesis, that a subpopulation of RG cells serves as precursors of postnatal adult stem cells (B1 cells) in the V-SVZ. Determining the identity and characteristics of these distinct RG, as well as defining the progeny that they make, will help us to understand the mechanisms that lead to the formation of adult neural stem cells.

OLIGODENDROGLIOGENIC AND NEUROGENIC ADULT SUBEPENDYMAL ZONE NEURAL STEM CELLS CONSTITUTE DISTINCT LINEAGES AND EXHIBIT DIFFERENTIAL RESPONSIVENESS TO WNT SIGNALING

Berninger, Benedikt[1], Ortega, Felipe[1], Gascón, Sergio[2], Masserdotti, Giacomo[2], Deshpande, Aditi[2], Simon, Christiane[2], Fischer, Judith[3], Dimou, Leda[2], Lie, Dieter Chichung[4], Schröder, Timm[5]
[1]Institute of Physiological Chemistry, University Medical Center Johannes Gutenberg University Mainz, Mainz, Germany, [2]Physiological Genomics, Ludwig-Maximilians University Munich, Munich, Germany, [3]Institute of Stem Cell Research, Helmholtz Zentrum München, Munich, Germany, [4]Institute of Biochemistry, Emil Fischer Center, University Erlangen-Nürnberg, Erlangen, Germany, [5]Institute of Stem Cell Dynamics, Helmholtz Zentrum München, Munich, Germany

The adult mouse subependymal zone (SEZ) harbors adult neural stem cells (aNSCs) that give rise to neuronal and oligodendroglial progeny. However it is not known whether the same aNSC can give rise to neuronal and oligodendroglial progeny or whether these distinct progenies constitute entirely separate lineages. Continuous live imaging and single cell tracking of aNSCs and their progeny isolated from the mouse SEZ revealed that aNSCs exclusively generate oligodendroglia or neurons, but never both within a single lineage. Moreover, activation of canonical Wnt signaling selectively stimulated proliferation within the oligodendrogliogenic lineage, resulting in a massive increase in oligodendrogliogenesis without changing lineage choice or proliferation within neurogenic clones. In vivo activation or inhibition of canonical Wnt signaling respectively increased or decreased the number of Olig2 and PDGFR-α positive cells, suggesting that this pathway contributes to the fine tuning of oligodendrogenesis in the adult SEZ.

VCAM1 IS A NICHE FACTOR IN THE DEVELOPING MOUSE TELENCEPHALON ACTING THROUGH BOTH CELL AUTONOMOUS AND NON-AUTONOMOUS EFFECTS

Hu, Xiao-Ling[1], Wang, Yue[2], Phoenix, Timothy N.[2], Temple, Sally[2], Shen, Qin[1]
[1]Tsinghua University, Beijing, China, [2]Neural Stem Cell Institute, Rensselaer, NY, USA

It has been recognized long time ago that in the developing mammalian cerebral cortex neurogenesis is accompanied by progressive patterning of blood vessels in the brain parenchyma. However, we have just begun to understand the molecular interaction between neural cells and the vasculature during embryonic development. Recently, we showed that vascular cell adhesion molecule 1 (VCAM1) or cluster of differentiation 106 (CD106), a cell surface sialoglycoprotein first identified on cytokine-activated endothelium, is expressed on the endfeet of Type B cells in the center of pinwheels in the adult neurogenic niche, the subventricular zone. Here we found that VCAM1 is expressed on radial glia cells and becomes gradually enriched in the apical and basal end feet during the development of the telencephalon. Knockdown of endogenous VCAM1 in neural progenitor cells in vitro affects neural progenitor cell proliferation and differentiation. Interestingly, conditional deletion of VCAM1 using Nestin-Cre or Emx1-Cre mouse lines led to reduced vessels density and abnormal blood vessel patterning in the cerebral cortex. We found that embryonic brain endothelial cells express high level of known VCAM1 receptors such as alpha4 and alpha9 integrins as well as VCAM1 interacting protein Sparc. Overexpression of VCAM1 in NSCs significantly promotes endothelial cells proliferation, sprouting and tube formation in vitro. To understand the molecular mechanism underlying VCAM1-mediated interaction between radial glial cells and endothelial cells, we compared the gene expression profiles in embryonic telencephalon between VCAM1 cKO and control mice using RNA-seq and identified potential signaling pathways involved in this process. Our study suggests that VCAM1 is a critical niche factor in the developing brain, regulating radial glia development and their interaction with the developing vasculature.

Poster Briefs

INTEGRATION OF GENOME-WIDE APPROACHES IDENTIFIES LNCRNAS OF ADULT NEURAL STEM CELLS AND THEIR PROGENY IN VIVO

Ramos, Alexander D.[1], Diaz, Aaron[2], Nellore, Abhinav[2], Delgado, Ryan N.[1], Park, Ki-Youb[1], Gonzalez-Roybal, Gabriel[1], Oldham, Michael C.[3], Song, Jun S.[4], Lim, Daniel A.[1]
[1]Department of Neurological Surgery, Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, UCSF, San Francisco, CA, USA, [2]Institute for Human Genetics, UCSF, San Francisco, CA, USA, [3]Department of Neurology, Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, UCSF, San Francisco, CA, USA, [4]Institute for Human Genetics, Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, UCSF, San Francisco, CA, USA

MITOCHONDRIAL P53-DEPENDENT REGULATION OF MITOCHONDRIAL DAMAGE AND NEURAL STEM CELL FATE

Solá, Susana[1], Xavier, Joana[2], Morgado, Ana Luísa[1], Rodrigues, Cecília MP[1]
[1]Department of Biochemistry and Human Biology, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal, [2]Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal

INDUCTION OF HUMAN PLURIPOTENT STEM CELLS TO A PRE-NEUROEPITHELIAL STATE TO GENERATE FLOOR PLATE AND ROOF PLATE PROGENITORS

Denham, Mark[1], Hasagawi, Kouichi[2], Zhang, Dong[3], Hough, Shelley[1], Menheniott, Trevelyan[3], Leung, Jessie[1], Rollo, Ben[3], Newgreen, Don[3], Pera, Martin[1], Dottori, Mirella[1]
[1]University of Melbourne, Parkville, Australia, [2]Institute for Integrated Cell-Material Sciences, Kyoto, Japan, [3]Murdoch Childrens Research Institute, Melbourne, Australia

A NOVEL NEURAL STEM CELL FACTOR WITH POTENT ROLES IN REGULATING BRAIN SIZE

Goetz, Magdalena[1], Stahl, Ronny[1], Walcher, Tessa[1], deJuan Romero, Camino[2], Pilz, Gregor[1], Silvia Cappello, Silvia Cappello[1], Beckers, Johannes[3], Irmler, Martin[4], Blum, Robert[5], Borrell, Victor[6]
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Organ size is regulated by balancing stem cell self-renewal versus the generation of differentiated progeny or transit-amplifying progenitors to enlarge the progeny number. This is of particular relevance in brain development as the evolution of the mammalian brain encompassed a remarkable increase in size of specific brain regions, such as the cerebral cortex. This process encompasses expansion in the tangential (larger brain area) and radial (higher number of neurons per brain area) dimension. However the mechanisms underlying these key features are still largely unknown. Here, we identified the novel DNA associated protein Trnp1, which is highly conserved only in mammals, as a regulator of neural stem cell self-renewal and mammalian cerebral cortex expansion. Its dynamic regulation during brain development together with gain and loss of function experiments in the mouse cerebral cortex in vivo demonstrate that higher Trnp1 levels promote neural stem cell self-renewal and tangential expansion. In contrast, lower levels promote radial expansion with a potent increase of the number of intermediate progenitors and basal radial glial cells leading to folding of the otherwise smooth murine cerebral cortex. Remarkably, TRNP1 expression levels exhibit regional differences in the cerebral cortex of human fetuses anticipating radial or tangential expansion. Thus, the dynamic regulation of Trnp1 is critical to regulate tangential expansion by promoting neural stem cell self-renewal while its reduction results in rapid amplification of transient progenitor lineages causing a fast increase in neuron numbers and their appropriate guiding structures. As Trnp1 is also expressed in other organs, this exciting and potent new factor may play similar roles in other mammalian stem cell lineages.

Thursday, June 13, 2013 1:15 PM - 3:05 PM

Room 258

Concurrent IC: Stem Cells and Tissue Engineering

BIOENGINEERED STRATEGIES TO PROMOTE TISSUE AND FUNCTIONAL REPAIR IN THE CENTRAL NERVOUS SYSTEM

Shoichet, Molly S.[1], Cooke, Michael J.[1], Ballios, Brian G.[1], Wang, Yuanfei[1], Tam, Roger Y.[1], Mothe, Andrea[2], Tuladhar, Anup[1], Fuehrmann, Tobias[1], Tator, Charles H.[2], van der Kooy, Derek[1], Morshead, Cindi M.[1]
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Stem cell strategies to promote tissue repair after disease or injury provide significant promise and can be achieved through both exogenous stem cell transplantation and endogenous stem cell stimulation. We have designed an innovative hydrogel comprised of hyaluronan and methyl cellulose (HAMC) that itself has some therapeutic benefit and can be combined with both cells and/or biomolecules to promote tissue regeneration and functional repair. We have explored the use of this biomaterial for both stem cell and biomolecule delivery to the retina, brain and spinal cord with the goals of ultimately overcoming blindness, stroke and spinal cord injury, respectively.
For retina repair, we explored the transplantation of adult retinal stem cell-derived rod photoreceptors in the HAMC hydrogel. In early studies, we found that the hydrogel promoted the distribution of stem cell progeny in the subretinal space after transplantation, thereby enhancing greater interaction with host tissue. Successful transplantation of the rods was dependent on the maturity of the differentiated cells as well as their co-delivery in HAMC and a small molecule gliotoxin. Using this multi-pronged strategy, we achieved significantly greater cell survival and integration with the host tissue. Importantly, the HAMC itself was shown to have a pro-survival effect on the photoreceptor rods. This exciting finding has been studied with other stem cells and their differentiated progeny as well.
For spinal cord repair, we examined the survival and efficacy of adult brain derived neural stem/progenitor cells injected within a platelet-derived growth factor (PDGF-A)-modified HAMC into a subacute, clinically-relevant clip compression model of rat spinal cord injury (SCI). Relative to controls, SCI rats transplanted with NSPCs in HAMC-rPDGF-A showed a significant reduction in cavitation, sparing of perilesional host oligodendrocytes and neurons and improved behavioral recovery, demonstrating the added benefit of the bioengineered hydrogel for the co-delivery of growth factors and stem cells.
For tissue repair after stroke, we investigated endogenous stem cell stimulation of the adult neural stem/progenitor cells of the subventricular zone that line the lateral ventricles. By taking advantage of the success with sequential delivery of epidermal growth factor (EGF) and erythropoietin (EPO) directly into the ventricles in a rodent model of stroke, we designed a minimally-invasive strategy where these drugs were encapsulated in polymeric nanospheres, dispersed in HAMC and applied directly on the mouse brain cortex. In this way, the blood-brain barrier was circumvented and the invasive strategy associated with intraventricular delivery obviated. Here, HAMC was shown to attenuate the inflammatory response and the local release of EGF and EPO promoted tissue repair.
These three examples of exogenous stem cell transplantation and endogenous stem cell stimulation demonstrate the promise of stem cells for regenerative medicine and the key role that bioengineering plays in bringing this promise to fruition.

INJECTION OF PEPTIDE NANOGELS AUGMENTS AND PROLONGS THE THERAPEUTIC EFFICACY OF AUTOLOGOUS BONE MARROW CELLS IN CARDIAC REPAIR

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Background_Because hearts have a limited capacity of self-healing, stem cell therapy has emerged as a promising approach for cardiac repair. However, numerous clinical trials showed only modest and transient benefits and thus impedes clinical realization. Poor cell retention is one of the major hurdles to overcome. Moreover, absence of microenvironmental support largely lessens the therapeutic utility of the remained cells. In this regards, we have previously demonstrated that intramyocardial injection of self-assembling peptide nanofibers (NFs) increases the retention and vascular differentiation of transplanted bone marrow mononuclear cells (MNCs) in pigs. Accordingly, the combined therapy significantly improves cardiac performance at 1 month after myocardial infarction (MI). In the present study, we further test whether the combined therapy remains effective in a long-term manner.
Materials & Results_A total of 26 mature minipigs were divided into 5 groups: sham, MI + normal saline (NS), MI + NFs, MI + MNCs, and MI + MNCs/NFs ($n \geq 5$ in each group). MI was induced by coronary occlusion followed by intramyocardial injection of 2 mL NS or 1% NFs with or without 1×108 isolated autologous MNCs over the infarct and peri-infarct areas. Cardiac functions were assessed by echocardiography immediately before and after MI, 1 and 2 months thereafter, and together with catheterization 3 months post-MI. Although injection of MNCs alone slightly improved cardiac systolic function at 1 month post-MI, this benefit was not found beyond the 2-month time point. In contrast, injection of MNCs with NFs not only provided greater cardiac benefit but also persisted for at last 3 months (ejection fraction: 42.9±2.5 in MI + NS and 52.1±1.2 in MI + NFs/MNCs, $p < 0.001$; +dP/dt: 1305±41 in MI + NS and 1959±184 in MI + NFs/MNCs, $p < 0.05$). Furthermore, there were more endogenous α-smooth muscle actin (SMA)-positive cells recruited to the NF injected region at 3 months after MI, which play a role in preserving cardiac diastolic function (-dP/dt: -1038±186 in MI + NS, -1773±187 in MI + NFs and 1859±143 in MI + NFs/MNCs, $p < 0.05$ compared to MI + NS) and preventing pathological remodeling (infarct size: 29.6±1.4 in MI + NS, 21.5±1.4 in MI + NFs and 18.4±2.5 in MI + NFs/MNCs, $p < 0.01$ compared to MI + NS). Importantly, these SMA-positive cells can be favourable for forming durable arteries and arterioles when MNCs were co-transplanted with NFs (artery density: 2.3±0.5 in MI + NS and 6.4±1.2 in MI + NFs/MNCs, $p < 0.01$; arteriole density: 16.8±1.4 in MI + NS and 36.6±3.5 in MI + NFs/MNCs, $p < 0.001$).
Conclusion_To our knowledge, this is the first study demonstrating the

inadequacy of stem cell treatment alone can be improved and prolonged through combined injection of stem cells and nanogels in a large animal model. We expect the unprecedented study offer a potential solution for conventional stem cell therapy, which could be translated into therapeutic application in the foreseeable future.

TETHERED EPIDERMAL GROWTH FACTOR ENHANCES BONE MARROW-DERIVED HUMAN CONNECTIVE TISSUE PROGENITOR COLONY FORMATION IN VITRO AND PROGENY SURVIVAL POST-TRANSPLANTATION

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Connective Tissue Progenitors (CTPs), a heterogeneous population of stem and progenitor cells found in native tissue, play an essential role in the repair of critical-sized bone defects, as evidenced by poor outcomes in patients with CTP deficiencies. Their low prevalence in bone marrow (~1:20,000 nucleated cells) presents a challenge towards their efficient use as a single point-of-care treatment. When used therapeutically, their post-transplantation survival is low due to the hypoxic, pro-apoptotic microenvironment of the bone wound. Extracellular ligands that can increase survival and expansion of CTPs in vivo should advance regeneration. One such ligand that is capable of sustaining CTP colony formation and growth in vitro is the Epidermal Growth Factor (EGF). This growth factor, when presented in a tethered format (tEGF), can induce additional adhesive and cytoprotective effects on CTPs and their progeny. Our overall goal is to test whether delivery of CTPs along with an EGF-tethered osteoconductive carrier (Beta-tricalcium phosphate; βTCP) can enhance the transplantation and survival of these cells. Osteoconductive calcium phosphate matrices such as βTCP lack functional groups for bioconjugation. For this reason, our EGF-tethering strategy involves the use of a high-affinity βTCP binding peptide that was identified by phage display and fused to EGF (βTCPbp-EGF). Binding of βTCPbp-EGF to βTCP was achieved by incubation of the protein solution with the substrate for a period of 24-36 hours. Tethered substrates stored in PBS retained more than 75% of the initially-bound βTCPbp-EGF (tEGF) after a 7-day, 37˚C incubation. These EGF-tethered βTCP substrates induced a 2.5-fold increase in the number of CTP-derived progeny (low-passage human Mesenchymal Stromal Cells; MSCs) after 7 days of culture ($N=4$, $p<0.01$). Bone-marrow samples from 8 patients were assayed in vitro for their potential to form osteogenic CTP colonies in the presence of tEGF using βTCP coverslips. On average, a 60% increase in CTP osteogenic colony forming efficiency was seen under tEGF conditions ($N=3$, $p=0.01$). Further testing in a heterotopic xeno-transplantation mice model revealed an increase in early and late-stage survival of transplanted MSCs (T-MSCs) in EGF-tethered BTCP carriers. At 2 days post-transplantation, a 4-fold higher number of T-MSCs was observed in the tEGF explants ($N=3$ mice per condition; $p<0.001$). After 3 weeks, the number of T-MSCs in the tEGF condition was 15-fold higher than controls ($N=3$ mice per condition; $p<0.05$). Aside from the survival benefits of tEGF, we observed qualitative increase in the vascularity of the tEGF explants. No inflammatory response towards the recombinant BTCPbp-EGF protein was observed. Overall, these results show that tEGF delivered along with a BTCP carrier has the potential to greatly enhance the survival of CTPs both in vitro and in vivo, leaving room for future testing in a more relevant bone defect model.

IN VITRO RECONSTITUTION OF THE EMBRYONIC NEURAL TUBE FROM MOUSE EMBRYONIC AND MOUSE INDUCED PLURIPOTENT STEM CELLS

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Center for Regenerative Therapies Dresden, TU Dresden, Dresden, Germany

Our goal is to develop a three-dimensional (3D) culture system for neuroepithelial cells derived from mouse embryonic stem (mES) or induced pluripotent stem (iPS) cells that will reproduce embryonic growth and patterning of the neural tube in vitro. We developed a novel 3D culture protocol that enables the generation of a polarized neuroepithelium from single mES cells. Using a reporter cell line for neural differentiation (46C), in which green fluorescent protein (GFP) is expressed under the control of the sox1-promoter, we obtained neural cysts showing uniform expression of GFP. Neural cysts grow clonally as evidenced by time-lapse analysis and form round spheres harboring a single lumen and resemble morphologically a developing neural tube. During their formation neural cysts first differentiate into primitive ectoderm before giving rise to neuroectoderm. Immature neural cysts do not express any differentiation markers after 6 days in 3D culture. However, after longer culture periods (10 days) we observe the generation of neurons. Neural cysts also show important hallmarks of a true neuroepithelium, like apical-basal polarity and interkinetic nuclear migration. Regarding regional identity along the neuraxis neural cysts acquire by default an anterior phenotype. Addition of retinoic acid results in posteriorization to cervical levels of the spinal cord. With respect to the dorsal-ventral identity we tested, if neural cysts can respond to morphogens that are critical for neural patterning. Addition of sonic hedgehog induced robust expression of the floor plate marker HNF-3b, whereas the roof plate markers msx1 and LMX1a are expressed by default. We also applied the same protocol to iPS cells that were derived from neural progenitors reprogrammed with 2 factors (Kim JB. et al, Nature 2008; 454(7204):646). We were able to obtain neural cysts that display the same morphological and molecular marker characteristics as neural cysts derived from mES cells. This is the first time that a polarized 3D neuroepithelial structure was created in vitro that closely resembles a developing neural tube in vivo.

Poster Briefs

FUNCTIONALITY AND DURABILITY OF ENGINEERED HUMAN VASCULAR NETWORKS FROM ENDOTHELIAL PROGENITOR CELLS IN A DEEP THERMAL WOUND.

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DERIVATION OF METABOLICALLY FUNCTIONAL HEPATOCYTE LIKE CELLS FROM HUMAN PLURIPOTENT STEM CELLS

Ogawa, Shinichiro[1], Surapisitchat, James[1], Ogawa, Mina[1], Sugamori, Kim S.[2], Wang, Shung[2], Laposa, Rebecca R.[2], Tyndale, Rachel F.[2], Grant, Denis M.[3], Keller, Gordon[1]
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ALGINATE ENCAPSULATION AND DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS TO ISLET CELL TYPES

Richardson, Thomas[1], Kumta, Prashant N.[2], Banerjee, Ipsita[1]
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ENGINEERING OF 3D PLURIPOTENT STEM CELL MICROENVIRONMENTS VIA BIOMATERIALS INCORPORATION

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Pluripotent stem cells (PSCs) can be differentiated to derivatives of all three germ lineages as self-assembled 3D cell aggregates commonly referred to as "embryoid bodies" (EBs). In addition to serving as a unique in vitro model system to study embryonic development and morphogenesis, suspension cultures of EBs represent an inherently scalable approach for the biomanufacturing of PSC-derivatives and modular tissue engineered constructs. We have previously demonstrated the ability to entrap microparticles (MPs) composed of synthetic degradable polymers to control the release of small molecules within the 3D microenvironment of EBs and induce morphogenesis (Carpenedo et al., Biomaterials, 2009; Carpenedo et al., JBMR A, 2010). More recently, we examined the effects of incorporating ECM-based MPs on mouse EB phenotypes (Bratt-Leal et al., Biomaterials, 2011), and exogenous growth factor delivery from entrapped MPs to promote EB hemogenic differentiation (Purpura et al., Biomaterials, 2012). Thus, the objective of these studies was to expand upon the utility of biomaterials-based approaches to engineer the biochemical and biophysical properties of 3D PSC environments.
Gelatin and heparin-conjugated gelatin MPs were fabricated using water-in-oil emulsion techniques similar to previously described methods. Heparin was conjugated to gelatin using EDC/NHS chemistry following gluteraldehyde cross-linking of the MPs. MPs were incorporated within EBs via forced aggregation with mouse ES cells (D3) in PDMS microwells (AggreWells™, STEMCELL Technologies). After 24-48 hours of formation, the resulting ESC/MP aggregates were subsequently cultured for up to 2 weeks using rotary orbital suspension culture (Carpenedo et al., Stem Cells, 2007). The temporal and spatial patterns of differentiation were assessed by a combination of gene expression, flow cytometry, confocal microscopy and immunohistochemistry.
Delivery of BMP-4 or Noggin from gelatin MPs stimulated opposing differentiation trajectories, promoting mesoderm or ectoderm morphologies and gene expression, respectively, at comparable levels to soluble treatment controls (10 ng/ml). The percentage of Brachyury-T+ cells (early mesoderm marker) was increased with BMP-4 treatment in a comparable manner with MP or soluble delivery methods. Introduction of unloaded heparin-conjugated and gelatin MPs significantly affected the secreted morphogen profile (BMP4, IGF2, VEGF) of EBs, suggesting that the presence of the materials sequestered endogenous growth factors capable of influencing cell fate decisions. In further support of this notion, divergent gene expression patterns were observed between EBs containing incorporated heparin-gelatin and gelatin MPs. In order to spatially pattern cell phenotypes in 3D, EBs with and without BMP-4 laden MPs were merged together to form more complex multicellular aggregates and exhibited enhanced mesoderm differentiation (Brachyury T+ cells) in hemispheres containing the BMP-4 loaded materials. Overall, these results demonstrate novel applications of engineered micron-scale biomaterials to engineer 3D PSC microenvironments for scalable directed differentiation. Enabling biomaterial technologies are anticipated to yield more robust, reproducible and cost-efficient strategies to produce stem cell derivatives and tissue engineered constructs for regenerative medicine and in vitro diagnostic applications.

Thursday, June 13, 1:15 PM - 3:05 PM

Room 205

Concurrent ID: Stem Cell Signaling and Niches

BLOODLESS: THE CRITICAL ROLE OF ASYMMETRIC DIVISION IN BLOOD DEVELOPMENT AND CANCER

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Our research focuses on the signals that control hematopoietic stem cell self-renewal and differentiation, how these signals are utilized during regeneration and how their dysregulation may lead to cancer. Using a multitude of genetic models, we have shown that classic developmental signaling pathways such as Wnt, Hedgehog and Notch play key roles in stem cell growth and regeneration and are dysregulated during leukemia development. In addition, we have used real-time imaging strategies to show that hematopoietic stem cells have the capacity to undergo both symmetric and asymmetric division, and that shifts in the balance between these modes of division are controlled by the microenvironment and subverted by oncogenes. Importantly, we have shown that regulators of this process, including the cell fate determinant Musashi, can promote aggressive cancers and could serve as critical targets for diagnostics and therapy. Most recently, we have developed a high resolution in vivo imaging system that has allowed us to begin to map the behavior and interactions of hematopoietic stem cells with the microenvironment within living animals, and to define how these change during regeneration and cancer formation.

NFIB: A GOVERNOR OF EPITHELIAL-MELANOCYTE STEM CELL BEHAVIOUR IN A SHARED NICHE

Chang, Chiung-Ying[1], Pasolli, H. Amalia[1], Giannopoulou, Eugenia G.[2], Guasch, Geraldine[3], Gronostajski, Richard M.[4], Elemento, Olivier[2], Fuchs, Elaine[1]
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Adult stem cells (SCs) reside in specialized niches where they receive environmental cues to maintain tissue homeostasis. In mammals, the SC niche within hair follicles (HFs) is home to two distinct SC populations—epithelial HFSCs and melanocyte SCs (McSCs), which behave cooperatively to sustain cyclical bouts of hair regeneration and pigmentation. To generate pigmented hairs, synchrony is achieved such that upon initiation of a new hair cycle, SCs of each type activate lineage-commitment. Dissecting the inter-SC crosstalk governing this intricate tango has been difficult, since mutations affecting one lineage typically affect the other. Here we identify NFIB, a transcription factor expressed by HFSCs, as an unanticipated coordinator of SC behaviour. HFSC-specific conditional targeting of Nfib uncouples SC synchrony. Remarkably, this happens not by perturbing hair cycle and follicle architecture, but rather by promoting McSC proliferation and precocious differentiation. The early production of melanin is restricted to McSCs near the dermal papilla (DP) just below the quiescent SC niche. There,

pigment is inappropriately transferred to neighboring HFSCs, promoting their apoptotic death. McSCs more distant from the DP are unscathed, thereby preventing hair graying and loss typical of McSC-differentiation mutants. Digging into the mechanism through chromatin-immuno-precipitation-high-throughput-sequencing (ChIP-seq) and transcriptional profiling, we identify endothelin-2 as a critical NFIB target aberrantly activated in NFIB-deficient HFSCs. Ectopically induced Endothelin-2 recapitulates NFIB-deficient phenotypes in wild-type mice; conversely, endothelin-receptor antagonists block precocious McSC differentiation in the NFIB-deficient niche. Thus, by repressing endothelin-2 in HFSCs, NFIB overrides precocious McSC proliferation and DP-dependent differentiation, forcing McSCs instead to rely upon environmental signals that naturally synchronize McSCs and HFSCs. Our findings provide new insights into how McSC and HFSC behaviours maintain reliance upon cooperative factors within the niche, and how this can be uncoupled in injury, stress and disease states.

LUNG ENDOTHELIAL CELLS DRIVE LINEAGE SPECIFIC DIFFERENTIATION OF LUNG EPITHELIAL STEM CELLS VIA BMP4-TSP1 REGULATION

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The adult mammalian lung is a highly vascularized organ comprised of numerous types of epithelial cells and stromal components. In the murine lung, Bronchioalveolar Stem Cells (BASCs) have been implicated in epithelial repair in the bronchiolar and alveolar compartments. Employing a three-dimensional (3D) co-culture system, we defined primary lung murine endothelial cells (LuMECs) as a critical stromal cell type supporting the self-renewal and differentiation of BASCs. BASCs cultured with LuMECs exhibited 7.2% colony forming efficiency in limiting dilution assays with serial passage. H&E and immunofluorescence analysis on BASC colonies demonstrated distinct types of differentiated epithelial colonies arose in LuMEC co-cultures; 24.3% of colonies were bronchiolar, containing cells positive for the bronchiolar Clara cell marker CCSP; 51.4% of colonies were alveolar, containing cells expressing the alveolar type II (AT2) cell marker SP-C; and 20.2% of colonies were bronchioalveolar, containing both CCSP-positive and SPC-positive cells. Single CD31-CD45-EpCAM+SCA1lowCD24low BASCs gave rise to bronchioalveolar colonies in co-culture with LuMECs. Serial passage of individual bronchiolalveolar colonies with LuMECs showed characteristics of multipotent stem cells, giving rise to bronchiolar and alveolar colonies. Subcutaneous co-injection of dissociated individual bronchioalveolar colonies with LuMECs generated bronchiolar- and alveolar-like structures in vivo. Whereas LuMECs induced bronchiolar and alveolar differentiation of BASCs, endothelial cells from different organs such as liver (LiMECs) and brain induced only bronchiolar differentiation. Thrombospondin1 (Tsp1), a secreted angiogenesis inhibitor highly expressed in lung endothelial cells, was a candidate regulator of differentiation in LuMECs. In response to bronchiolar injury using naphthalene, Tsp1-null mice exhibited enhanced injury repair; 49.3% of Clara cells were regenerated 5 days after injury in Tsp-1 null mice vs. 19.06% in wild-type (p<0.01). In contrast, Tsp1-null mice showed impaired regeneration of alveolar epithelia after bleomycin treatment; 4.4-fold fewer AT2 cells were present in Tsp1-null lungs 21 days after injury, p<0.01 vs. wild-type). 3D co-culture of BASCs with Tsp1-null LuMECs produced 3.2-fold more bronchiolar colonies and 3.5-fold less alveolar colonies than BASCs with wild-type LuMECs (p<0.01). In vivo subcutaneous transplantation of individual bronchioalveolar colonies with Tsp1-null LuMECs increased bronchiolar-like structure formation (2.6-fold more, p<0.03) at the expense of alveolar-like structures (4.1-fold less, p<0.02) compared to co-injection with wild-type LuMECs. Bmp4 treatment in co-cultures increased alveolar differentiation (51.4% in control vs. 91% in Bmp4, 1.6-fold, p<0.01) by induction of Tsp1 expression in LuMECs, whereas adding Bmp4 in 3D culture of BASCs with LiMECs or Tsp1-null LuMECs showed no discernable changes. We have shown the first 3D, in vitro and in vivo multipotent differentiation of single lung stem cells. These experiments demonstrate the importance of the microenvironment in modulating lineage-specific differentiation of BASCs, and specifically reveal an organ-specific, Bmp4-Tsp1 axis in endothelial cells regulating BASC differentiation. On a larger scale, this work identifies exogenous factors that can direct lineage-specific differentiation towards mature lung epithelial cells.

NEURAL CREST-DERIVED STEM CELLS CONTRIBUTE TO HYPOXIA-INDUCED PHYSIOLOGICAL ANGIOGENESIS IN THE ADULT MAMMALIAN CAROTID BODY.

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Previous observations in our laboratory identified the mammalian carotid body (CB) as a neurogenic niche in the adult peripheral nervous system (PNS). This chemoreceptor organ is responsible for the detection of hypoxemia and is able to adapt to a persistent stimulus by increasing the number of neuronal, type I cells. We have previously shown that this neurogenic process depends on a subpopulation of neural crest-derived, glia-like stem cells, or type II cells (expressing glial fibrillary acidic protein, GFAP), which become activated under hypoxia, proliferating and changing their phenotype to nestin positive intermediate progenitors.
In addition to neurogenesis, the CB exhibits a profound angiogenic process under hypoxia, bringing blood through newly formed vessels to neuronal cells, to contribute to the structural and functional adaptation of the organ to persistence of the stimulus. Since neural crest-derived stem cells have been shown to give rise to mesenchymal derivatives during development, we have tested whether adult CBSCs retain multipotent capacity and contribute to angiogenesis by differentiating into vascular cell types in response to hypoxia.
In the normoxic-resting situation, quiescent CBSCs are in intimate contact to neuronal cells and away from blood vessels. Therefore, we decided to first study whether hypoxia was inducing structural changes and cellular movements within the CB niche to facilitate the role of neural progenitors in angiogenesis. By both electron and confocal microscopy (EM and CM respectively), we have observed that the expression change in cytoskeleton intermediate filaments (from GFAP to nestin), occurring upon progenitor activation, is associated to short migratory movements. These cells are mobilized by hypoxia, moving away from neuronal glomeruli and closer to surrounding blood vessels. In order to study the physiological capacity of CBSCs to contribute to vascular cell types, we have performed a cell fate mapping approach using GFAP-cre/ROSA26-LacZ transgenic mice. By tracking differentiation of GFAP+ cells, we have demonstrated that CBSCs give rise to endothelial cells within the adult CB parenchyma, contributing to the formation of new vessels in the hypoxic organ.
Finally, we have analyzed the molecular mechanism by which CBSCs become endothelial cells. We have studied mesenchymal differentiation of CBSCs in vitro by exposing them to different adherent substrates and vascular factors. We have demonstrated that adhesion per se induces differentiation of CB progenitors into smooth muscle cells and, to a lesser extent, into endothelial cells. However, endothelial differentiation is significantly exacerbated when cells are cultured in hypoxia or in the presence of vascular factors such as VEGF, EPO, PDGF, and ET-1. We also confirmed multipotency of CBSCs in vitro by performing single cell experiments showing the presence of neuronal and endothelial cells within the same clones.
This work shows a remarkable plasticity of an adult population of neural stem cells in the PNS. CBSCs retain multipotent capacity to give rise to both neuronal and vascular cell types during organ adaptation to a persistent hypoxemia. Understanding the physiology of CBSCs within their niche is crucial not only to learn more about adult neurogenic niches but also to understand the pathophysiology of the organ and to improve the use of these cells for therapeutic purposes.

Poster Briefs

MTOR MAINTAINS LGR5+ INTESTINAL STEM CELLS AND REGULATES SECRETORY INTESTINAL EPITHELIAL CELL DIFFERENTIATION THROUGH A WNT-INDEPENDENT MECHANISM

Sampson, Leesa L., Grogg, Matthew, Zheng, Yi
Experimental Hematology and Cancer Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

EXPRESSION OF EBF2 IN OSTERIX-POSITIVE IMMATURE OSTEOBLASTIC CLELS DEFINES A NICHE FOR HEMATAPOIETIC STEM CELLS

Hinzen, Christoph, Zimber-Strobl, Ursula, **Kieslinger, Matthias**
Institute of Clinical Molecular Biology and Tumor Genetics, Helmholtz Zentrum München, Munich, Germany

IN-VIVO SINGLE CELL RNA-SEQ ANALYSIS OF OSTEO-LINEAGE CELLS WITHIN THE HSPC NICHE IDENTIFIES INTERLEUKIN-18 AS A NOVEL HEMATOPOIEITIC REGULATOR

Silberstein, Lev[1], Kharchenko, Peter[2], Osawa, Masatake[1], Lin, Charles[1], Mercier, Francois[1], Kfoury, Youmna[1], Lo Celso, Christina[3], Scadden, David T.[1]
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BIOENGINEERED NICHES IDENTIFY REGULATORS THAT REJUVENATE THE FUNCTION OF AGED MUSCLE STEM CELL POPULATIONS

Blau, Helen M., Cosgrove, Ben, Gilbert, Penney
Stanford University Sch of Medicine Baxter Lab for Stem Cell Biology, Stanford, CA, USA

Tissue-specific stem cells with potent regenerative properties are present in many adult tissues including blood and muscle, but their 'stemness' is rapidly lost upon culture in traditional plastic dishes, limiting therapeutic applications. We hypothesized that by deconstructing the muscle stem cell (satellite cell) niche, we could overcome this limitation and screen for drugs that enhance stem cell function, expansion and rejuvenation. To meet this goal, we bioengineered substrates which recapitulate key biophysical and biochemical niche features. Using a novel hydrogel culture substrate in conjunction with time-lapse microscopy and a highly automated data analysis algorithm, we tracked the behavior of clones derived from single muscle stem cells in culture and assessed stem cell self-renewal. We then subjected them to a stringent assay of function: transplantation into mouse muscles followed by a quantitative assessment of regeneration by noninvasive bioluminescence imaging coupled with assays of muscle strength. We found that stem cells cultured on a substrate with the elastic modulus of muscle tissue and tethered with a niche extracellular matrix protein laminin maintain regenerative capacity, illustrating the power of biomaterials to direct stem cell fate and overcome roadblocks to stem cell therapeutic utility. Using this platform we have developed a screen and discovered biochemical cues and drugs that enhance stem cell function, expansion and rejuvenation of aged muscle stem cells. Such studies are of fundamental interest and will aid in the treatment of muscle wasting disorders, such as injury related muscle weakness in the aged.

1:15 PM - 3:05 PM

Ballroom West

Concurrent IE: Stem Cells, Injury and Regeneration

Supported by StemCells, Inc

SKELETAL MUSCLE DEDIFFERENTIATION DURING ADULT LIMB REGENERATION

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The possibility that progenitor cells during appendage regeneration in salamanders are derived by cellular dedifferentiation has been studied and discussed for decades. Due to the lack of appropriate fate mapping technologies it was not possible to conclusively test this hypothesis.
Using a novel tracing strategy in adult newts, we can demonstrate that dedifferentiation of differentiated myofibres is an integral part of limb regeneration. By exclusively labelling myonuclei we see that fragmentation of myofibres results in proliferating mononuclear cells in the blastema, which is a growth zone that gives rise to the new limb. Cell cycle re-entry is a post-fragmentation event, myofibre-derived progeny remain in the myogenic lineage and contribute to skeletal muscle along the entire proximo-distal limb axis in the new limb. The results highlight the distinction between cell cycle and cell lineage plasticity, and provide new tools for systematic analyses of dedifferentiation during naturally occurring limb regeneration.
Using these tools we addressed how injury leads to dedifferentiation. We identified a direct link between programmed cell death (PCD) and cellular dedifferentiation. We can show that inducing a PCD response in skeletal muscle cells brings about the reversal of terminal differentiation. Blocking PCD inhibits the multinucleate-to-mononucleate transition both in vivo and in vitro. After gaining a temporal correlation between PCD and dedifferentiation in individually traced cells, we were able to derive proliferating progeny from postmitotic myotubes by first inducing and subsequently intercepting the PCD response. Our data indicate that PCD is a multifunctional process, and we conclude that its diversion is an instrument to achieve cellular dedifferentiation.

ACTIVATION OF PROXIMO-DISTAL POSITIONAL INFORMATION MOLECULES, MEIS AND HOX, DURING AXOLOTL LIMB REGENERATION

Nacu, Eugeniu, Quang, Huy Le, Glausch, Mareen, Zhang, Yang, Damanik, Febriyani Fiain Rochel, Schüz, Maritta, Tanaka, Elly Margaret
Center for Regenerative Therapies Dresden / Max Planck Institute of Mol Cell Biology and Genetics, Dresden, Germany

Urodeles poses the unique capacity to regenerate lost limbs. This happens by the formation of an amorphous mass of proliferating cells called the blastema, which are primarily of myogenic, connective tissue (CT) and Schwann cell origin. Upon proliferation the cells will differentiate and pattern into a functional replica of the missing structure. The ability to pattern and integrate with the existing organs is one aspect that differentiates a regenerating blastema from an amorphous proliferating tumour and understanding it is crucial for future implementation of regenerative therapies.
We investigated limb patterning in regeneration, decoding the molecular machinery behind the ability of blastema cells to know which limb elements need to be regenerated. This ability was termed positional information (PI). There are two types of positional information: 1) proximo-distal (PxDs) and 2) circumferential. PxDs PI runs along the limb from the shoulder to the fingers, shoulder - proximal, fingers - distal. Circumferential PI is along the circumference of the limb with

4 coordinates: anterior, posterior, dorsal, and ventral. PxDs PI dictates which limb elements will be regenerated distal of the amputation plane. Known molecules involved in PxDs specification of limb elements are: MEIS - upper arm, HOXA11 - lower arm, HOXA13 - hand. These molecules are expressed in limb development and reinduced upon amputation. We previously showed that CT-derived blastema cells are determining the pattern of the limb. Therefore it is important to study these molecules in CT cells.
To investigate the mechanism of induction of these molecules in regeneration we looked at the function of the nerve and interaction between cells with different circumferential PIs, called positional discontinuity (PD). It was previously shown that a minimal amount of innervation is required for regeneration and a nerve deviated to a simple anterior lateral wound can induce the cells to acquire characteristics of blastema cells. However, a limb does not regenerate from a lateral wound with nerve, unless a piece from the posterior part of the limb is transplanted to the wound, creating PD. This indicates that PD is necessary for limb regeneration. To test if nerves and PD are necessary for MEIS and HOXA11 upregulation, we analyzed three situations: 1) a lateral wound that lacks PD and deviated nerves; 2) a lateral wound with deviated nerves and 3) an amputated limb that has nerves and PD at the amputation plane.
We found that MEIS was upregulated in CT cells upon upper arm amputation and in an upper arm lateral wound with deviated nerves but not in a wound without nerves. Thus, MEIS upregulation is independent of circumferential PD but dependent on presence of nerves. In contrast, HOXA11 upregulation was observed in CT cells in all three conditions, indicating that a simple injury without nerves or PD is sufficient for HOXA11 upregulation. The difference in regulation between MEIS, an upper arm identity regulator, and HOXA11, a lower arm marker, could be due to MEIS and HOXA genes being regulated differently or there is a difference between the regulation of PI molecules in the lower and upper arm. To distinguish between these hypotheses we looked at regulation of HOXA9 which is associated with upper limb identity and found that HOXA9 upregulation upon upper arm amputation is independent of nerves. This suggests that MEIS and HOX genes are regulated differently and that there are several levels at which PxDs PI of cells is determined.

YAP REPRESSES WNT-INDUCED INTESTINAL STEM CELL EXPANSION AND ORGAN GROWTH DURING REGENERATIVE STRESS

Barry, Evan[1], Morikawa, Teppei[2], Butler, Brian[1], de la Rosa, Rosemarie[1], Shrestha, Kriti[1], Yan, Kelley[3], Fuchs, Charles[4], Magness, Scott[5], Smits, Ron[6], Ogino, Shuji[4], Kuo, Calvin[3], Camargo, Fernando[1]
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Regulation of organ size is one of the least understood phenomena in stem cell biology, particularly during emergency growth after injury. The Hippo Signaling Pathway is implicated in organ size "sensing", however for most organs, the mechanisms involved are still poorly understood. The processes responsible for halting organ growth and regeneration are likely mutated in cancer, therefore understanding this event is of utmost importance. The intestine is the most rapidly self-renewing organ in mammals. At the heart of this regenerative capacity is the Wnt signaling pathway, which instructs stem cells at the base of the crypts of Lieberkuhn to retain their stemness. The Wnt signaling program is well known to be critical for intestinal homeostasis and regeneration, however the mechanisms involved in regulating Wnt output during different regenerative states is virtually completely unknown. Unexpectedly, we find that the Hippo effector YAP represses Wnt signaling in the mammalian intestine. Overexpression of YAP specifically in the intestinal epithelium inhibits homeostatic self-renewal, leading to near complete loss of intact epithelium. Transcriptome analysis demonstrates that YAP rapidly inhibits the Wnt/Beta-Catenin and intestinal stem cell gene expression signatures 2 days after YAP induction. Consistent with YAP repressing Wnt signaling, intestine-specific loss of Yap leads to Wnt hypersensitivity and expansion of intestinal stem cells. Treatment of YAP mutants with adenovirus expressing the Wnt agonist R-spondin1 leads to extensive crypt hyperplasia and formation of microadenomas resembling loss of the tumor suppressor Apc. In addition, YAP mutant crypts become massively overgrown after tissue injury, and exhibit upregulated Wnt signaling. We find that cytoplasmic YAP at the crypt/villus junction inhibits Wnt responsiveness, in part through sequestering DVL2 in the cytoplasm in parallel to the Beta-catenin destruction complex. Furthermore, activation of the Hippo kinases MST1/2 (phospho-MST) at the crypt/villus junction appears necessary to regulate overall levels of YAP protein. Indeed, loss of MST1/2 leads to crypt loss and Wnt repression, likely through an overall increase in YAP protein levels. Consistent with YAP repressing Wnt independent of the destruction complex, loss of YAP in APC-null adenomas leads to hyperactive Wnt/Beta-catenin signaling in mouse colorectal tumors. In vitro, loss of YAP is synergistic to APC loss, and expression of a dox-inducible YAP phospho-mimic inhibits the growth of human colorectal cancer cells in tumor xenograft assays through repression of the Wnt gene expression signature. In human colorectal cancer patients (n=672), YAP staining is lost in the most aggressive tumors and patients with stage IV disease. Together, our data demonstrate that the Hippo pathway inhibits Wnt signaling in vivo by sequestering YAP in the cytoplasm and that loss of YAP results in Wnt hypersensitivity and uncontrolled organ growth during times of regenerative stress. Therefore, we identify an important mechanism for controlling stem cell output after injury, and that this mechanism is mutated in cancer.

FGF9 FROM DERMAL GAMMA DELTA T CELLS INDUCES HAIR FOLLICLE NEOGENESIS AFTER WOUNDING

Gay, Denise[1], Kwon, OhSang[2], Zhang, Joshua[1], Spata, Michelle[1], Plikus, Maksim[3], Holler, Phillip[1], Ito, Mayumi[4], Kim, Chang Deok[5], Wang, Fen[6], Baratono, Sheena[1], Ornitz, David[7], Millar, Sarah[1], Cotsarelis, George[1]
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Understanding molecular mechanisms for regeneration of hair follicles during wound healing provides new opportunities for developing treatments for hair loss and other skin disorders. Here we show that Fibroblast Growth Factor 9 (Fgf9) modulates hair follicle regeneration following wounding of adult mice. Inhibition of Fgf9 during wound healing severely impedes this wound-induced hair follicle neogenesis (WIHN). Conversely, overexpression of Fgf9 results in a 2-3 fold increase in the number of neogenic hair follicles. Remarkably, γδ T cells in the wound dermis are the initial source of Fgf9. Deletion of the fgf9 gene in T cells in Lck-Cre;floxed Fgf9 mice results in a marked reduction in WIHN. Similarly, mice lacking γδ T cells demonstrate impaired follicular neogenesis. We found that Fgf9, secreted by γδ T cells, initiates a regenerative response by triggering Wnt expression and subsequent Wnt activation in wound fibroblasts. Employing a unique feedback mechanism, activated fibroblasts then express Fgf9, thus amplifying Wnt activity throughout the wound dermis during a critical phase of skin regeneration. Strikingly, humans lack a robust population of resident dermal γδ T cells, potentially explaining their inability to regenerate hair. These findings, which highlight the essential relationship between the immune system and tissue regeneration, establish the importance of Fgf9 in hair follicle regeneration and suggest its applicability for therapeutic use in humans.

Poster Briefs

MYOD AND MYF5 HAVE ESSENTIAL BUT OVERLAPPING FUNCTIONS IN ADULT MYOGENESIS

Legendre, Nicholas P., Yamamoto, Shoko, Yamamoto, Masakazu, **Goldhamer, David J.**
MCB, University of Connecticut, Storrs, CT, USA

DIRECT EVIDENCE BY IN VIVO CLONAL ANALYSIS THAT PROXIMAL TUBULE EPITHELIA DEDIFFERENTIATE TO MEDIATE REPAIR AFTER KIDNEY INJURY

Kusaba, Tetsuro, Lalli, Matthew, Kobayashi, Akio, Humphreys, Benjamin D.
Renal Division, Brigam and Women's Hospital, Boston, MA, USA

LOOKING BEHIND THE CURTAIN: PLASTICITY OF POSTNATAL CARDIOMYOCYTES OF THE MOUSE

Raulf, Alexandra[1], Geisen, Caroline[1], Grünberg, Sabine[1], Freitag, Patricia[1], Klein, Alexandra[1], Roell, Wilhelm[2], Fleischmann, Bernd Kurt[1], Hesse, Michael[1]
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REGENERATION OF HAIR CELLS FROM COCHLEAR STEM CELLS

Shi, Fuxin, Bramhall, Naomi, **Edge, Albert**
Harvard Medical School, Boston, MA, USA

The vestibular and mammalian auditory organs have a limited ability to replace damaged cells, but our laboratory has recently identified cells in the cochlear sensory epithelium with stem cell properties. Located in the organ of Corti, this epithelium consists of hair cells, the receptor cells for sound, and surrounding supporting cells. In an attempt to identify cochlear stem cells, we have isolated cells with distinct phenotypes by flow cytometry. Whereas all supporting cells express Sox2, a specific subset expresses Lgr5, a downstream target of the Wnt pathway and a protein that marks intestinal epithelial stem cells. We show that cochlear Lgr5-expressing cells account for new hair cell generation in vitro and in vivo. Lgr5-expressing supporting cells after sorting gave rise to self-renewing neurospheres. The Lgr5-expressing neurospheres could be induced to differentiate to hair cells, and Lgr5pos cells differentiated to hair cells at a higher rate than total Sox2pos cells. Hair cells did not differentiate from Lgr5neg cells. The Lgr5-expressing cells, in contrast to cochlear cells in previous studies, showed a capacity for transdifferentiation to hair cells after damage to the cochlea. This was the first time that hair cell regeneration could be seen in in vivo studies in postnatal animals. The demonstration of hair cell regeneration was made possible by the sensitivity of lineage tracing of Lgr5-expressing cells, which provided analysis of cell fate at a single-cell level. A further increase in hair cells in the postnatal animal after damage was induce by treatment with a gamma-secretase inhibitor that blocked Notch signaling within the epithelium. The new hair cells generated by this inhibitor came from Lgr5 expressing cells, consistent with these cells playing a role as hair cell progenitors. Upregulation of Wnt signaling specifically targeted the Lgr5-expressing cells, leading to proliferation in the normally post-mitotic postnatal ear. The dividing cells increased expression of transcription factor, Atoh1, which we have previously shown to be a downstream target of Wnt, and transdifferentiated to hair cells. Thus, Lgr5-expressing cells at neonatal ages show a capacity to replace hair cells. Signaling pathways responsible for the proliferation and differentiation of these cells could be manipulated to provide a source of new hair cells to restore hearing.
Supported by grant DC007174 from the National Institutes of Health.

4:00 PM - 5:50 PM

Ballroom East

Concurrent IIA: Pluripotent Stem Cells II

Supported by Fluidigm Corporation

EPIGENETIC MAPPING OF NORMAL AND DISEASE-SPECIFIC IPSC DIFFERENTIATION

Loring, Jeanne F.
The Scripps Research Inst, La Jolla, CA, USA

Our approach to understanding pluripotency and differentiation is to map global genomic and epigenetic characteristics of human pluripotent stem cells. We analyze large groups of cell lines in order to identify characteristics that are common to pluripotent and differentiated states. In hundreds of analyses, we have found no significant genomic or epigenetic characteristics that consistently distinguish normal hESC and iPSC lines. We have extended these studies to analysis of single cells by RNA sequencing as a measure of heterogeneity in differentiating populations. To gain insight into the underlying mechanisms of disease, we are mapping the epigenetics of lineage-specific differentiation, and determining at what stage of differentiation disease-specific iPSCs begin to diverge from typical normal cells. All of our results are incorporated into a growing database of molecular phenotypes of undifferentiated and differentiated pluripotent cells and fetal and adult tissues. This database of thousands of samples, called the "Stem Cell Matrix", helps to provide insights into the mechanisms underlying normal development and developmental disorders.

NANOG-DEPENDENT FUNCTION OF TET1 AND TET2 IN ESTABLISHMENT OF PLURIPOTENCY

Wang, Jianglong[1], Costa, Yael[2], Ding, Junjun[1], Theunissen, Thorold W.[3], Faiola, Francesco[1], Hore, Timothy A.[4], Shliaha, Pavel V.[5], Fidalgo, Miguel[1], Saunders, Arven[1], Lawrence, Moyra[3], Dietmann, Sabine[3], Das, Satyabrata[6], Levasseur, Dana N.[6]
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Molecular control of the pluripotent state is thought to reside in a core circuitry of master transcription factors including the homeodomain-containing protein Nanog, which plays an essential role in establishing ground state pluripotency during somatic cell reprogramming. While the genomic occupancy of Nanog has been extensively investigated, comparatively little is known about Nanog-associated proteins and their contribution to the Nanog-mediated reprogramming process. Using enhanced purification techniques and a stringent computational algorithm, we identified 27 high-confidence protein interaction partners of Nanog in mouse ES cells. These consist of 19 novel partners of Nanog that have not been reported before including the Ten eleven translocation (Tet) family methylcytosine hydroxylase Tet1. We confirmed physical association of Nanog with Tet1, and demonstrated that Tet1, in synergy with Nanog, enhances the efficiency of reprogramming. We also found

physical association and reprogramming synergy of Tet2 with Nanog, and demonstrated that knockdown of Tet2 abolishes the reprogramming synergy of Nanog with a catalytically deficient mutant of Tet1 (Tet1Mut). These results indicate that the physical interaction between Nanog and Tet1/2 proteins facilitates reprogramming in a manner that is dependent on Tet1/2's catalytic activity. Tet1 and Nanog co-occupy genomic loci of genes associated with both maintenance of pluripotency and lineage commitment in ES cells, and Tet1 binding is reduced upon Nanog depletion. Co-expression of Nanog and Tet1 results in expression priming of and increased 5hmC levels at top ranked common targets Esrrb and Oct4 before reprogramming to naïve pluripotency. We propose that Tet1 is recruited by Nanog to enhance the expression of a subset of key reprogramming target genes. These results provide an insight into the reprogramming mechanism of Nanog and uncover a novel role for 5mC hydroxylases in the establishment of naïve pluripotency.

PROTEIN DEGRADATION BY THE UBIQUITIN PROTEASOME SYSTEM PLAYS A KEY ROLE IN MAINTAINING AND INDUCING PLURIPOTENCY

Buckley, Shannon M., Aranda-Orgilles, Beatriz, Strikoudis, Alexandros, Aifantis, Iannis
NYU School of Medicine, New York, NY, USA

Pluripotency is regulated by a core network of transcription factors that also play a key role in cellular reprogramming of somatic cells. Our laboratory has strong data indicating that post-translational modifications of proteins, specifically by the ubiquitin-proteasome system (UPS), controls stem cell fate decisions and pluripotency. The UPS regulates the abundance of protein by adding or removing ubiquitin through substrate recognition by E3 ligases, which can lead to poly-ubiquitin chains that target proteins for proteolysis by the proteasome. Using proteomic approaches in embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC) we have determined that a number of key pluripotency factors (including Nanog, Oct4, and Rex1) are targeted for protein degradation by the UPS. Also, RNAi screens in both pluripotent ESC and during ESC differentiation have revealed a number of substrate recognizing E3 ligases as novel regulators of ESC cell fate decisions. Further characterization of E3 ligases identified in ESC based RNAi screens identified Socs3 and Ubr5 to regulate pluripotency. Depletion of Ubr5, a HECT family E3 ligase, leads to up-regulation of early ectodermal markers in ESC, and inhibits cellular reprogramming. Whereas silencing of Socs3, part of an E3 ligase complex and inhibitor of the Jak/Stat signaling, delays differentiation of ESC, and promotes a 2-fold increase in generation of iPSC. Moreover, overexpression of Socs3 in ESC leads to loss of pluripotency markers. During reprogramming expression patterns of both members of the core and the lid of the proteasome clearly divide iPSC and intermediate cells poised for reprogramming from mouse embryonic fibroblasts (MEF) and subsets of refractory cells. Consistent with these findings partial inhibition of the proteasome completely inhibits generation of iPSC. These studies suggest active ubiquitination (and de-ubiquitination) and protein degradation is required for proper induction and maintenance of pluripotency. Further detailed knowledge of the identity of key ubiquitin ligases and their substrates will further unravel mechanisms of pluripotency, and reprogramming.

TRANSCRIPTION FACTORS INTERFERING WITH DEDIFFERENTIATION INDUCE CELL TYPE-SPECIFIC TRANSCRIPTIONAL PROFILES

Masui, Shinji
CiRA, Kyoto University, Kyoto, Japan

Transcription factors (TFs) are able to regulate differentiation-related processes, including dedifferentiation and direct conversion, through the regulation of cell type-specific transcriptional profiles. However, the functional interactions between the TFs regulating different transcriptional profiles are not well understood. Here, we show that the TFs capable of inducing cell type-specific transcriptional profiles prevent the dedifferentiation induced by TFs for pluripotency. Of the large number of TFs expressed in a neural-lineage cell line, we identified a subset of TFs that when overexpressed, strongly interfered with the dedifferentiation triggered by the procedure to generate induced pluripotent stem cells (iPSCs). This interference occurred through a maintenance mechanism of the cell type-specific transcriptional profile. Strikingly, the maintenance activity of the interfering TF set was strong enough to induce the cell line-specific transcriptional profile when overexpressed in a heterologous cell type. In addition, the TFs that interfered with dedifferentiation in hepatic-lineage cells involved TFs with known induction activity for hepatic-lineage cells. Our results suggest that dedifferentiation needs to suppress a cell type-specific transcriptional profile, which is primarily maintained by a small subset of TFs capable of inducing direct conversion. We anticipate that this functional correlation might be applicable in various cell types and might facilitate the identification of TFs with induction activity in efforts to understand differentiation.

Poster Briefs

IN SILICO PREDICTION AND VALIDATION OF PLURIPOTENT STATE TRANSITIONS AND SINGLE CELL HETEROGENEITY

Yachie, Ayako, Onishi, Kento, Zandstra, Peter W.
Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada

EVALUATING THE LANDSCAPE OF SPECIFICITY OF CRISPR-CAS SYSTEMS FOR RNA-GUIDED HUMAN GENOME ENGINEERING

Mali, Prashant, Aach, John, Church, George M.
Harvard Medical School, Boston, MA, USA

SCREENING EMBRYONIC STEM CELL FACTORS IDENTIFIES A NOVEL CLASS OF PROTEINS INVOLVED IN REPROGRAMMING

Wang, Stan, Miyamoto, Kei, Gurdon, John B.
Wellcome Trust/CRUK Gurdon Institute, University of Cambridge, Cambridge, United Kingdom

DECONSTRUCTION OF THE DYNAMIC PLURIPOTENT STEM CELL TRANSCRIPTION PROGRAM

Kumar, Roshan M.[1], Cahan, Patrick[2], Shalek, Alex[3], Satija, Rahul[3], DaleyKeyser, AJay[1], Li, Hu[1], Pardee, Keith[1], Gennert, David[3], Yosef, Nir[3], Ferrante, Thomas C.[1], Regev, Aviv[3], Daley, George Q.[2], Collins, James J.[4]
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Recent findings paint pluripotency as a fluid construct composed of multiple sub-states, with some biased towards self-renewal and others towards differentiation. The regulatory circuitry underlying pluripotency is the product of multiple interacting pathways, but it is unclear how these factors conspire to generate a dynamic cell state. We set out to deconstruct the transcriptional program underlying pluripotency and explore the mechanisms that give rise to population heterogeneity by performing single-cell expression profiling, using both QPCR and RNA-Seq, on pluripotent stem cells (PSCs) subjected to a variety of chemical and genetic perturbations. This allowed us to map gene expression variability across the genome, connect the activity of individual regulatory pathways to modulation of variable expression patterns, identify discrete cellular sub-states within populations of cells, and define the state of the pluripotency regulatory network within individual cells. Many genes displayed bimodal expression patterns, on in some cells and off in others. As a class, transcription factors and signaling molecules showed more variable expression than did genes involved in metabolism and structural functions. Importantly, quantitation of absolute transcript levels indicated that bimodal expression was not simply a result of stochasticity due to low expression levels, but rather reflects fluctuations in expression between a high and a low state. Perturbation of epigenetic regulatory pathways altered the distribution of bimodal gene expression patterns. Deletion of polycomb repressive complexes resulted in more disperse populations of cells, while inhibition of DNA methylation and histone deacetylase activity resulted in more uniform populations. The transcriptional program of PSCs in the presence of serum, where some level of spontaneous differentiation is observed, was more complex than those cultured in the presence of Erk and Gsk3 inhibitors (2i conditions) that promote self-renewal and block differentiation. Surprisingly, this low-noise ground state of PSC self-renewal resembled the state of DGCR8-deficient PSCs which lack mature miRNAs, with the similarity driven largely by the absence of a novel regulatory circuit containing c-myc and lin28a that appears to be specific to states primed for differentiation. Quantitative protein immunofluorescence revealed that the pluripotency regulatory network adopts distinct configurations in the ground state and in primed states, with Oct4 acting as a stable node across states, while Tcfcp2L1, Nanog, Esrrb, Rex1, Zfp281, and Dax1 are expressed in a greater fraction of cells in the ground state, and Sall4 expression is more uniform in the presence of serum. This suggests that Oct4 may act as a core factor at PSC enhancers, while the presence or absence of stochastically expressed accessory factors may recruit additional transcriptional machinery to set the expression state of common target genes. Our finding that distinct perturbations result in common modes of PSC self-renewal suggests that PSCs can transition between discrete states through the activation or suppression of insulated gene expression circuits. The approach taken here provides a digital view of complex transcriptional programs and allows for their deconvolution into component modules.

HIGH-THROUGHPUT SINGLE CELL RNA-SEQ TO DEFINE STEM CELL POPULATION HETEROGENEITY

Soumillon, Magali, Cacchiarelli, Davide, Mikkelsen, Tarjei S.
Harvard Stem Cell Institute and Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA

Transcriptome profiling is a key method for functional characterization of cells and tissues. Due to technical limitations, traditional methods for whole transcriptome analysis have been restricted to either population averages or to a limited number of single cells. However, both stochastic variation in gene expression between individual cells and the existence of distinct subpopulations have been proposed to be key factors in driving cellular differentiation and tissue homeostasis. To fully characterize any cell population, it is therefore essential to characterize gene expression at the single-cell scale.

Here, we describe the development and application of a new protocol for whole-genome digital gene expression profiling at single cell resolution. The protocol incorporates unique molecular identifiers (UMIs) as a robust guard against amplification biases, efficient transposon-based fragmentation for high yield, as well as two levels of barcode-based multiplexing, which allows us sample thousands to tens of thousands of cells in a cost-efficient manner (at present, ~$2 per cell).

As a proof of principle, we have generated extensive RNA-seq data for human adipose tissue-derived stromal/stem cells (hADSCs), both from undifferentiated cultures and during directed differentiation towards an adipogenic fate. In a single experiment, we quantified the gene expression profiles of more than 9,000 individual cells, collected at 8 different time points across the 14 days of the differentiation process (~1100 cells per time point). This extensive dataset has allowed us to detect and quantify distinct sub-populations by clustering cells with similar gene expression levels. Moreover, by analysing changes in gene expression over time, we could being to infer the fate of these different subpopulations and predict the basis for the incomplete response of hADSCs to a common differentiation cocktail. These results establish single cell RNA-Seq as a powerful tool for analysis and rational improvement of directed differentiation protocols for tissue engineering and regenerative medicine.

Thursday, June 13, 4:00 PM - 5:50 PM

Room 258

Concurrent IIB: New Technologies for Controlling and Observing Stem Cell Behavior

OPTICAL DECONSTRUCTION OF FULLY-ASSEMBLED BIOLOGICAL SYSTEMS

Deisseroth, Karl
Stanford University, Stanford, CA, USA

This talk will focus on the development and application of the field of optogenetics, a technology that uses light to precisely control activity patterns in genetically defined cells. This technology has been applied to a range of different stem cell types as well as to differentiated cellular elements within intact biological systems including freely-moving mammals. The talk will cover development of light sensitive proteins found in pond algae and salt lake bacteria, and applications of these tools to probe the causal dynamics of neural circuits in health and disease, including questions relating to reward, motivation, and depression. Also discussed will be a new anatomical method called CLARITY, which enables high-throughput whole-brain anatomical analysis at cellular resolution, with potential implications for stem cell research in intact biological systems.

FUNCTIONAL SCREENING OF PLURIPOTENT STEM CELLS FOR CHIMERA FORMING ABILITY

Masaki, Hideki, Kato, Megumi, Umino, Ayumi, Sato, Hideyuki, Yamaguchi, Tomoyuki, Nakauchi, Hiromitsu
Stem Cell Therapy, Institute for Medical Science, Tokyo, Japan

Our group has reported a system to derive transplantable organs by forming chimeras with organ-deficient host animals (Kobayashi et al., Cell, 2012). To transfer this technology from rodent to human, one of the critical problems to be solved is chimera formation with non-rodent pluripotent stem cells (PSCs), not successfully achieved to date.

It is known that human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have more characteristics in common with mouse epiblast stem cells (mEpiSCs) than do rodent ESCs/iPSCs. As mEpiSCs are a type of PSC that cannot contribute to chimera formation ('non-contributors'), human ESCs/iPSCs are also expected to be non-contributors. However, due to ethical concerns, injection of these PSCs into human blastocysts to test chimera-forming ability is not possible. To circumvent this issue, we aimed to establish an in vitro functional assay utilizing interspecific blastocyst injection and in vitro culture (interspecies in vitro chimera assay).

Several groups recently reported generation of human PSC lines with characteristics similar to those of mouse ESCs/iPSCs by modified reprogramming procedures such as additional gene transduction and/or different culture conditions (Li et al. 2009; Buecker et al. 2010; Hanna et al. 2010). We first tried to reproduce these workers' methods of establishing modified human PSC lines and then subjected cells from these lines to interspecies in vitro chimera assay, injecting them into

mouse blastocysts and culturing them in vitro up to 6 days. The resultant embryos were examined for chimerism. Most PSC lines thus established did not contribute to chimera formation. However, some lines showed contribution to epiblast-stage mouse embryos developed from blastocysts in vitro. These cells were either cultured under special conditions or genetically modified prior to injection. Such treatment appeared to confer an ability to contribute to chimera formation.
For further clarification, we treated mouse and rat EpiSCs similarly, and then injected them into mouse blastocysts to assay chimera formation in vivo. Surprisingly, these mouse and rat EpiSCs contributed to chimera formation just as well as did mouse ESCs/iPSCs. Now we are proceeding to apply promising treatments to non-rodent animal PSCs. Our results suggest that interspecies in vitro chimera assay is useful in evaluating the ability of non-rodent PSCs to contribute to chimera formation. The system may also be useful for screening factors/conditions that enable transition of PSCs from non-contributor to contributor status.

PREDICTING STEM CELL FATE CHANGES BY DIFFERENTIAL CELL CYCLE PROGRESSION PATTERNS

Roccio, Marta[1], Schmitter, Daniel[2], Knobloch, Marlen[3], Okawa, Yuya[2], Sage, Daniel[2], Lutolf, Matthias[2]
[1]Department of Clinical Research, University of Bern, Bern, Switzerland, [2]Federal Institute of Technology Lausanne, Lausanne, Switzerland, [3]University of Zurich, zurich, Switzerland

Stem cell self-renewal, commitment and reprogramming rely on a poorly understood coordination of cell cycle progression and execution of cell fate choices. Using existing experimental paradigms it has not been possible to probe this relationship systematically in live stem cells in vitro or in vivo. Alterations in stem cell cycle kinetics probably occur long before changes in phenotypic markers are apparent and could be used as predictive parameters to reveal changes in stem cell fate. To explore this intriguing concept, we developed a single-cell tracking approach that enables automatic detection of cell cycle phases in live (stem) cells expressing fluorescent ubiquitylation-based cell-cycle indicator (FUCCI) probes. Using this tool, we have identified distinctive changes in lengths and fluorescence intensities of G1 (red fluorescence) and S/G2-M (green) that are associated with self-renewal and differentiation of single murine neural stem/progenitor cells (NSCs) and embryonic stem cells (ESCs). We further exploited these distinctive features using fluorescence-activated cell sorting to select for desired stem cell fates in two challenging cell culture settings. First, as G1 length was found to nearly double during NSC differentiation, resulting in progressively increasing red fluorescence intensity, we successfully purified stem cells from heterogeneous cell populations by their lower fluorescence. Second, as ESCs are almost exclusively marked by the green (S/G2-M) FUCCI probe due to their very short G1, we substantially augmented the proportion of reprogramming cells by sorting green cells early on during reprogramming from a NSC to an induced pluripotent stem cell state.
Taken together, our studies begin to shed light on the crucial relationship between cell cycle progression and fate choice, and we are convinced that the presented approach can be exploited to predict and manipulate cell fate in a wealth of other mammalian cell systems.

Poster Briefs

MONITORING PROTEIN SYNTHESIS IN LIVING CELLS WITH FLUORESCENT LABELED TRNA FRET PAIRS

Smilansky, Zeev[1], Barhoom, Sima[1], Farrel, Ian[1], Dahary, Dvir[1], Leask, Andrew[2], Vanderklish, Peter[3], Ehrlich, Marcelo[4], Cooperman, Barry S.[5], Elroy-Stein, Orna[6]
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MAPPING THE HEMATOPOIETIC HIERARCHY BY SINGLE CELL ANALYSIS OF THE CELL SURFACE REPERTOIRE

Guo, Guoji[1], Luc, Sidinh[1], Marco, Eugenio[2], Lin, Ta-Wei[3], Zou, Keyong[4], Yuan, Guo-Cheng[2], Orkin, Stuart H.[5]
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DYNAMIC PHOTOCONTROL OF CELL SIGNALING THROUGH INDUCIBLE PROTEIN CLUSTERING

Bugaj, Lukasz J.[1], Choksi, Atri[1], Mesuda, Colin[2], Kane, Ravi[3], Schaffer, David[4]
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A MICROFLUIDIC APPROACH TO IPS GENERATION

Zoldan, Janet[1], Sharei, Armon[2], Anderson, Daniel[1], Langer, Robert[1], Jensen, Klavs F.[2]
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Induced pluripotent stem cells (iPSCs) and their application to tissue engineering and disease modeling have great potential to change current medical practices. Current research is largely focused on devising efficient virus-free protocols to produce large numbers of iPSCs. Direct delivery of proteins obviates the risk of mutagenic insertion and enables more accurate control of the highly sensitive reprogramming process. However, protein delivery methods currently provide reprogramming efficiencies that are too low for clinical use. Here, We describe a microfluidic approach to protein delivery in which human fibroblasts are mechanically deformed as they pass through a constriction 30–80% smaller than the cell diameter. The resulting controlled application of compression and shear forces results in the formation of transient holes that enable the diffusion of reprogramming proteins from the surrounding buffer into the cytosol.
The current prototype is capable of delivering high throughput rates of 10,000-20,000 cells/s and can yield up to 1 million delivered cells per run. This combination of single-cell level control and macro-scale throughput places this device in a unique position relative to existing delivery methods. Indeed, the microfluidic devices produced a 10-fold improvement in colony formation relative to electroporation and cell-penetrating peptides. Using Nano-String NCounter system we monitored cell state, pluripotency, and differentiation ability of the generated IPSCs. Over all, generated IPSCs exhibited normal karyotyping and were able to differentiate into the three germ layers both in-vitro and in-vivo. Taken together, these developments can advance cell replacement therapies by providing a safe, efficient and robust method of producing patient specific cell lines.

Thursday, June 13, 2013 4:00 PM - 5:50 PM

Room 253

Concurrent IIC: Cell Fate Conversion

GENERATION OF INDUCED NEURONS VIA DIRECT CONVERSION IN VIVO AND IN VITRO

Parmar, Malin
Lund University, Lund, Sweden

By viral expression of neural fate determinants, it is possible to directly reprogram mouse and human somatic cells into functional neurons, termed induced neurons (iNs). The resulting cells are non-proliferating, and presents an alternative to induced pluripotent stem cells (iPS cells) for obtaining patient and disease specific neurons to be used for disease modeling and for development of cell therapy.
In this study, we show that transplanted human fibroblasts and human astrocytes that are engineered to express inducible versions of neural reprogramming genes convert into neurons in vivo, when reprogramming genes are activated after transplantation. Using a Cre-LoxP system to specifically direct expression of re-programming genes to parenchymal astrocytes residing in the striatum, we show that also endogenous mouse astrocytes can be directly converted into NeuN expressing neurons in situ. Taken together, our data provides proof-of-principle that direct neural conversion can take place in vivo, in normally non-neurogenic regions of the adult rat brain, both when using transplanted human cells and endogenous mouse cells as a starting cell for neural conversion.

GENERATION OF OLIGODENDROGLIAL CELLS BY DIRECT LINEAGE CONVERSION

Yang, Nan[1], Zuchero, Bradley J.[2], Ahlenius, Henrik[1], Marro, Samuele[1], Ng, Yi Han[1], Vierbuchen, Thomas[1], Hawkins, John[1], Geissler, Richard[1], Uchida, Nobuko[3], Barres, Ben A.[2], Wernig, Marius[1]
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Transplantation of oligodendrocyte precursor cells (OPCs) is a promising potential therapeutic strategy for diseases affecting myelin, such as Pelizaeus-Merzbacher disease (PMD), other inherited leukodystrophies, and possibly also Multiple Sclerosis and spinal cord injury. However, the derivation of engraftable OPCs from human pluripotent stem cells has not been successful to date and primary OPCs are not readily available. Here we report the generation of induced OPCs (iOPCs) by direct lineage conversion. Forced expression of the three transcription factors Sox10, Olig2 and Zfp536 was sufficient to reprogram mouse and rat fibroblasts into iOPCs with morphologies and gene expression signatures resembling primary OPCs. More importantly, iOPCs gave rise to mature oligodendrocytes that could ensheath multiple host axons when co-cultured with primary dorsal root ganglion cells and formed myelin after transplantion into shiverer mice. We propose direct lineage reprogramming as a viable alternative approach for the generation of OPCs for use in disease modeling and regenerative medicine.

REGULATORS OF GLOBIN SWITCHING IN THE ZEBRAFISH EMBRYO

Ganis, Jared J.[1], Wiley, David M.[1], Riley, Elizabeth B.[1], Satishchandran, Sruthi[1], Palis, James[2], Zon, Leonard I.[1]
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The switching of the globin genes involves complex regulation of critical transcriptional regulators such as BCL11A, EKLF and SOX6, and the induction of fetal globin has been shown to ameliorate the symptoms of diseases such as sickle cell anemia. Recently, there has been interest in driving iPS cells to produce mature red cells that express adult globin genes in an attempt to make these cells therapeutically useful.
To understand hemoglobin switching and the molecular pathways that allow the establishment of an adult fate in embryonic tissues, we utilized screening approaches in the zebrafish model. Using a mouse microarray database in combination with GO term analysis we identified 24 genes that are selectively expressed in embryonic red blood cells. Morpholinos were used to knock down these 17 genes in zebrafish embryos, and the expression of adult globin gene αa1 was analyzed by in situ hybridization. We identified 4 morpholinos, Tcf7l2, Hif1al, E2F5, and Ncoa1 which significantly increased the number of adult globin positive cells. Pairwise knockdown of these genes demonstrated that Tcf7l2, a member of the canonical Wnt pathway, and Ncoa1, a nuclear hormone receptor coactivator, had the largest effect on αa1 expression. Since the genes that we identified did not fall into a single pathway or provide a clear mechanism we decided to screen over 3000 chemical compounds for inductors of adult globin. Interestingly, the top three hits in this large scale chemical screen were regulators of the nuclear hormone receptor (NHR) pathway; the top hit was a PPAR-γ antagonist, in addition to the thyroid hormones T3 and T4. Given this convergence on the NHR pathway between both the chemical and the morpholino screen, we focused our analysis on the NHR pathway. To test which thyroid hormone receptor was responsible for the phenotype, we knocked-out each receptor individually in the presence and absence of T4. We observed that Thraa and Thrb block the T4 induction of adult globin, but Thrab independently increases adult globin, and synergizes with T4. Chip-Seq analysis of Ncoa1 occupancy in the erythroid cell line K562 was performed to examine potential mechanisms of action. Significant binding was observed at the enhancers of the α- and β-globin loci, indicating that the nuclear hormone receptor pathway may be acting directly on the globin loci to modulate globin expression patterns. Our studies have impact on the understanding of globin switching in vertebrates, and could establish new methods to activate specific globins clinically, and to make iPS cells form adult-type tissues.

REPROGRAMMING TO PLURIPOTENCY USING DESIGNER TALE TRANSCRIPTION FACTORS

Liu, Pentao, Gao, Xuefei, Yang, Jian, Tsang, Jason
Wellcome Trust Sanger Institute, Cambridge, United Kingdom

Designer Transcription Factors (dTFs) based on Transcription Activator-Like Effectors (TALEs) of Xanthomonas bacteria can be made to bind a specific DNA sequence and to regulate gene expression, and provide a powerful genetic tool for studying gene regulation and functions. We have developed a new system to rapidly assemble dTFs and to test their functions in regulating key pluripotency loci. We find that a dTF binding to the distal enhancer of the Oct4 (Pou5f1) locus induces rapid epigenetic changes, efficiently activates Oct4 expression, and substitute exogenous Oct4 in reprogramming mouse embryonic fibroblast cells to induced pluripotent stem cells (iPSCs). Similarly, another dTF activator specific for a Nanog enhancer activates its expression and reprograms epiblast cell stem cells (EpiSCs) to iPSCs. Conversely, dTF repressors to the same genetic elements inhibit the endogenous gene expression,

and effectively block reprogramming. These results demonstrate that directly regulating key pluripotency gene loci is an alternative approach for reprogramming to pluripotency, and that dTFs can be used to dissect mechanisms of cellular reprogramming.

Poster Briefs

SINGLE-CELL ANALYSIS OF MYOGENIC DIFFERENTIATION REVEALS A BISTABLE LINEAGE COMMITMENT SYSTEM

Gibson, Tyler M., Gersbach, Charles A.
Biomedical Engineering, Duke University, Durham, NC, USA

FORCED EXPRESSION OF SOX17 CONVERTS MOUSE EMBRYONIC STEM CELLS (MESCS) INTO FUNCTIONAL EXTRAEMBRYONIC ENDODERM STEM (XEN) CELLS

McDonald, Angela C.H.[1], Biechele, Steffen[1], Stanford, William L.[2], Rossant, Janet[1]
[1]Developmental and Stem Cell Biology, The Hospital for Sick Children, Toronto, ON, Canada, [2]Sprott Centre for Stem Cell Research, Ottawa Hospital Research Institute, Ottawa, ON, Canada

A SYSTEMS BIOLOGY APPROACH TO GUIDE DIRECT CONVERSION STRATEGIES AND ASSESS CELL IDENTITY

Morris, Samantha A.[1], Cahan, Patrick[1], Li, Hu[2], Fink, Emma C.[1], Reichel, Chloe E.[1], Collins, James J.[2], Daley, George Q.[1]
[1]Boston Children's Hospital, Harvard Medical School, Boston, MA, USA, [2]Department of Biomedical Engineering and Center for BioDynamics, Boston University, Boston, MA, USA

EFFICIENT GENERATION OF SAFE INDUCED DOPAMINERGIC NEURONAL (IDAN) CELLS FROM ADULT HUMAN FIBROBLASTS BY DIRECT REPROGRAMMING

Broccoli, Vania[1], Caiazzo, Massimiliano[1], Dell'Anno, Maria Teresa[1], Curreli, Sebastiano[2], Novara, Francesca[3], Leo, Damiana[2], Bespalov, Maxim M.[1], Sun, Alfred[4], Crabtree, Gerald R.[5], Gainetdinov, Raul[2], Pezzoli, Gianni[6], Zuffardi, Orsetta[3], Dityatev, Alexander[2]
[1]San Raffaele Scientific Institute, Milan, Italy, [2]Department of Neuroscience and Brain Technologies, Istituto Italiano di Tecnologia, Genoa, Italy, [3]Medical Genetics, University of Pavia, Pavia, Italy, [4]Department of Developmental Biology and Department of Pathology, Stanford University, Stanford, CA, USA, [5]Stanford University, Stanford, CA, USA, [6]Parkinson Institute, Istituti Clinici di Perfezionamento, Milan, Italy

Forced expression of developmental transcription factors (TFs) has shown an unpredictable capacity to govern the conversion of differentiated somatic cell types into functional induced neuronal (iN) cells. We previously identified a minimal set of three TFs (Ascl1, Nurr1 and Lmx1a) sustaining conversion of mouse dermal fibroblasts into functional induced dopaminergic neuronal (iDAN) cells. However, the same procedure resulted inefficient at reprogramming human fibroblasts. Here, we report new conditions that strongly enhance the reprogramming of adult human fibroblasts into functional induced dopaminergic neuronal (iDAN) cells. We have developed a unique combination of improved culture conditions, small-molecule treatments and microRNA supplementation to produce morphological and functional mature human iDAN (hiDAN) cells with high efficiency. In addition, we have disclosed a new molecular pathway that has a major influence in promoting human iDAN cell reprogramming restraining cell senescence while sustaining mitochondrial activity. Finally, we provide evidence that this procedure preserves genomic integrity of the reprogrammed cells. Importantly, these hiDAN cells are resistant to tumorigenic growth in immunodeficient mice. These conditions can accelerate the use of human iDAN as well as other induced neuronal cells for in vitro disease modeling and lay the foundation for exploiting their potential in cell replacement regenerative therapy.

4:00 PM - 5:50 PM

Ballroom West

Concurrent IID: Modeling Human Disease

MODELING HUNTINGTON'S DISEASE WITH INDUCED PLURIPOTENT STEM CELLS

Ellerby, Lisa
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Huntington's Disease is caused by a CAG expansion in the huntingtin gene. A polyglutamine expansion of over 42 repeats in the N-terminus of the huntingtin protein causes the disease and results in progressive loss of neurons in the striatum and cortex of Huntington's disease patients. In order to model HD and eventually utilize stem cells for therapeutics in this disease, we created an isogenic induced pluripotent stem cell model from HD patient cells using homologous recombination. We will present the characterization of this isogenic HD model including mitochondrial bioenergetics, metabolomics and RNA-seq analysis. We find that the neural stem cells from HD-IPSCs models many of the features of HD and genetic correction of these cells results in reversal of the phenotypes.

NOONAN SYNDROME ASSOCIATED-RAF1 MUTANT EVOKES HYPERTROPHIC CARDIOMYOPATHY FEATURES IN HUMAN CARDIOMYOCYTES IN VITRO

Araki, Toshiyuki[1], Tiburcy, Malte[2], Yin, Jiani[3], Dubois, Nicole[4], Mital, Seema[5], Kotton, Darrell[6], Keller, Gordon[4], Neel, Benjamin[1], Zimmermann, Wolfram[2]
1Ontario Cancer Institute, Toronto, ON, Canada, 2University of Gottingen, Gottingen, Germany, 3University of Toronto, Toronto, ON, Canada, 4University Health Network, Toronto, ON, Canada, 5Hospital for Sick Children, Toronto, ON, Canada, 6Boston University, Boston, MA, USA

Noonan syndrome (NS) and its related disorders, which are now called "RASopathies", are caused by aberrant activation of RAS/ERK pathway. Most RASopathies feature proportional short stature, facial dysmorphia, cognitive impairment and cardiac defects. The cardiac manifestations in RASopathies vary widely, but hypertrophic cardiomyopathy (HCM) is found in virtually all NS cases caused by RAF1 allele that encode a hyperactive kinase mutant. HCM also is common in LEOPARD syndrome and Costello syndrome. We reported previously that a mouse model of NS caused by a kinase-activated Raf1 mutant recapitulates major features of NS, including HCM. Importantly, these features were normalized by post-natal treatment of MEK inhibitor.
To extend these mouse studies to pre-clinical human models to better identify detail molecular basis and signaling as well as to aid in the development of new therapies for RASopathies, we have generated reprogramming factor-free human induced pluripotent stem cells (hiPSCs) from fibroblasts of multiple RASopathy patients. We also have generated human embryonic stem cells (hESCs) bearing RAF1 mutations by gene targeting. By forming embryoid bodies with cytokine cocktail, we successfully differentiated our hiPSCs into cTnT positive cardiomyocytes efficiently (50~80%). We found that a kinase-activating RAF1 mutant causes increased cell size (up to ~20%) of cardiomyocytes differentiated from hiPSCs compared with normal hiPSCs. To obtain more mature cardiomyocytes, we cultured differentiated cardiomyocytes as engineered heart tissue (EHT), which allows us to apply mechanical forces on these cardiomyocytes. As expected, cardiomyocytes form sarcomere structure in both normal and mutant EHT. We also found that cardiomyocytes in EHT show increased calcium sensitivity and lower response to b adrenergic stimulation in RAF1 mutant EHT. Importantly, we also observed the similar features in EHT from hESCs bearing RAF1 mutations when compared with their isogenic control. These data indicate that the features we observed in hiPSC-derived cardiomyocytes are not due to clonal variations among hiPSCs lines, and that NS-associated RAF1 mutants can evoke functional abnormalities in vitro.
We next attempted to normalize these features by pharmacological approach. Most importantly, we found that MEK inhibitor normalize functional abnormalities we observed in EHT. Our data show that NS associated RAF1 mutant can cause HCM phenotypes in vitro and provide a potential pre-clinical system for testing new therapies.

ATTITUDES OF PATIENTS TOWARD THE DONATION OF BIOLOGICAL MATERIALS FOR THE DERIVATION OF INDUCED PLURIPOTENT STEM CELLS

Jeremy Sugarman, Juli Bollinger, Ishan Dasgupta, Debra JH Mathews
Berman Institute of Bioethics, Johns Hopkins University, Baltimore, MD, United States

Background: Although there has been considerable discussion of the ethics of induced pluripotent stem cells (iPSC) in the popular and academic literatures, there is a paucity of data regarding the attitudes of patients toward the donation of biological materials for this research. Furthermore, while informed consent plays a central role in research, the informational needs of patients regarding iPSC donation for research are unclear. The purpose of this project is to gather such information to inform the development of appropriate policies for the consent, collection and use of biological materials being procured to derive induced pluripotent stem cells.
Methods: We conducted 3 focus groups with English speaking adult patients who receive outpatient care at Johns Hopkins. Following informed consent, a trained focus group moderator elicited participants' baseline knowledge about stem cells. A short background presentation on iPSCs was then provided. The moderator subsequently ensured coverage of all the domains in a focus group guide that was developed by the project team to include areas of potential concern that have been raised in the academic literature: research-related issues (e.g., immortalization, animal research, tissue type, data sharing); donor concerns (e.g., rights, confidentiality, incidental findings, clinical use, creation of gametes); and commercial-related issues (e.g., intellectual property, material sharing). Audio recordings from the focus groups were transcribed. The project team met to review the major themes that emerged in the groups. Two members of the team then independently reviewed the transcripts to identify additional themes and nuance. Major themes were selected for this preliminary analysis.
Results: Participants expressed considerable degrees of altruism towards the donation of biological materials for iPSC research, such that there was an overwhelming desire to donate as means of helping others in the future. This was especially salient concerning particular diseases and conditions with which they were personally familiar. Some participants expressed an interest in learning the results of research. Participants' ethical concerns focused primarily on the creation of gametes and commercialization, with the latter raising questions about compensation. Overall, they were less troubled by iPSC research than human embryonic stem cell research. Ultimately, participants had high degrees of trust in the research enterprise to manage its inherent ethical aspects. Transparency and obtaining informed consent for collection were generally believed to be necessary components of maintaining trust.
Discussion: Despite an array of ethical concerns related to stem cell research in general, our preliminary data indicate that they should not impede the collection of biological materials to create iPSCs. This assumes, of course, that the research system is trustworthy and has established procedures for navigating the related ethical issues. Moreover, participants believed that obtaining informed consent for collection was both necessary and appropriate. These findings have implications regarding the appropriateness of using banked collections of biological materials for iPSC and related research when specific consent has not been obtained. Future work will be directed at conducting additional focus groups to ensure informational redundancy as well as formal coding and analyses to help inform future policy development.

DISCOVERING AN EARLY NEURAL PHENOTYPE OF FAMILIAL ALZHEIMER'S DISEASES USING INDUCED PLURIPOTENT STEM CELLS

Jin, Ying[1], Yang, Juan[1], Ma, Yu[1], Zhao, Hanzhi[1], Shi, Guilai[1], Li, Ting[1], Tang, Fan[1], Gu, Junjie[1], Liu, Nan[2], Zhang, Xiaohui[2], Le, Weidong[1]
[1]Institute of Health Sciences, Shanghai, China, [2]Institute of Neuroscience, Shanghai, China

Alzheimer's disease (AD) is the most common form of age-related dementia with two pathological hallmarks: amyloid plaques and neurofibrillary tangles in the brain. The neurodegeneration in AD patients is progressive, beginning many years before clinically obvious symptoms emerge. The concept of a preclinical stage of AD is widely accepted. Currently, our understanding of AD etiology and pathogenesis is restrained by the unavailability of live neurons from patients and animal models fully recapitulating human AD pathogenesis. Recently, two independent studies reported modeling AD using induced pluripotent stem cells (iPSCs) from AD patients to derive neurons, providing proof of principle that iPSCs can serve as useful model to study patient specific AD pathology in vitro. Clearly, there remain many more questions such as how to use iPSCs to probe AD and how to find early AD phenotypes. In the present study, we generated iPSC lines from fibroblasts of two patients suffering from autosomal dominant early-onset familial AD (FAD) with mutations in presenillin (PS1) (A246E) or (S169del), respectively. Apart from having characteristic properties of human pluripotent stem cells, all AD iPSC lines were capable of differentiating into neurons that exhibited normal electrophysiological activity and expressed markers of various neuronal subtypes. However, neural cells generated from FAD iPSCs exhibited a significantly elevated Aβ42 to Aβ40 ratio, a hallmark feature of FAD with PS1 mutation. Moreover, these AD iPSC-derived neuronal cells had higher percentages of apoptotic cells and lower rates of proliferation, as compared to normal control cells differentiated from human ESCs or normal iPSCs cells. Most importantly, we found early phenotypes specific to neural cells differentiated from FAD prior to the detection of elevated ratios of Aβ42 to Aβ40, increasing the possibility of recapitulating early AD pathogenesis and identifying new strategies to interfere with FAD at an earlier stage. Genetic correction and verification of the early phenotypes specific to FAD neural cells in vivo are undergoing. Collectively, our study not only further demonstrates that FAD-iPSCs can be used as a valid model to study AD but also uncovers a previously unappreciated early neural alteration in FAD iPSC-derived neural cells. The established patient specific FAD iPSC lines will aid our understanding of the molecular mechanisms underlying AD pathogenesis and can be used in screening for new drugs for AD therapy.

PROGRAM AND ABSTRACTS

Poster Briefs

ACCELERATED NEURAL DIFFERENTIATION AND LOSS OF SELF-RENEWAL IN SMITH LEMLI OPITZ SYNDROME PATIENT IPS CELLS

Francis, Kevin, Wassif, Christopher, Ton, Amy, Westphal, Heiner, Porter, Forbes D.
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EFFICIENT AND REPRODUCIBLE MYOGENIC DIFFERENTIATION FROM HUMAN IPS CELLS CAN RECREATE A PATHOLOGICAL CONDITION OF MIYOSHI MYOPATHY

Sakurai, Hidetoshi[1], Tanaka, Akihito[1], Miyake, Katsuya[2], Woltjen, Knut[1], Hotta, Akitsu[1], Ikeya, Makoto[1], Shoji, Emi[1], Manabe, Yasuko[3], Yamamoto, Takuya[1], Era, Takumi[4], Sehara-Fujisawa, Atsuko[5], Kimura, En[6]
[1]Ctr for IPS Cell Res & Application (CiRA), Kyoto, Japan, [2]Department of Histology and Cell Biology, School of Medicine, Kagawa University, Kagawa, Japan, [3]Department of Health Promotion Sciences, Graduate School of Human Health Sciences, Tokyo Metropolitan University, Hachioji, Japan, [4]Institute of Molecular Embryology and Genetics, Kumamoto University, Kumamoto, Japan, [5]Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan, [6]Department of Neurology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan

IMPAIRED AUTOPHAGY IN A LIPID STORAGE DISORDER NIEMANN-PICK TYPE C1 DISEASE WHERE UPREGULATION OF AUTOPHAGY IS A POSSIBLE THERAPEUTIC STRATEGY

Sarkar, Sovan, Maetzel, Dorothea, Jaenisch, Rudolf
Whitehead Institute for Biomedical Research, Cambridge, MA, USA

HUMAN NEURONS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS REVEAL DETRIMENTAL EFFECTS OF APOLIPOPROTEIN E4: IMPLICATIONS FOR ALZHEIMER'S DISEASE

Wang, Cheng Zhong[1], Yoon, Seo Yeon[1], Walker, David[1], Jeong, Dah-eun[1], Xu, Qin[1], Balestra, Maureen[1], Hayashi, Yohei[2], Huang, Yadong[1]
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Alzheimer's disease (AD), one of the most common neurodegenerative disorders, causes progressive memory loss and other debilitating cognitive deficits. Clinically, most AD cases begin after age 65, referred to as late-onset AD (LOAD). A major genetic risk factor for LOAD is apolipoprotein (apo) E4. Human apoE has three isoforms (apoE2, apoE3 and apoE4), with apoE3/3 being the most common genotype in almost all populations. Expression of the apoE4 allele significantly increases disease occurrence and lowers the age of onset in a gene-dose-dependent manner. Induced pluripotent stem cells (iPSCs) can be used to model human diseases and to study their pathogenesis in culture. Here, we report the generation of several iPSC lines from human fibroblasts with different apoE genotypes (i.e., apoE3/3 and apoE4/4) and their differentiation into neurons in culture. We found that iPSC-derived neurons with an apoE4/4 genotype generated higher levels of neurotoxic apoE fragments than those with an apoE3/3 genotype. The ratio of phosphorylated-tau (AT8 positive) to total tau (Tau-5 positive) was also significantly higher in apoE4/4-neurons than in apoE3/3-neurons. Furthermore, the number of GABAergic neurons was much lower in apoE4/4-iPSC-derived neuron cultures than in apoE3/3-iPSC-derived neuron cultures, as determined by both immunostaining and western blot, although the total neuron numbers were similar, as determined by MAP2 or Tuj1 immunostaining and western blot. Interestingly, these detrimental effects of apoE4 could be rescued by treating neurons with an apoE4 structure corrector (PH002). We have also "corrected" apoE4 to apoE3 using zinc-finger-nuclease-mediated gene editing technology to confirm the specificity of apoE4's detrimental effects. Thus, these human iPSC lines with different apoE genotypes represent a critical advancement towards the accurate modeling of LOAD, and will be invaluable for detailed mechanistic studies, drug-screening efforts, and potential cell therapies.

Thursday, June 13, 2013 4:00 PM - 5:50 PM

Room 205

Concurrent IIE: Stem Cell Aging and Metabolism

REGULATION OF GROWTH BY THE MTOR PATHWAY

Sabatini, David
Whitehead Institute, Cambridge, MA, USA

mTOR is the target of the immunosuppressive drug rapamycin and the central component of a nutrient-and hormone-sensitive signaling pathway that regulates cell growth and proliferation. We now appreciate that this pathway becomes deregulated in many human cancers and has an important role in the control of metabolism and aging. We have identified two distinct mTOR-containing proteins complexes, one of which regulates growth through S6K and another that regulates cell survival through Akt. These complexes, mTORC1 and mTORC2, define both rapamycin-sensitive and insensitive branches of the mTOR pathway. I will discuss new results from our lab on the regulation and functions of the mTORC1 and mTORC2 pathways.

WHOLE CHROMOSOME INSTABILITY IS TOLERATED BY INTERFOLLICULAR EPIDERMAL CELLS, BUT NOT HAIR FOLLICLE STEM CELLS IN MOUSE SKIN

Foijer, Floris[1], Zhu, Yinan[1], DiTommaso, Tia[2], Donati, Giacomo[3], Hautaviita, Katta[4], Heath, Emma[3], Xie, Stephanie Z.[5], Smyth, Ian[2], Watt, Fiona M.[3], Sorger, Peter K.[6], Bradley, Allan[4]
[1]European Institute for the Biology of Ageing - University Medical Center Groningen, Groningen, Netherlands, [2]Monash University, Clayton, Australia, [3]Kings College, London, United Kingdom, [4]Wellcome Trust Sanger Institute, Hinxton, United Kingdom, [5]Princess Margaret and Toronto General Hospitals, Toronto, ON, Canada, [6]Harvard Medical School, Boston, MA, USA

Whole chromosome instability (wCIN) is a condition detrimental for the fitness and survival of normal cells, but also a hallmark of cancer cells. In our lab, we are interested in the consequences of wCIN for tissue and somatic stem cell integrity and the relationship between wCIN, ageing and cancer. The spindle assembly checkpoint (SAC) prevents wCIN by ensuring correct chromosome segregation during mitosis. Therefore, one way to provoke wCIN in the mouse is by inactivating this checkpoint. Homozygous inactivation of SAC genes unequivocally results in ES cell death and is therefore incompatible with early mouse development. To circumvent this limitation, we are developing mouse models in which we can inactivate the SAC in a conditional fashion, allowing us to provoke wCIN in tissues of choice during embryogenesis or in the adult.
To selectively induce wCIN and aneuploidy in the skin, we have recently generated a mouse model in which we can inactivate the SAC specifically in mouse epidermis using a conditional knockout of mouse Mad2, an essential component of the SAC signaling cascade. Whereas Mad2 deficiency results in rapid cell death in vitro, we found that despite dramatic aneuploidy, in vivo SAC inactivation is tolerated by the interfollicular epidermal (IFE) cells (basal and stratified cells in the skin). So these mice have a functional skin albeit without any hair. Consistent with aneuploid cell state, Mad2 deficient IFE cells in this epidermis exhibit abnormal transcription of metabolic genes and express high levels of p19ARF, suggestive of accelerated skin ageing. Conversely, the hair follicle bulge stem cells that are responsible for hair growth are completely absent, even though rudimentary hair follicles are continuously present, explaining the loss of hair in SAC-deficient skin.
Our results indicate that hair follicle stem cells are intrinsically more sensitive to chromosomal instability than (more differentiated) IFE cells. We are currently investigating the mechanisms that underlie this differential response, and whether similar differential responses also occur in other tissues. By understanding the molecular responses to aneuploidy in cell lineages that survive versus those that do not, we hope to uncover pathways that can be employed to specifically target aneuploid cell progeny in cancer.

PROLYL AND ASPARAGINYL HYDROXYLATION IN HYPOXIA-INDUCIBLE FACTOR PLAYS A CRITICAL ROLE IN HEMATOPOIETIC STEM CELL MAINTENANCE

Takubo, Keiyo, Suda, Toshio
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Mammalian hematopoietic stem cells (HSCs) reside in the hypoxic niche of the bone marrow. Lifelong maintenance of HSCs is supported by the fine-tuning of hypoxia response system including hypoxia-inducible factor (HIF)-1alpha. Under normoxic condition, HIF-1alpha is hydroxylated by prolyl hydroxylases (Phds) and an asparaginyl hydroxylase (FIH-1). Prolyl hydroxylated HIF-1alpha is recognized by an E3 ubiquitin ligase, VHL, resulting in protein degradation through ubiquitin-proteasome pathway. Asparaginyl hydroxylated HIF-1alpha by FIH-1 is unable to associate with coactivators. Thus hydroxylation mechanisms are crucial regulators for HIF-1alpha. In cells, FIH-1 is antagonized by Munc-18 Interacting Protein 3 (Mint3) through a protein sequestration mechanism. To clarify the functional significance of these hydroxylases in HSCs, prolyl hrdroxylase 2 (Phd2)- and Mint3-deficient HSCs from knockout mice were analyzed. Conditional Phd2 inactivation in HSCs induced an activation of HIF-downstream effectors. Transplantation of Phd2-deficient HSCs resulted in a suppression of peripheral blood chimerism. In contrast, an accumulation of Phd2-deficient HSCs was observed in the recipient mice, suggesting a crucial role of Phd2 in maintenance of primitive phenotype of HSCs. Mint3-deficient mice had an increased number of mononuclear cells and primitive hematopoietic cells including HSCs in the bone marrow. However, transplantation of Mint3-deficient HSCs showed poor reconstitution capacity. Suppression of prolyl and asparaginyl hydroxylases by a small molecule inhibitor reversibly suppressed proliferation of HSCs and maintained primitive phenotypes ex vivo. These data suggest important roles of the hydroxylation regulators for HIF in HSCs and the modulation of hydroxylation of HIF is a promising tool for HSC manipulation ex vivo.

HUMAN INDUCED PLURIPOTENT STEM CELLS EXHIBIT HIGH PKM2 LEVELS AND HIF-1 ALPHA DRIVEN EARLY RECONFIGURATION OF ENERGY METABOLISM

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Reprogramming somatic cells to a pluripotent state drastically reconfigures the cellular anabolic requirements, thus potentially inducing cancer-like metabolic transformation. Accordingly, we and others have previously shown that somatic mitochondria and bioenergetics are extensively remodeled upon generation of induced pluripotent stem cells (iPSCs), as the cells transit from oxidative to glycolytic metabolism. Here, we sought to identify possible regulatory mechanisms and porentially determine whether metabolic restructuring is a necessary step for the induction of pluripotency.
Using global gene expression analysis and targeted proteomics, we discovered that iPSCs express high levels of pyruvate kinase M2 (PKM2), whose elevation has been linked to the metabolic switch in tumor cells, and pyruvate dehydrogenase kinase (PDK) 1 and 3, all known downstream targets of hypoxia-inducible factor 1 alpha (HIF1α). The up-regulation of these HIF1α-related transcripts occurred during the initial reprogramming-initiation phase, when the genes dictating self-renewal and pluripotency have yet to be turned on. Moreover, this reprogramming-associated transcriptional reconfiguration of energy metabolism corresponded to metabolic alterations indicative of a glycoliytic shift. Finally, using lentiviral-mediated shRNA, we found that the ablation of the master metabolic regulator HIF1α or its target PDK1 dramatically hampered reprogramming efficiency. On the other hand, chemical HIF1α activation improved iPSC generation.
Taken together, HIF1α-mediated reconfiguration of glucose metabolism may represent an early enabling step of cellular reprogramming, a barrier that has to be overcome in order to allow somatic cells to sustain their newly acquired proliferative and biosynthetic needs. The findings imply a possible instructive role for metabolic regulation in cell fate conversion and may help to shed light on the mechanisms regulating the cellular plasticity required to reprogram adult somatic cells into self-renewing pluripotent stem cells.

Poster Briefs

CYCLES OF DIETARY RESTRICTION REDUCE INSULIN/IGF-1 SIGNALING TO PROMOTE STEM CELL-BASED REGENERATION AND REVERSE IMMUNODEFICIENCY

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THE ATM-BID-MTCH2 MITOCHONDRIAL PATHWAY REGULATES HAEMATOPOIETIC STEM CELL FATES

Maryanovich, Maria, Gross, Atan
Biological Regulation, The Weizmann Institute of Science, Rehovot, Israel

NUCLEAR MIRNAS REGULATE THE METABOLISM OF INDUCED PLURIPOTENT STEM CELLS IN PART THROUGH MITOCHONDRIAL GENES

Lee, Man Ryul, Charlie Mantel, Hal E. Broxmeyer
Immunology and Microbiology, Indiana University School of Medicine, Indianapolis, IN, United States

MODELING NEURONAL AGING TO STUDY LATE-ONSET NEURODEGENERATIVE DISEASES WITH IPSCS

Miller, Justine D.[1], Ganat, Yosif M.[1], Kishinevsky, Sarah[1], Mandal, Pankaj[2], Bowman, Robert L.[3], Tu, Edmund[4], Shim, Jae-won[1], Tomishima, Mark J.[4], Betel, Doron[5], Krainc, Dimitri[6], Rossi, Derrick J.[2], Studer, Lorenz[1]

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Induced pluripotent stem cells (iPSCs) represent a powerful technology for modeling human disease in vitro. We have previously demonstrated the use of iPSC technology for modeling early onset disorders such as familial dysautonomia or Herpes Simplex Encephalitis. Those studies led to novel insights into disease mechanisms and enabled the first high throughput drug screen in an iPSC-based disease model. Despite those early successes, there is a fundamental question as to how well iPSC-based approaches can model late-onset disorders such as Parkinson's disease (PD), which normally take decades to develop. Indeed current studies modeling genetic or sporadic forms of PD show mild phenotypes and have not yet recreated the severe degenerative pathology characteristic of the disease.

We sought to investigate whether age, the most significant risk factor in PD, is maintained during in vitro reprogramming of patient-specific fibroblasts, and during the subsequent re-differentiation of iPSCs into somatic cells, including midbrain dopamine (mDA) neurons. Previous studies have suggested that the reprogramming process may reset the molecular clock of the cell, making aged cells appear young. We first defined a set of molecular markers capable of stratifying healthy fibroblasts according to donor age. Those markers, related to age-dependent changes in nuclear morphology, chromatin state, DNA repair, and mitochondrial function, were fully reset after reprogramming to pluripotency, independent of fibroblast donor age. In addition, the re-differentiation into iPSC-derived fibroblasts did not re-establish those age-related markers, further suggesting that age was permanently erased during the reprogramming process.

In order to address this reset to a young/embryonic cell state, we explored several genetic strategies to induce rapid in vitro aging using synthetic mRNA technology. From those studies we were able to establish conditions that can re-induce the age-related signature in iPSC-derived fibroblasts, independent of fibroblast donor age. We next assessed the impact of our accelerated in vitro aging technology on iPSC-derived midbrain dopamine (mDA) neurons, the cell type responsible for key clinical symptoms in PD. While multiple phenotypes observed in "aged" iPSC-fibroblasts could be induced in "aged" iPSC-mDA neurons, mDA neurons also exhibited unique phenotypes suggestive of neuronal-specific decline, including dendrite degeneration and an increased rate of apoptosis. Finally, we used our newly developed in vitro aging protocols on mDA neurons derived from several PD-iPSC lines to model the late-onset aspects of the disease. The "aged" mDA neurons from PD-iPSCs displayed Akt dysregulation and accelerated degeneration indicative of frank disease, phenotypes which were not observed in control-treated cells. Our study presents a first attempt at controlling cellular age in vitro in combination with the modeling of genetic disease susceptibility. Directing in vitro neuronal aging may represent a key strategy towards establishing more relevant disease models of late-onset neurodegenerative disorders.

Friday, June 14, 2013, 9:00 AM - 11:20 AM

Plenary Hall B1

Plenary IV: Cell and Gene Therapy

Joint Session with the American Society of Gene & Cell Therapy and the European Society of Gene & Cell Therapy

INTEGRATING VIRAL VECTORS, GENE TRANSFER INTO HEMATOPOIETIC STEM CELLS AND THERAPIES IN HUMAN MONOGENIC DISEASES

Williams, David

Boston Children's Hospital, Boston, MA, USA

Since the early development of viral vectors, their use to increase gene transfer efficiency into rare populations of cells has been exploited. Gene transfer into rare, reconstituting hematopoietic stem cells using integrating viral vectors has developed as a therapeutic modality for monogenic diseases of the blood and some inflammatory conditions. Proof-of-principle successes showing phenotypic correction in multiple diseases have been complicated by integration-associated genotoxicity leading to serious adverse events. Subsequent improvement in vector designs in both gamma retrovirus and lentivirus platforms has led to new follow-on trials that seek to determine if these vectors improve safety while maintaining efficacy in X-linked severe combined immunodeficiency, Wiskott-Aldrich Syndrome and chronic granulomatous disease. These new human trials represent maturation of the field via bedside to bench reverse translation which has also been associated with renewed interest by biotech and pharmaceutical companies. Present and future trials seek to further refine lineage-specific transgene expression, introduce expression of shRNAs for gene knock-down and utilize sequence-specific gene targeting for clinical applications in definitive gene correction in some monogenic diseases.

PHASE I/II CLINICAL TRIAL OF HEMATOPOIETIC STEM CELL GENE THERAPY FOR THE TREATMENT OF METACHROMATIC LEUKODYSTROPHY

Biffi, Alessandra

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Metachromatic Leukodystrophy (MLD) is an autosomal recessive lysosomal storage disorder caused by Arylsulfatase A (ARSA) deficiency and leading to severe demyelination, neurodegeneration and premature death of the affected patients. Currently, no treatment can halt the progression of this devastating disease. According to preclinical data demonstrating the safety and efficacy of hematopoietic stem cell gene therapy in the animal model of the disease, and based on the experience we acquired on the natural clinical course of the disease and its instrumental and clinical monitoring, on March 2010 a clinical trial based on transplantation of autologous hematopoietic stem cells transduced with LVs encoding ARSA was approved by the Italian Regulatory Authorities. The clinical protocol foresees the enrollment of 6 late infantile (LI) and 2 early juvenile (EJ) patients, in pre- and, in the case of EJ patients, early-symptomatic stage, in order to provide them a reasonable expectation of clinical benefit. The study objectives are the evaluation of the safety of the treatment, related to the myeloablative conditioning regimen employed and to the use of LVs, and of its efficacy by measuring patients' motor abilities and demyelination occurring in the nervous system through the use of validated instrumental readouts.

Until now seven patients have been enrolled and treated. Six of them had a biochemical, molecular and familiar history compatible with a diagnosis of LI MLD and have been treated in a pre-symptomatic stage of their disease. Only one patient, with a disease onset compatible with the EJ form of the disease, was treated in an early symptomatic stage. Thus far, we can report a favorable outcome of the transplant procedure with a good bone marrow recovery and the short/medium-term safety of both the conditioning regimen and stem cell transduction with LVs in all the treated patients. Moreover, we report stable sustained ARSA gene replacement to nearly exhaustive levels in the reconstituted hematopoiesis of the patients, resulting in supra-normal ARSA activity throughout the hematopoietic lineages and its reconstitution in the cerebrospinal fluid, the latter thus far documented in the first three treated patients. These findings are associated with substantial therapeutic benefit. Indeed, the follow-up of the first three late infantile treated patients, in which the follow up time after the expected symptoms onset (as defined according to disease onset in the affected older siblings) the disease had not appeared/progressed; furthermore they are rather experiencing a continuous motor and cognitive development, at odds with the natural disease course and their sibling anamnesis, and have a normal quality of life. These data are extremely encouraging, even if only the long-term follow-up of all the treated patients will confirm this favorable preliminary indication.

ETHICAL ISSUES IN CELL- AND GENE-BASED RESEARCH AND THERAPY

King, Nancy

Wake Forest University Health Sciences, Winston-Salem, NC, USA

Gene transfer research and research on cell-based interventions have many overlapping scientific and ethical features, and the history of gene transfer research provides some important lessons from which cell-based intervention research can benefit. This presentation addresses some key considerations that apply to both research and treatment using cell and gene therapies. It begins with an overview of ethical considerations in the design of clinical and translational trials, and goes on to address a set of issues that arise in both fields. Some of these issues represent long-standing debates in research ethics; others are novel and continue to unfold along with the rapidly developing science. Relevant questions include: translation from preclinical studies to research with human subjects; how to measure success and failure in research and treatment; describing research interventions, especially in early-phase trials, when therapeutic misconception is likely; discussing research participation, especially when promising preliminary results may influence decision-making; and ensuring adequate follow-up when adverse events are rare but serious and long-term efficacy data are needed. Research involving both gene transfer and cell-based interventions has developed rapidly in a climate of great enthusiasm, based on the promising logic of the science and its potential application to many serious conditions, especially those for which few adequate alternatives exist. It is essential to consider whether novel biotechnologies like gene transfer and cell-based intervention research have altered our understanding of the nature and goals of first-in-human trials, the line between research and treatment, and the concept of normal human functioning. Research development and design decisions must take account of the balance between clinical urgency and scientific thoroughness, by ensuring that the trajectory of research is focused on learning as much as possible, moving to the next stage of research only when enough information has been gathered to justify a step forward, and minimizing the risks of harm to patient-subjects without expecting them to forgo potential benefit from standard or, in appropriate cases, experimental interventions. Researchers and oversight bodies in gene transfer and cell-based interventions can lead the way in addressing these critical and contested matters, as the science in these overlapping fields continues its rapid development.

TRANSDIFFERENTIATION AND ITS IMPLEMENTATION IN AUTOLOGOUS CELL REPLACEMENT THERAPY FOR DIABETES

Ferber, Sarah

Sheba Medical Center & Tel-Aviv University, Tel-Hashomer, Israel

Cellular differentiation and lineage commitment were considered irreversible processes. However, recent reports have indicated that differentiated adult cells can be reprogrammed to an embryonic like pluripotent state and in some cases directly to alternate committed lineages. Dominant instructive roles for transcription factors in reprogramming somatic cells have been suggested. However, while numerous somatic cell sources give rise to pluripotent cells, it is believed that committed lineages are generated either in developmentally related tissues or by the differentiation of pluripotent cells.

Here we challenge this view and suggest that adult cell reprogramming along alternate committed lineages can be a direct process, which crosses the boundaries of the distinct developmental germ layers, without the need for pre-induced pluripotency.

We demonstrate that the endoderm derived pancreatic lineage and β-cell-like function are induced in adult cells which are derived from all three germ layers, by ectopic expression of non-integrating pancreatic differentiation factors. The direct reprogramming process along the alternate committed lineage is fast, specific and relatively abundant, compared to reprogramming pluripotent cells. The mechanism which mediates the developmental process and the role of Epithelial-Mesenchyme transitions will be discussed.

Direct reprogramming of somatic cells carries important implications in developing patient specific regenerative medicine approaches which may prove safer than using ESC or iPS cells as progenitors. The generation of insulin producing cells by adult cells reprogramming allows the diabetic patient to serve also as the donor of his own therapeutic tissue.

REGENERATING THE HEART OF A NON-HUMAN PRIMATE

Murry, Charles

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The heart is among the body's least regenerative organs, which makes heart disease an important target for stem cell-based regenerative therapies. To date no adult stem cell population has exhibited a robust cardiogenic activity, and this has led our group to explore human embryonic stem cells (hESCs) as a source of cardiomyocytes for heart regeneration. We have developed techniques for the scalable production of more than one billion human cardiomyocytes per run and cryopreserving them with good viabilities. Based on success of hESC-cardiomyocytes in small animal models of cardiac repair (mouse, rat, guinea pig) we sought to test the ability of these cells to repair the heart of the species most likely to reflect the human response: the non-human primate.

Pig-tailed macaques (Macacca nemestrina) were given myocardial infarcts by balloon catheter inflation followed by reperfusion. Two weeks later we injected 1 billion hESC-cardiomyocytes, genetically modified to express the calcium reporter, GCaMP3, into the infarct and peri-infarct region. Control animals received a vehicle injection. Monkeys were immunosuppressed with cyclosporine A, corticosteroids and co-stimulatory blockade. All cell-treated monkeys experienced a transient period of ventricular arrhythmias that subsided by the third week. The human cells engrafted in the monkey hearts and beat in synchrony with the host tissue, indicating successful electromechanical integration. Histological evaluation showed large grafts of human myocardium, often remuscularizing half of the infarct region.

These experiments show that hESC-derived cardiomyocytes can partially regenerate the heart of primate, suggesting that they can integrate and contribute to systolic function in a human heart as well. Additional studies need to be done to determine the source of arrhythmias and how they can be suppressed.

1:15 PM - 3:05 PM

Ballroom East

Concurrent IIIA: Hematopoietic Stem Cells

PROSTAGLANDIN-MODULATED UMBILICAL CORD BLOOD HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Slow engraftment and incomplete immunologic reconstitution associated with low hematopoietic stem cell (HSC) numbers limit the effectiveness of umbilical cord blood (UCB) transplantation. We previously identified 16,16 dimethyl prostaglandin E_2 ($dmPGE_2$) to be a critical regulator of hematopoietic stem cell homeostasis. We hypothesized that a brief ex vivo modulation of HSCs with $dmPGE_2$ prior to transplantation would enhance UCB engraftment by increasing the "effective dose" of hematopoietic stem cells in UCB units. METHODS: A phase I trial was performed to evaluate the safety and efficacy of ex vivo modulation of a single UCB unit using $dmPGE_2$ prior to reduced-intensity double UCB transplantation, with a goal of enhancing UCB engraftment. The primary endpoints were neutrophil engraftment and $dmPGE_2$-derived donor chimerism. RESULTS: A total of 21 patients were enrolled. Under initial $dmPGE_2$ modulation conditions (10 µM $dmPGE_2$, 4°C, 60 minutes), no favorable effect on engraftment or chimerism was noted in the first 9 patients treated. Molecular characterization experiments revealed that the prostaglandin pathway was not being activated in the treated HSCs under these conditions. Once the optimized ex vivo modulation conditions were determined (10 µM $dmPGE_2$, 37°C, 120 minutes) 12 additional patients were enrolled. This cohort demonstrated accelerated neutrophil engraftment (17.5 vs. 21 days, p=0.045) and chimerism dominance of $dmPGE_2$-modulated unit (10 of 12, p=0.039), which persisted through 27 months from transplantation. CONCLUSIONS: Ex vivo modulation of the prostaglandin pathway with $dmPGE_2$ prior to UCB transplantation leads to physiologic changes that enhance engraftment and favor sustained hematopoiesis in a competitive transplantation experiment.

NOTCHLESS AT THE CROSSROAD BETWEEN RIBOSOME BIOGENESIS AND ADULT HEMATOPOIETIC STEM CELL MAINTENANCE

Le Bouteiller, Marie[1], Souilhol, Celine[1], Beck-Cormier, Sarah[1], Stedman, Aline[1], Burlen-Defranoux, Odile[2], Vandormael-Pournin, Sandrine[1], Bernex, Florence[3], Cumano, Ana[2], **Cohen-Tannoudji, Michel[1]**
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Recent studies have revealed that stem cells may differ from their progenies at the level of constitutive cellular processes such as response to DNA damage or energy metabolism regulation. From this perspective, we are addressing the role of Notchless (Nle) in mouse. Nle ortholog in yeast has been shown to be involved in the large ribosomal subunit biogenesis, and we showed that this role is conserved in mouse. Ubiquitous inactivation of Nle in adult induced the death of the mice within 12 days, which is preceded by a severe disturbance of hematopoietic tissues. In the bone marrow, we observed a rapid and drastic exhaustion of stem cells and multipotent progenitors. Non-competitive and competitive transplantations showed that Nle is required for HSC maintenance in a cell autonomous manner both at steady-state and under stress. Thus, our data identified Nle as an important factor for adult HSC regulation. Strikingly, Nle is not required in more committed hematopoietic cells, despite its role in the basic process of ribosome biogenesis. Indeed, using either ex vivo or in vivo approaches, we showed that Nle is dispensable for the differentiation and proliferation of myeloid progenitors and B cells respectively. We developped a FISH technique coupled with hematopoietic immunostainings to monitor accumulation of immature rRNA in hematopoietic cell nucleoli, which allowed us to correlate ribosome biogenesis defects with the phenotype induced by Nle deletion. These results suggest that there may be alternative pathways for ribosome biogenesis, either Nle-dependent or Nle-independent, which could be used differently by stem/progenitor cells and more committed cells. Together with other reports, our results suggest that ribosomes and ribosome biogenesis may have a regulatory rather than a constitutive role. To our knowledge, we provide evidence for the first time that ribosome biogenesis may have a regulatory role in stem cell maintenance.

NUCLEAR TRANSLOCATION OF GATA3 NEGATIVELY REGULATES SELF-RENEWAL IN ACTIVATED LONG-TERM HEMATOPOIETIC STEM CELLS

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Transcript-level expression of the Gata3 transcription factor has been described in hematopoietic fractions enriched in long-term hematopoietic stem cells (LT-HSC). However, conditional deletion is reported to have little if any significant impact in adult HSC. The findings suggest that Gata3 is either not expressed in LT-HSC but rather in contaminating cells present in the purified fractions previously tested, or is expressed but like many transcription factors plays a role only when activated by upstream signalling.
In order to establish LT-HSC-specific expression of Gata3, we made use of a Gata3 mutant mouse in which GFP is expressed under the control of the endogenous Gata3 promoter. When marrow from these mice was fractionated according to GFP fluorescence and tested in reconstitution assays, long term engraftment was obtained only from GFP+ cells. We used immunofluorescence microscopy on purified HSC populations to determine if Gata3 mRNA was translated into protein. Gata3 protein was detected in 30% of $Rho^{lo}Kit^{+}Sca^{+}Lin^{-}CD49b^{lo}$ and 85% of $Rho^{lo}Kit^{+}Sca^{+}Lin^{-}CD49b^{lo}CD150^{hi}$ LT-HSC fractions, percentages that corresponded to the number of cells able to long-term graft in those fractions, reinforcing the evidence that Gata3 is expressed specifically in LT-HSC.
Confocal microscopy localized Gata3 in the cytoplasm of quiescent LT-HSC suggesting that Gata3 may not play a role in steady state conditions. To determine if Gata3 could be activated by upstream signalling, we cultured LT-HSC with cytokines and observed that Gata3 translocated entirely into the nucleus after 2 days. Translocation was repressed by pharmacological inhibition of p38/MAPK, and activation of p38 was confirmed in cultured HSC. We also observed nuclear translocation of Gata3 in vivo in LT-HSC 10 days after induction with poly(I:C), a double-stranded RNA analog and Toll-like receptor 3 agonist with multiple downstream effects including induction of Type I interferon. The translocation was reversible, Gata3 was again cytoplasmic 5 months after poly(I:C) treatment. LT-HSC harvested 10 days after poly(I:C), when Gata3 was still nuclear, had sharply reduced capacity for long-term engraftment. In contrast, long-term reconstituting activity of LT-HSC assayed 5 months after treatment was robust revealing a correlation between nuclear localization of Gata3 and negative regulation of self-renewal .
To test directly for Gata3 involvement in self-renewal we assessed the effects of conditional deletion of Gata3 using M×1-cre deleter mice. We showed, as expected, that Gata3 deletion did not affect steady state hematopoiesis. However, Gata3-deleted HSC were resistant to the depleting effect of poly(I:C), exhibited strikingly enhanced expansion of HSC numbers after transplant into irradiated mice, and, unlike normal controls, maintained input levels of long-term engraftment capacity through 7 days of culture. Ki67/Hoechst staining of deleted and control LT-HSC showed no differences in cell cycle status either in steady state marrow or in cycle entry in vivo following poly(I:C) treatment, and deleted cells had growth kinetics in culture identical to controls.
Our results reveal a novel role for Gata3 that is specific to LT-HSC. Gata3 protein is cytoplasmic in quiescent LT-HSC but relocates to the nucleus in response to activation by p38/MAPK. Deletion experiments reveal a significant negative regulatory role in HSC self-renewal that occurs without direct influence on cell cycle state.

THE INTRINSIC APOPTOSIS CASPASE CASCADE REGULATES HEMATOPOIETIC STEM CELL HOMEOSTASIS AND FUNCTION

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Since the seminal observation that overexpression of pro-survival Bcl-2 causes an expansion of hematopoietic stem cell (HSC) number and repopulation potential (Domen et al. 2000 J Exp Med), it has become widely accepted that apoptotic death is a common fate for HSCs. Along with self-renewal and differentiation, programmed cell death is believed to be critical for regulating the size of the HSC pool. Consistent with this idea, mice lacking the apoptotic effector caspase, Caspase-3, which operates downstream of Bcl-2-regulated mitochondrial damage, were reported to have increased numbers of immunophenotypic long-term repopulating HSCs (Janzen et al. 2008 Cell Stem Cell). Intriguingly; however, this expansion was ascribed to perturbations in cytokine signaling, rather than impaired HSC apoptosis.
To examine the role of the intrinsic apoptosis pathway in HSC homeostasis and function in more detail, we generated bone marrow chimeras lacking the critical pro-apoptotic proteins Bak/Bax, Apaf-1, Caspase-9 or Caspase-3/7. Surprisingly, we found that loss of Bak and Bax - the essential initiators of the intrinsic apoptosis pathway - had a minor impact on HSC number and function. In contrast (but consistent with Janzen et al.) bone marrow chimeras lacking the downstream effectors Apaf-1, Caspase-9 or Caspase-3/7 displayed a 10-fold expansion of the HSC compartment. Casp9-/- bone marrow exhibited an impaired ability to reconstitute lethally irradiated recipients, suggesting that Caspase-9 regulates HSC function, and in its absence, a dysfunctional pool of HSCs accumulates. Whereas, apoptosis mediated by Bak and Bax appears to be dispensable for HSC homeostasis. This raised several questions: 1) Is the HSC expansion in Caspase-9-deficient mice intrinsic to the HSCs themselves? 2) Is the function of Caspase-9 (and the rest of the apoptotic cascade) in HSCs to promote cell death, or is it to catalyze some other cellular process? 3) If Caspase-9 is inducing apoptosis in HSCs, can it really be operating independently of Bak and Bax?
To answer these questions, we firstly generated bone marrow chimeras containing 50% wild-type and 50% Casp9-/- cells. In these animals, wild-type HSCs exhibited the same expansion and proliferation as Casp9-/- HSCs. Thus, the HSC expansion evident in Caspase-9 deficient mice is the result of HSC extrinsic factors. We then tested the relationship between Caspase-9-deficient HSC expansion and Bak/Bax-mediated apoptosis by generating Bak-/- Bax-/- Casp9-/- mice. Deletion of Bak and Bax completely rescued the HSC phenotype evident in Caspase-9 deficient mice. This suggests that the intrinsic caspase cascade is essential for normal Bak/Bax-mediated cell death in the hematopoietic system, and in its absence, aberrant cell death feeds back to drive HSC expansion and dysfunction. We embarked on a search for extrinsic factors in Caspase-9 deficient mice, and found evidence of up-regulated interferon signaling. Our hypothesis is that the failure of Bak/Bax-mediated apoptosis (due to the loss of Caspase-9 and the apoptotic cascade) in an as-yet unidentified hematopoietic cell promotes the production of interferons, which feeds back to the HSC compartment.
Our study demonstrates that Bak/Bax-mediated cell death of HSCs is dispensable for the maintenance of the HSC pool at steady state. This raises the question of whether HSCs die via alternative cell death pathways, or, whether programmed cell death is a much more infrequent HSC fate than previously believed.

Poster Briefs

NEW INSIGHTS INTO HEMATOPOIETIC STEM AND PROGENITOR CELL LINEAGE CONTRIBUTIONS AND KINETICS VIA CLONAL BARCODING IN A NON-HUMAN PRIMATE MODEL

Li, Brian[1], Wu, Chuanfeng[1], Lu, Rong[2], Jares, Alexander[1], Donahue, Robert E.[1], Weissman, Irving[2], Dunbar, Cynthia E.[1]
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MIR-126 CONTROLS HEMATOPOIETIC STEM CELL FUNCTION

Boccalatte, Francesco E.[1], Gentner, Bernhard[1], Giustacchini, Alice[1], Saini, Massimo[1], Restuccia, Umberto[2], Garcia Manteiga, Jose M.[3], Lechman, Eric R.[4], Van Galen, Peter[4], Stupka, Elia[3], Bachi, Angela[2], Dick, John E.[4], Naldini, Luigi[1]
[1]Telethon Institute for Gene Therapy, Milan, Italy, [2]Biomolecular Mass Spectrometry unit, San Raffaele Scientific Institute, Milan, Italy, [3]Center for Translational Genomics and Bioinformatics, San Raffaele Scientific Institute, Milan, Italy, [4]Ontario Cancer Institute, University Health Network, Toronto, ON, Canada

OMICS LANDSCAPE OF HEMATOPOIETIC STEM CELLS AND MULTIPOTENT PROGENITORS

Cabezas-Wallscheid, Nina[1], Klimmeck, Daniel[1], Hansson, Jenny[2], Dohrn, Lisa[1], Reyes, Alejandro[2], Huber, Wolfgang[2], Krijgsveld, Jeroen[1], Trumpp, Andreas[1]
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HUMAN DEFINITIVE AND PRIMITIVE HEMATOPOIESIS ARE SPECIFIED SIMULTANEOUSLY FROM HUMAN PLURIPOTENT STEM CELLS

Sturgeon, Christopher M., Ditadi, Andrea, Awong, Geneve, Kennedy, Marion, Keller, Gordon
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The generation of the hematopoietic stem cell (HSC) from human pluripotent stem cells (hPSC) is a major goal for regenerative medicine. HSCs derive from a progenitor population known as hemogenic endothelium (HE) that is specified at the onset of definitive hematopoiesis. To generate HSCs from hPSCs, it will be important to recapitulate these developmental steps in stem cell differentiation cultures. We have recently identified a hPSC-derived CD34+ definitive hematopoietic progenitor that has characteristics of HE and displays T lymphoid, myeloid and erythroid potential. We used this approach to study the developmental progression of human hematopoiesis, and found that these different developmental programs can be easily separated by the expression of KDR and CD235a. Functional analyses revealed that the KDR+CD235a+ population contained hemangioblasts and gave rise to primitive erythroid progenitors, as well as a CD34+ HE population that gave rise to erythroid and natural killer (NK) progenitors but not T cell progenitors. In contrast, the KDR+CD235a- lacked hemangioblast potential, but instead gave rise to CD34+ HE that generates T cell, NK cell, and erythroid progenitors. These observations strongly suggest that the KDR+CD235a+ and KDR+CD235a- cells represent progenitors of the primitive and definitive hematopoietic programs, respectively. However,

both CD34+ populations equally express hemogenic endothelium markers such as CD144, CD117, GATA2, SCL, LMO2 and RUNX1, suggesting these markers may not be informative in discerning the earliest progenitors of primitive versus definitive hematopoiesis. Interestingly, development of the KDR+CD235a+ progenitors is dependent on Activin/nodal signaling, whereas the simultaneous specification of the definitive KDR+CD235a- progenitors is independent of Activin/nodal signaling. The simultaneous differentiation of both human hematopoietic programs via KDR+ progenitors represents a novel insight into their development, highlighting our need for a greater understanding of the specification of primitive and definitive HE in order to properly address the in vitro generation of the hematopoietic stem cell.

Friday, June 14, 2013 1:15 PM - 3:05 PM

Room 258

Concurrent IIIB: Epigenetics of Stem Cells

CYTOSINE-5 RNA METHYLATION IN NORMAL TISSUES AND DISEASES

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Cytosine-5 methylation (m5C) is a widespread modification in both DNA and RNA and the corresponding methyltransferases share many structural features. Whereas the functions of m5C in DNA have been extensively studied, the cellular and molecular functions of the same modified nucleobase in RNA remain unclear. We found that m5C is a common post-transcriptional modification in transfer RNA (tRNA) and other non-coding RNA species. RNA-methylation pathways are important regulators of stem cell differentiation in skin and loss-of-function mutations in the cytosine-5 RNA methylase NSun2 causes neurodevelopmental diseases in humans. Depletion of the m5C modification in tRNAs causes their cleavage and the cleaved tRNA fragments are directly implicated in the reduction of protein translation rates under stress. Thus, post-transcriptional cytosine-5 methylation is an important and unexpected regulatory pathway to control cellular behavior.

DNA METHYLTRANSFERASE 3 DEPENDENT NON RANDOM SEGREGATION OF DNA STRANDS IN DIFFERENTIATING EMBRYONIC STEM CELLS

Elabd, Christian, Cousin, Wendy, Chen, Robert Y., Chooligan, Marc S., Pham, Joey T., Conboy, Irina M., Conboy, Michael J.
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Non-random template segregation (NRTS) of chromosomes, in which template DNA chromosomes are segregated by age, has been shown during asymmetric cell divisions in phylogenetically divergent organisms such as plants, fungi, and mammals. The molecular mechanism and purpose of NRTS have remained unknown and theories of Immortal DNA genome maintenance, preventing mutations or epigenetic changes, have so far lacked direct evidence. Asymmetry of cell fate, where one daughter cell self-renews while the other differentiates, is a fundamental characteristic of stem cells. NRTS has been associated with asymmetric division of some tissue-specific stem cells. However, prior to this work asymmetric inheritance of chromatids has never been demonstrated in embryonic stem cells. We unambiguously demonstrate high occurrence of NRTS in asymmetrically-dividing, differentiating human and mouse embryonic stem cells through the use of conventional microscopy as well as time-lapse imaging. We show that NRTS is linked to cell fate of early mesodermal and primitive endodermal lineages. Moreover, using both pharmacological and genetic ablation, we show that NRTS is dependent on DNA methylation and on DNA methyltransferase 3 (Dnmt3), uncovering the molecular mechanism that regulates this phenomenon.
Our data suggest NRTS as a potential way for differentiating stem cells to pass on differential epigenetic information to daughter cells: retention of chromatids with the "old" template DNA preserves the epigenetic memory of cell fate, while localization of "new" DNA strands and de novo Dnmt to the lineage-destined daughter cell facilitates epigenetic commitment to a new cell fate.

DURING REPROGRAMMING, AID STABILIZES A STEM CELL PHENOTYPE BY REMOVING EPIGENETIC MEMORY OF SECONDARY PLURIPOTENCY NETWORK GENES

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DNA methylation is thought to represent a major barrier to cellular reprogramming, as it has the capacity to impose an epigenetic memory that functions to stabilize cell fate and phenotype. Recently there has been progress demonstrating the true dynamics of DNA methylation, with the discovery of active mechanisms for reversing previously established methylation patterns, thereby altering epigenetic memory. One major pathway is controlled by the activation-induced cytidine deaminase (AID) enzyme that is highly expressed in germinal center B cells and modifies the immunoglobulin locus. Cytidine deamination can promote the generation of mutations that help increase antibody diversity. However, it has been suggested that AID may also regulate gene expression epigenetically, irrespective of mutator activity, by directly deaminating 5-methylcytosine (5mC), in concert with base excision repair glycosylases to exchange unmethylated cytosine. For example, AID promotes active demethylation of the genome in primordial germ cells. However, whether or not AID promotes pluripotency has been controversial. Different studies have suggested either a requirement or a lack of function for promoting pluripotency in somatic nuclei following fusion with embryonic stem cells (ESC). We tested directly whether AID regulates epigenetic memory, by comparing the relative ability of cells lacking AID to reprogram from a differentiated cell type to an induced pluripotent stem cell (iPSC). We show that loss of AID impacts two distinct steps of reprogramming. First, AID-null cells are hyper-responsive to the reprogramming process. This is a somewhat subtle and transient phenotype that may reflect aberrant methylation patterns that normally provide a brake to the reprogramming machinery in differentiated cells. More importantly, we find that while AID-null cells initiate expression of pluripotency genes including Oct4 and Nanog, they fail to stabilize the pluripotent state. The genome of AID-null cells remains globally hyper-methylated in reprogramming cells, and hypermethylated genes associated with pluripotency fail to be stably up-regulated. Hyper-methylation is enriched in gene bodies, rather than distal regulatory elements, consistent with known targeting mechanisms for AID. MYC target genes are highly enriched in the set of genes that are hyper-methylated and under-expressed in reprogramming cells lacking AID. The AID-dependent defects in methylation and gene expression for secondary pluripotency genes, including Cbx7, Dpp3, Gdf3, and others, are seen in bulk colonies and also in individual isolated iPSC clones. When isolated clones are carefully passaged in reprogramming conditions, some of them can stabilize as iPSCs, and these clones form normal embryoid bodies capable of differentiating along all three germ layer fates. AID is expressed starting 1-2 weeks into the reprogramming process, and rescue studies conclude that AID does not function early in the process. Thus, AID is not essential for reprogramming, but significantly impacts the efficiency of stabilizing the pluripotent state, by removing epigenetic memory at a relatively late stage from secondary pluripotency network genes.

COMPARATIVE AND SPATIOTEMPORAL EPIGENOMIC ANNOTATION OF REGULATORY DNA

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Despite the explosive growth of genomic data, functional annotation of the regulatory sequences remains difficult. Here we introduce 'comparative epigenomics' and 'spatiotemporal clustering' as novel approaches for annotation of the regulatory genome. 'Comparative epigenomics' is an interspecies comparison of epigenomes. 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 embryonic stem cells into mesendoderm cells. Thus, comparative epigenomics reveals regulatory features of the genome that cannot be discerned from sequence comparisons alone. In addition to the comparative epigenomics across species, we developed a probabilistic model to learn from the temporal changes of the epigenome. We cluster genomic sequences based on the similarity of temporal changes of multiple epigenomic marks during a cellular differentiation process. Genomic sequences were clustered based on the spatiotemporal epigenomic information. These clusters not only clearly distinguished gene bodies, promoters, and enhancers, but also were predictive of bidirectional promoters and piRNAs. A Sox17 enhancer containing a FOXA2 binding site was identified, and vice versa, suggesting a positive feedback loop between the two mesendoderm transcription factors. These data illustrate the power of using epigenome comparison and dynamics to investigate regulatory functions.

Poster Briefs

DYNAMICS OF X-CHROMOSOME INACTIVATION IN HUMAN EMBRYOS AND NEWLY DERIVED HUMAN PLURIPOTENT STEM CELLS

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GENOME ORGANIZER SATB1 REGULATES DIFFERENTIATION-ASSOCIATED SILENCING OF THE NANOG LOCUS BY MODULATING ITS NUCLEAR POSITIONING IN HUMAN EMBRYONIC STEM CELLS

Ahmed, Mohammed[1], Harrison, Neil J.[2], Stavish, Dylan[2], Unger, Christian[2], Mardaryev, Andrei N.[1], Fessing, Michael Y.[1], Poterlowicz, Krzysztof[1], Andrews, Peter W.[2], **Botchkarev, Vladimir A.[3]**
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RNF12 IS ESSENTIAL FOR X INACTIVATION IN FEMALE MOUSE EMBRYONIC STEM CELLS AND IS REQUIRED FOR FEMALE MOUSE DEVELOPMENT

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IDENTIFICATION OF SISTER CHROMATIDS IN CELLS USING DNA TEMPLATE STRANDS

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Following replication of parental DNA, sister chromatids are expected to have exactly the same DNA sequence except for mutations resulting from DNA replication. How epigenetic marks are duplicated and transmitted to daughter cells during and following DNA replication is less clear. Given the plethora of possible epigenetic modifications, it seems unlikely that all parental chromatin marks are faithfully copied onto both sister chromatids. Epigenetic marks that are not lost during mitosis are either distributed randomly between sister chromatids or assigned to a specific sister chromatid e.g. depending on whether leading or lagging strand replication was used to replicate the parental DNA template strands. Alternatively, sister chromatids could be imprinted in a strand-specific, recombination based process. Whether and how epigenetic marks on one sister chromatid are copied onto the other following replication is another interesting research area. Independent of their origin, epigenetic differences between sister chromatids could contribute to the well documented, "stochastic", differences in gene expression between daughter cells. Epigenetic differences between sister chromatids could also direct cell fate more directly. For example, microtubules from the "mother' centrosome could prefer one sister centromere over the other in asymmetrically dividing cells. These ideas represent the basic elements of the "silent sister" hypothesis, which we proposed as an alternative to the "immortal strand" hypothesis. In order to test the "silent sister" hypothesis, it is necessary to reliably identify sister chromatids. We initially reported that FISH with PNA probes recognizing unidirectional arrays of repetitive DNA template strand sequences can be used for this purpose. More recently, we expanded this approach to a sequencing method. With our current Strand-seq method all sister chromatids in a cell can be identified with unprecedented precision. Strand-seq has a number of applications, including the fine-tuning of reference genomes, haplotyping and the analysis of genomic alterations in single cells.

Friday, June 14, 2013 1:15 PM - 3:05 PM

Ballroom West

Concurrent IIIC: Stem Cell Therapies

SURGICAL APPROACHES TO HUMAN SPINAL CORD STEM CELL TRANSPLANTATION IN AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease resulting in the progressive loss of motor neurons in the brain and spinal cord resulting in the death of patients over two to five years. Preclinical proof of principle studies demonstrated that spinal cord transplantation of fetal spinal cord neural progenitor cells, NSI-566RSC, derived from

PROGRAM AND ABSTRACTS

laminar cultures by NeuralStem Inc prolonged survival and protected motor neurons in the SOD1 mouse. Human translation of this approach required the development of safe, accurate, and reproducible methods for spinal cord stem cell transplantation. Several principles guided the development of these methods and the devices designed to execute them. First, the shallow depth of penetration to the ventral horn location of motor neurons in the spinal cord dramatically increases the potential for reflux of the cellular payload. Reflux can be minimized through gradual injection of the cell suspensions. However, gradual injection requires that the cannula remain in the spinal cord tissue for increasing periods of time. To prevent cord injury, immobilization of the injection cannula with respect to the patient was required. Moreover, to insure accurate and reproducible targeting to the specific depth of the ventral horn, a cannula was designed with a fixed block at 4 mm. Finally, because the spinal cord's position within the canal fluctuates with the heart beat and ventilation, this cannula was designed with a flexible shaft to allow the tip to float with the spinal cord. To achieve these ends a series of three devices were prototyped with successive improvements. The most region of the spinal platform is mounted to percutaneous posts that are anchored to the spine. The frame that mounts to these posts, allows for the cannula z drive to be positioned in the ideal location sagittal and coronal location. The z drive is then used for controlled placement of the cannula into the spinal cord, penetrating 1 mm medial to the dorsal root entry zone. Following placement of the cannula tip, its outer sheath is pulled back to convert the cannula from rigid to highly flexible, allowing the tip to float with the cord. This device was tested initially in Gottingen Minipigs, demonstrating that NSI-566RSC could be delivered to the ventral horn accurately and safely in both the lumbar and cervical spinal cord. Pigs were kept alive for three weeks, receiving a triple immunosuppression regimen of Basiliximab, Tacrolimus, and Mycophenolate mofetil. Behavioral analysis demonstrated no neurological deficits following surgery, while histological analysis revealed accurate graft placement into the ventral horns without obvious damage. Based on these data, the FDA approved an 18 patient open label first in human clinical trial of NSI-566RSC transplantation. This trial utilized a novel "risk escalation" design, beginning with lumbar injections in nonambulatory patient, proceeding to lumbar injections in ambulatory patients, and finishing with cervical injections in ambulatory patients. Enrollment and surgeries were completed safely in October of 2012.

HUMAN IPSC-DERIVED OLIGODENDROCYTE PROGENITOR CELLS CAN EFFICIENTLY AND COMPLETELY RESTORE CENTRAL MYELIN IN ANIMAL MODELS OF BOTH CONGENITAL AND ACQUIRED DEMYELINATION

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Neonatal engraftment by human fetal tissue-derived oligodendrocyte progenitor cells (OPCs) permits the myelination of congenitally dysmyelinated brain (Nature Medicine, 2004; Cell Stem Cell, 2008). To establish a potential autologous source of these cells for therapeutic implantation into adults with acquired demyelination, we developed a strategy by which to differentiate human induced pluripotential stem cells (hiPSCs) into OPCs. From 3 hiPSC lines, as well as from human embryonic stem cells (hESCs), we generated highly enriched OLIG2+/PDGFRα+/NKX2.2+/SOX10+ hOPCs, which could be further purified using fluorescence-activated cell sorting. hiPSC OPCs efficiently differentiated into both myelinogenic oligodendrocytes and astrocytes, in vitro and in vivo. In myelin-deficient shiverer mice, neonatally engrafted hiPSC OPCs robustly myelinated both the brain and spinal cord, and substantially increased the survival of these mice. In adults demyelinated by cuprizone ingestion, resident hiPSC OPCs that had been introduced neonatally were recruited to generate new oligodendrocytes, which efficiently remyelinated the demyelinated host axons. These mobilized hiPC-derived OPCs ultimately mediated the complete remyelination of the adult-demyelinated brain, by oligodendroglia newly generated from the activated pool of resident hiPSC OPCs. In each model of neonatally-engrafted hiPSC OPC mice, including both congenitally hypomyelinated shiverers and adult cuprizone-demyelinated adults, the speed and efficiency of myelination by hiPSC OPCs was at least as high as that previously observed using fetal tissue-derived OPCs; critically, no donor-derived tumors were ever noted in either model, in animals studied as long as 9 months after neonatal transplant. These results strongly suggest both the safety and efficacy of human iPSC-derived OPCs in treating both congenital and acquired disorders of myelin loss.
Supported by the National Multiple Sclerosis Society, New York State Stem Cell Science (NYSTEM), NINDS R01NS39559, the Mathers Charitable Foundation, and the Adelson Medical Research foundation.

AUTOGENOUS POINT OF CARE BONE MARROW CONCENTRATE (BMC) FOR THE TREATMENT OF LUMBAR DEGENERATIVE DISC DISEASE: IRB CONTROLLED PROSPECTIVE STUDY

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The Orthopedic Stem Cell Institute, Loveland, CO, USA

Background: Current operative treatment options for moderate to severe symptomatic lumbar degenerative disc disease include fusion versus non-fusion technology. The use of autogenous BMC may provide a non-surgical option for this condition. This is the very first report of the safety and efficacy utilizing autogenous BMC for the treatment of lumbar degenerative disc disease. Purpose: The purpose of this study is to evaluate the safety and efficacy of autogenous BMC for the non-surgical treatment of moderate to severe lumbar degenerative disc disease. Study Design: This is a prospective non-randomized IRB controlled study representing class II data. Patient Sample: Twenty-six patients were prospectively injected with autogenous BMC. Thirteen patients were injected at one level (5 males, 8 females, avg age 38yrs) and 13 patients were treated at two levels (6 males, 7 females, avg age 37yrs). The Pfirrmann grades in the one-level group were three patients with a Pfirrmann grade-4, three patients with a Pfirrmann grade-5, and seven patients with a Pfirrmann grade-6. In the two-level group, two levels were a Pfirrmann grade-3, one level was a Pfirrmann grade-4, nine levels were Pfirrmann grade-5, and 14 levels were Pfirrmann grade-6. Outcome Measures: Every patient had a pre-procedure oswestry disability index (ODI), visual analog scale (VAS), physical examination, and MRI scanning. The patients were followed prospectively at six weeks, three months, and six months with repeat MRI scanning at six months. Methods: The procedure takes 30 minutes and consists of IV sedation utilizing Versed and IV Fentanyl with the patient in the prone position. Percutaneous aspiration of the posterior iliac wing is performed to obtain 60ml of bone marrow aspirate followed by concentration utilizing the Spine Smith ART-21 system. Depending upon the number of lumbar discs to be injected, the volume of BMC was either 3ml or 6ml. Typically, 2-3ml of BMC was injected into each symptomatic lumbar nucleus with a standard two-needle discography technique. The symptomatic discs were diagnosed based on MRI scanning or by discography in six patients. Results: There have been no complications associated with the iliac wing aspirate or disc injection. The single-level patients had an average pre-treatment ODI of 54.9% which improved to 24% at six months (p-value < 0.0001). The pre-treatment VAS averaged 79 which improved to 23.3 at six months (p-value < 0.0001). The two-level patients had a pre-treatment ODI of 58% which improved to 23.4% at six months (p-value < 0.0001). The pre-treatment VAS averaged 79.5 and improved to 25.9 at six months (p-value < 0.0001). Three patients have met criteria for reinjection at six months by not having greater than 25% improvement in symptoms. One patient has elected to proceed with lumbar fusion. Conclusion: These preliminary results utilizing autogenous BMC in a prospective IRB controlled study in 26 patients with minimum six-month follow-up indicate safety and very statistical efficacy. Only one patient has elected to proceed with a surgical procedure following the injection. These six-month follow-up results indicate autogenous BMC has clinical efficacy for the non-operative treatment of degenerative disc disease in the lumbar spine. These patients will continue to be followed for a minimum of two years.

OUGHT PATIENTS TO PAY TO PARTICIPATE IN CELL TRANSFER CLINICAL TRIALS?

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Biomedical research has traditionally been funded either publicly through government agencies, or privately, sponsored by a pharmaceutical, biotechnology, or device company. In the paradigm of patient-funded research (PFR), by contrast, eligible study participants share some or most of the costs of participation. PFR shifts the ethical focus from unduly coercing subjects into participating (whether they should be paid) to requiring payment from them in order to participate (whether they should pay 'us').
In the 1980s, several companies began offering - for a fee - unproven experimental cancer, Alzheimer's Disease, and Parkinson's Disease treatments to patients (Lind 1984; Morreim 1991; Kolata 1992); this trend continues, especially in both oncology and stem cell biology (Lindvall and Hyun 2009), where patients are especially desperate, clinical trials are expensive, and promised therapies have been slow to materialize. The lines between approved clinical treatments, innovative practices, and experimental therapies are blurrier than ever before, thrusting the entire clinical and translational research enterprise into a conceptual and methodological murkiness threatening progress.
Two putative advantages of PFR are as follows: it is an economically advantageous pathway to clinical translation, and the validity of the results is likely to be higher due to improved adherence and appropriate subject inclusion. But there are also putative disadvantages of PFR, including the claims that requiring participants to pay to take part in a study is exploitative, and that PFR threatens to confuse research with clinical care in ways that are harmful to participants.
In this presentation, I explore the following question: How do these putative benefits and risks balance against each other in the protection of patient autonomy, the maximization of beneficence, the minimization of maleficence, and the promotion of justice, in pursuit of medical progress?

Poster Briefs

REVERSIBLE CELL IMMORTALIZATION ALLOWS HAC-MEDIATED GENE CORRECTION OF HUMAN DYSTROPHIC MYOGENIC PROGENITORS

Benedetti, Sara[1], Hoshiya, Hidetoshi[1], Ragazzi, Martina[1], Kazuki, Yasuhiro[2], Oshimura, Mitsuo[2], Messina, Graziella[3], Cossu, Giulio[1], **Tedesco, Francesco Saverio[1]**
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THE EFFECTIVNESS OF ALLOGENIC MESENHYMAL STROMAL CELLS OF BONE MARROW IN PATIENTS WITH REFRACTORY CROHN'S DISEASE

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HUMAN INDUCED PLURIPOTENT STEM CELLS DIFFERENTIATE INTO PURE POPULATIONS OF ENDOTHELIAL CELLS MEDIATED BY SET SIMILAR PROTEIN THROUGH VE-CADHERIN TRANSCRIPTIONAL ACTIVATION

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EXPLOITING ARTIFICIAL NUCLEASES FOR TARGETED TRANSGENE INTEGRATION IN HUMAN HEMATOPOIETIC STEM CELLS AND INDUCED PLURIPOTENT STEM CELLS DERIVED FORM NORMAL DONORS AND SCID-X1 PATIENTS

Lombardo, Angelo[1], Genovese, Pietro[2], Firrito, Claudia[1], Di Tomaso, Tiziano[1], Escobar, Giulia[2], Schiroli, Giulia[2], Gennery, Andrew R.[3], Sergi Sergi, Lucia[1], Gregory, Philip D.[4], Holmes, Michael C.[4], Naldini, Luigi[2]
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The development of artificial nucleases has brought the possibility of targeted integration and gene correction within the reach of gene therapy. To this end we have exploited engineered Zinc Finger Nucleases (ZFNs) to induce homology-driven transgene insertion into a predetermined genomic site and used this strategy to insert transgene expression cassettes and correct mutations in Hematopoietic Stem/Progenitor Cells (HSPC) and induced Pluripotent Stem Cells (iPSC) from normal donors and X-linked Severe Combined Immunodeficiency (SCID-X1) patients, respectively. This disease, which is caused by mutations in the Interleukin-2 Receptor Common Gamma Chain (IL2RG) gene, is an ideal candidate to test the efficacy and safety of this novel approach, as HSPC-based gene therapy trials performed with randomly integrating vectors showed clinical benefits but also a high rate of leukemia due to insertional mutagenesis and uncontrolled transgene expression. To achieve ZFN-mediated targeted insertion in HSPC, we developed a combined gene delivery protocol based on integrase-defective lentiviral vectors and mRNA nucleofection that provides the donor template DNA for homologous recombination while transiently expressing the ZFNs. By using this protocol we can either target a transgene expression cassette into the putative "safe harbor" AAVS1 locus or insert a functional corrective IL2RG cDNA downstream of its endogenous promoter with unprecedented efficiency and high specificity in HSPC. Gene edited HSPC generated both erythroid and myeloid colonies in vitro and, upon transplantation into NSG mice, reproducibly gave rise to both myeloid and lymphoid lineages. Importantly, gene-targeted cells were also found in the mice within the primitive human hematopoietic compartment, thus demonstrating editing of the long-term repopulating stem cell. In parallel, we have explored the use of patient-derived iPSC as a potentially unlimited source of gene-corrected HSPC. We have established a strategy that allows correction of the IL2RG gene and safe reprogramming of these cells. Using ZFN technology we inserted the corrective IL2RG cDNA in fibroblasts from SCID-X1 patients with high efficiency. Since fibroblasts do not express IL2RG, to identify the gene-corrected cells we included downstream of the corrective cDNA a loxP-flanked drug-selection cassette, and efficiently reprogrammed

the selected cells to iPSC by using a single-copy, Cre-excisable Lentiviral Vector (LV) expressing the reprogramming factors. Transient Cre delivery resulted in near complete excision of the reprogramming LV and the selector cassette from the iPSC genome, demonstrating the feasibility of generating gene-corrected and reprogramming factor-free iPSC from SCID-X1 patients. Overall, these studies provide proof-of-principle for ZFN-mediated gene targeting and correction in wild-type and patient-derived HSPC and iPSC, and provide a path to the development of a more precise and safe gene therapy strategy for SCID-X1 and, conceivably, several other diseases.

Friday, June 14, 2013 1:15 PM - 3:05 PM

Room 253

Concurrent IIID: Chemical Control of Stem Cell Behavior

INNOVATING PRECLINICAL DRUG DEVELOPMENT & HUMAN CELL THERAPY

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There has been significant interest in the therapeutic and scientific potential of stem cells since reconstitution of the haematopoietic system was first realized by bone marrow transplantation in the 1960s. The isolation of tissue-specific, multipotent stem cells from adult organs and the derivation of pluripotent human embryonic stem cells offer the potential for regeneration of a number of different tissues and organs susceptible to age-related degenerative conditions and traumatic injury. In the not-too-distant future, it will be possible to repair heart tissue damaged by myocardial infarction, to replace neuronal cells lost in Parkinson's and Alzheimer's diseases, to transplant new insulin producing cells for diabetics and myelinating cells for individuals afflicted with multiple sclerosis, and to replace bone and cartilage lost through aging and inflammatory disease. In addition, the generation of specific populations of defined subtypes of human cells has tremendous potential to revolutionize the fields of drug discovery and investigation into the cellular bases of human disease. The newly emerging field of Regenerative Medicine will fundamentally alter clinical medicine and significantly influence our perceptions of aging, health and disease, with a myriad of consequences for society at large.

HIGH-THROUGHPUT SCREENING IDENTIFIES A NOVEL OLEATE SYNTHESIS INHIBITOR THAT SELECTIVELY ELIMINATES HUMAN PLURIPOTENT STEM CELLS AND PREVENTS TERATOMA FORMATION

Ben-David, Uri[1], Gan, Qing-Fen[2], Golan-Lev, Tamar[1], Arora, Payal[2], Yanuka, Ofra[1], Oren, Yifat[3], Leikin-Frenkel, Alicia[4], Graf, Martin[5], Garippa, Ralph[2], Boehringer, Markus[5], Gromo, Gianni[5], Benvenisty, Nissim[1]
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Human pluripotent stem cells (hPSCs) hold great promise for cell therapy treatments, as they can differentiate into all the cell types of the human body. However, the clinical use of hPSCs is hindered by the tumorigenic risk from residual undifferentiated cells. We performed a high-throughput screen of undifferentiated hPSCs with over 52,000 small molecules, which were selected to represent a chemical library of over one million chemical entities. Compounds found to be cytotoxic towards human embryonic and induced pluripotent stem cells were counter-screened against a large array of progenitor and differentiated cells of all germ layers and developmental stages. 15 pluripotent-specific inhibitors (which we termed "PluriSIns") were identified, 9 of which share a common structural moiety.
The effect of the PluriSIns is highly selective, as they eliminate hPSCs rapidly and robustly while completely sparing the other cell types. Cellular, molecular and bioinformatic analyses demonstrated that the most selective compound, PluriSIn#1, induces ER stress, protein synthesis attenuation, and apoptosis in hPSCs. Direct biochemical characterization identified this molecule as an inhibitor of stearoyl-coA desaturase (SCD1), the key enzyme in oleate biosynthesis, thus revealing a previously unknown unique role for lipid metabolism in hPSCs. Interestingly, SCD1 was reported to be essential for the survival of some cancer cell lines, but its function in hPSCs has not been explored before. We show that pharmacological and genetic ablation of SCD1 activity recapitulate the cellular effects of PluriSIn#1. Remarkably, exogenous supplementation of oleate completely rescued the PluriSIn#1-induced cell death, demonstrating that oleate depletion is the direct cause of hPSC ablation following exposure to PluriSIn#1. Of note, structurally-similar PluriSIns were found to exert their cytotoxic effect on hPSCs through the same mechanism of action, while structurally-unrelated PluriSIns act through different and surprising mechanisms that we now begin to unravel. Importantly, PluriSIn#1 was also cytotoxic to mouse pluripotent stem cells and to mouse blastocysts, indicating that the dependence on oleate is evolutionarily-conserved, and is inherent to the pluripotent state. Finally, application of PluriSIn#1 to cell cultures prevented teratoma formation from tumorigenic undifferentiated hPSCs. This novel technique to eliminate undifferentiated cells from culture should thus enable the generation of pure differentiated cultures and increase the safety of hPSC-based therapies.
** The first parts of this study have been recently accepted for publication:*
Ben-David et al. Cell Stem Cell 2013 (in press)

IDENTIFICATION OF SMALL MOLECULES FOR MATURATION OF IPS-DERIVED HEPATOCYTE-LIKE CELLS

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Liver disease is an important clinical problem, impacting over 30 million Americans and over 600 million people worldwide; it is the 12th leading cause of death in the United States and 16th worldwide. Due to liver organ paucity, several thousand Americans die yearly while waiting for liver transplantation. Human embryonic stem (hES) cells and induced pluripotent stem cells (iPS) derived hepatocyte-like cells would enable cell based therapeutics, the study of the mechanisms of human disease and human development as well as provide a platform for pharmacology and toxicology drug screening. iPS cells can be differentiated in a step-wise fashion with high efficiency and reproducibility into hepatocyte-like cells with the morphologic and phenotypic characteristics of hepatocytes, but only with some of the functional characteristics as well. iPS derived hepatocyte-like cells have functional activity as they secrete urea (~44µg/million cells/day), alpha-1-antitrypsin (~2 µg/million cells/day), and albumin (~4 µg/million cells/day) However, iPS derived hepatocyte-like cells exhibit an immature hepatic phenotype more closely resembling fetal hepatocytes rather than adult hepatocytes. Specifically, iPS derived hepatocyte-like cells express fetal markers such as alpha fetoprotein (AFP) and lack key mature hepatocyte functions, as reflected by drastically reduced activity (0.1%) of many detoxification enzymes (i.e. CYP2A6, CYP3A4). These key differences between iPS derived hepatocyte-like cells and adult hepatocytes have limited the use of stem cells as a renewable source of functional adult human hepatocytes for in vitro and in vivo applications. To overcome this limitation, we developed a high-throughput liver screening platform to identify small molecules that can be used to promote differentiation of iPS-derived hepatocytes, toward a more mature phenotype. Using these small molecules, we were able to differentiate iPS derived hepatocyte-like cells into adult hepatocyte-like cells as evidenced by improved albumin secretion, loss of AFP secretion (a fetal marker) and acquisition of CYP2A6 and CYP3A4 enzyme activity (adult cytochrome P450 enzyme). The identification of these small molecules helps to address a major challenge impacting many facets of liver research including cell-based therapeutics, ADME/T testing and modeling of human disease.

DEVELOPMENT OF HIGH-CONTENT IMAGING ASSAY TO EVALUATE DIFFERENTIATION TOWARDS FUNCTIONAL BROWN ADIPOSE TISSUE IN ADIPOSE AND SKELETAL MUSCLE PROGENITORS

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Due to the increasing epidemic of obesity and metabolic diseases, there has been significant interest in adipose tissue as a therapeutic target. 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 enhancing the differentiation of adipose derived stem cells to brown adipose as well as increasing activity of the existing brown adipose tissue attractive therapeutic targets. However, the issue is complicated by 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 9 days 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, 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 have demonstrated 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.

Poster Briefs

CONTROL THE BEHAVIOR OF INTESTINAL STEM CELLS

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EFFICIENT DIFFERENTIATION OF HUMAN EMBRYONIC AND INDUCED PLURIPOTENT STEM CELLS INTO TARGETED MESODERM AND ENDODERM CELL FATES

Lam, Albert Q., Freedman, Benjamin S., Morizane, Ryuji, Valerius, Todd, Bonventre, Joseph V.
Renal Division, Brigham and Women's Hospital, Boston, MA, USA

CHEMICAL GENETIC IDENTIFICATION OF MOLECULES THAT POTENTIATE HEPATIC MATURATION OF A HUMAN IPS-DERIVED HEPATOCYTES BASED SCREENING SYSTEM AS A TOOL FOR LOOKING INTO THE MECHANISM OF HEPATIC MATURATION

Yamazoe, Taiji[1], Shiraki, Nobuaki[1], Uesugi, Motonari[2], Kume, Kazuhiko[1], Kume, Shoen[1]
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COMBINATORIAL SMALL MOLECULE MODULATION OF HESC-DERIVED PANCREATIC MULTIPOTENT PROGENITOR CELLS

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Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA

Type 1 Diabetes is characterized by the immune destruction of insulin-producing pancreatic beta cells, resulting in dysregulation of blood glucose levels. As such, the identification of small molecules capable of efficiently inducing the differentiation of human embryonic stem cells (hESC) to insulin producing beta cells may provide a route to cell transplantation therapies for diabetic patients. Toward this end, several protocols have been developed to differentiate hESC toward the pancreatic lineage. One of the major principles behind these protocols is the use of chemical and growth factor signals that mimic those present in the developing pancreas. Although this approach is promising, existing protocols fall short of producing functional beta cells in vitro, perhaps due to the fact that they fail to recapitulate the complexity of interactions present in the developing pancreas. Traditional one-at-a-time screening approaches have been used to identify signals that are important in directing the cell fate decisions of cells along the pancreatic path, but by definition this approach fails to capture synergistic interactions between signals. Herein, we report the development of a combinatorial screening approach capable of identifying combinations of signals that trigger cell fate decisions. We assembled a library of small molecules with activity in key developmental signaling pathways including EGF, MAPK, SHH, mTOR, TGFbeta, Notch and RA signaling. Using design-of-experiment (DOE) principles and statistical optimization, this library was evaluated

at multiple concentrations to identify the pairwise, third and forth order interactions critical for the induction of the pancreatic multipotent progenitor population as defined by the induction of the critical pancreatic transcription factor Nkx6.1. In total ~680 small molecule combinations were evaluated to identify main effectors and to establish optimal conditions for induction of the multipotent progenitor cell fate. These results demonstrate the importance of combinatorial screening as an approach to elucidating the pathways necessary to drive desired cell fate transitions in vitro.

Friday, June 14, 2013 1:15 PM - 3:05 PM

Room 205

Concurrent IIIE: Germ Cell Biology and Artificial Gametes

GENERATION OF MAMMALIAN HAPLOID EMBRYONIC STEM CELLS

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Haploid cells are amenable for genetic analysis. Recent success in the derivation of mouse haploid embryonic stem cells (haESCs) via parthenogenesishas enabled genetic screening in mammalian cells. However, successful generation of live animals from these haESCs, which is needed to extend the genetic analysis to the organism level, has not been achieved. Here, we report the derivation of haESCs from androgenetic blastocysts. These cells, designated as AG-haESCs, partially maintain paternal imprints, express classical ESC pluripotency markers, and contribute to various tissues, including the germline, upon injection into diploid blastocysts. Strikingly, live mice can be obtained upon injection of AG-haESCs into MII oocytes, and these mice bear haESC-carried genetic traits and develop into fertile adults. Furthermore, gene targeting via homologous recombination is feasible in the AG-haESCs. Our results demonstrate that AG-haESCs can be used as a genetically tractable fertilization agent for the production of live animals via injection into oocytes.

REBUILDING PLURIPOTENCY FROM MOUSE PRIMORDIAL GERM CELLS

Leitch, Harry G.[1], McEwen, Kirsten R.[2], Nichols, Jennifer[1], Turp, Aleksandra[2], Mulas, Carla[1], Martello, Graziano[1], Grabole, Nils[3], Nashun, Buhe[2], Surani, Azim[3], Hajkova, Petra[2], Smith, Austin[1]
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In the preimplantation mouse embryo, naïve pluripotency is established in the mature epiblast cells of the inner cell mass (ICM). Cultured epiblast gives rise to embryonic stem (ES) cells in vitro. However, primordial germ cells (PGCs) can also give rise to naïve pluripotent stem cells called embryonic germ (EG) cells. Whereas ES cell derivation has been optimised and characterised at the single cell level, the process of EG cell derivation has hitherto been ill-defined and inconsistent. Furthermore, EG cells have been reported to retain elements of their germline origin, for instance by exhibiting erasure of genomic imprints and DNA hypomethylation. We have established a robust system for conversion of PGCs to EG cells which allows continuous imaging of single cells. In defined conditions without feeders, 20% of isolated PGCs consistently become EG cells. We also report the first derivation of EG cell lines from newly specified PGCs at E7.5. Using this defined system we establish that conversion is driven by leukaemia inhibitory factor (LIF) and the downstream transcription factor Stat3 and does not require fibroblast growth factor. Notably, Stat3 targets are also upregulated in pluripotent germ cell tumours suggesting dysregulation of this pathway may underlie teratocarcinogenesis. We propose that LIF/Stat3 stimulation reconstructs the germline's latent pluripotency and instils self-renewal.
We have also measured gene expression and global DNA methylation in a large cohort of genetically matched EG and ES cell lines. We report that EG cell lines can be derived with genomic imprints intact, contrary to previous findings. Thus, imprint erasure is not a defining feature of EG cells. Furthermore, we demonstrate that established EG cell lines are indistinguishable from ES cells at the transcriptome level and exhibit equivalent global DNA methylation. Finally we show that inhibition of MAP kinase signalling and Gsk3 in 2i medium leads to a pronounced reduction in global DNA methylation in ES and EG cells, to a level similar to that measured in the ICM. This DNA hypomethylation is driven by Prdm14 and is associated with down-regulation of Dnmt3a and 3b. These results establish that EG cells and ES cells reach an equivalent naïve pluripotent state in 2i/LIF, and that this is associated with global DNA hypomethylation.

THE TSC2-MTORC1 SIGNALING AXIS CONTROLS GERMLINE STEM CELL FATE

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The balanced adoption of self-renewing and differentiating fates among adult stem cell daughters is critical for tissue maintenance. While factors that regulate self-renewal of male germline stem/progenitor cells (spermatogonial progenitor cells; SPCs) in the mouse remain poorly defined, we have previously implicated the growth-promoting mTORC1 pathway in this process. The mTORC1 complex is a key regulator of mRNA translation plus cell growth and its activity is perturbed in a number of pathological conditions including cancer. We find that mTORC1 hyperactivation in SPCs by conditional deletion of the upstream inhibitor Tsc2 throughout the SPC pool using Vasa-Cre triggers aberrant differentiation commitment and progressive germline degeneration. Treatment with rapamycin rescued SPC depletion, thus confirming involvement of the mTORC1 pathway. Interestingly, Tsc2 ablation within a subset of SPCs using Stra8-Cre did not compromise SPC function. SPC activity also appeared unaffected by Tsc2 deletion within somatic cells of the niche. Importantly, those SPCs insensitive to Tsc2 deletion are physically larger than the remaining SPC pool as a result of mTORC1 activation. We therefore define mTORC1 as a key regulator of SPC fate and identify a subpopulation of SPCs displaying elevated mTORC1 activity that presumably represent the committed SPC pool. We propose that mTORC1 acts as a regulator of SPC fate by functionally defining phenotypically distinct SPC subpopulations with varying propensities for self-renewal and differentiation. Continuing studies are aimed at elucidating key downstream targets of the mTORC1 pathway in SPCs and their relevance to germline maintenance.

IDENTIFICATION AND PLURIPOTENCY OF MOUSE SPERMATOGONIAL STEM CELLS

Azizi, Hossein[1], Conrad, Sabine[2], Hajizadeh Moghaddam, Akbar[3], Skutella, Thomas[1]
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Spermatogonial stem cells (SSCs) are able to differentiate to sperm cells in order to transfer genetic information to the next generation. In the seminiferous tubules of the testis SSCs, sertoli cells and differentiating cells during spermatogonesis are present. In our studies we successfully established two types of SSCs with morphology-based selection (type I and type II) from testicular culture both from neonate and old Oct4-GFP transgenic mice. We have been successful to expand type I SSCs on mouse embryonic feeder (MEF) and type II SSCs on both SNL and primary testicular stroma cells (TSCs) feeder for long term culture. Immunocytochemistry, Flowcytometry and Fluidigm real time RT-PCR results showed that type I SSCs clearly express germ cells markers while type II SSCs partially expressed the typical germ cell profile of SSCs. Electron microscopic analysis revealed that type I SSCs have similar or homogenous morphology comparable with undifferentiated SSCs that are localized on the basement membrane of the seminiferous tubules (high nucleus/cytoplasm ratio) while type II SSCs have a different morphology (small nucleus/cytoplasm ratio). After transplantation of type II SSCs in busulphan treated NOD SCID mice we observed localization of GFP labeled cells in the basal compartment of the seminiferous tubule and observed GFP labeled sperms in the epididymis. The most obvious molecular differences between type I and type II SSCs were reprogramming to mouse embryonic stem cells-like cells that occurred only during a critical time period after initiation of type I SSCs culture.
These different types of SSCs could provide an ideal cell system for studying both germ and pluripotency profiles and provide a new strategy for isolation of SSCs from neonate and old mice.

Poster Brief

EXOGENOUS SUPPLEMENTATION WITH ACTIVIN A BOOSTS GERM CELL DIRECTED DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

Duggal, Galbha[1], Heindryckx, Bjorn[1], Van der Jeught, Margot[1], Lierman, Sylvie[1], Deroo, Tom[1], Deforce, Dieter[2], Chuva de Sousa Lopes, Susana[3], De Sutter, Petra[1]
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YEAST GENETICS IN MAMMALIAN STEM CELLS

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Some organisms such as yeast or social insects arehaploid, i.e. they carry a single set of chromosomes. Organisms with a single copy of their genome provide a basis for genetic analyses where any recessive mutation of essential genes will show a clear phenotype due to the absence of a second gene copy. Haploidy in yeast has been therefore utilized to identify fundamental mechanisms of biology. Such recessive genetic screens have elucidated a wide variety of biological processes over the last century and markedly contributed to our understanding of normal development, basic physiology, and disease. However, all somatic mammalian cells carry two copies of chromosomes (diploidy) that obscure mutational screens. Although deemed impossible, we were able to generate the first mammalian haploid embryonic stem cells from parthenogenetic mouse embryos. Haploid mouse cells show stable growth and maintain haploidy over multiple passages, can be efficiently subcloned, and, importantly, can be readily mutagenized at the haploid state to generate complete, homozygous mutants for reverse genetics and forward genetic screens.

Friday, June 14, 2013 4:00 PM - 5:50 PM

Ballroom West

Concurrent IVA: Stem Cells in Organ Development

SHAPE IS FATE

Semb, Henrik
Danish Stem Cell Center, University of Copenhagen, Copenhagen, Denmark

Our work focuses on how tissue architecture controls cell fate specification and how this knowledge can be translated into more reliable strategies in regenerative medicine, in this case human pluripotent stem cell-based cell therapy in diabetes. During development the three-dimensional organization of tissues and organs set the physical and biomechanical properties of distinct cellular microenvironments. These forces will affect the shape of cells at individual as well as multicellular level, which strongly influence proliferation and cell fate. Our recent results show that the Rho GTPase family members are indirectly (by controlling tissue architecture) and directly (via cortical actin dynamics and cell polarity) required for pancreatic beta cell differentiation and maturation in vivo. Recent attempts to translate new knowledge and concepts for how tissue architecture and biomechanics instruct beta cell fate in vivo into production of pancreatic cell lineages from human embryonic stem cells will be discussed.

PAX2 MAINTAINS THE NEPHRON LINEAGE BY REPRESSING STROMAL CELL FATES IN NEPHRON PROGENITOR CELLS DURING MAMMALIAN KIDNEY DEVELOPMENT

Kobayashi, Akio, Naiman, Natalie
Harvard Medical School/Brigham and Women's Hospital, Boston, MA, USA

The functional unit of the kidney, the nephron, is repetitively generated during mammalian kidney development. Previously, our fate map analysis revealed that the Six2+ cap mesenchyme represents a multipotent, self-renewing nephron progenitor population throughout kidney development (Kobayashi et al., 2008, Cell Stem Cell). Currently, it is not well known how the nephron progenitor population is maintained during kidney organogenesis. A homeodomain transcription factor, PAX2, plays important roles in urogenital development.
Renal-coloboma syndrome (RCS) is a congenital and developmental disorder characterized by renal hypodysplasia with reduced nephron numbers caused by haploinsufficient PAX2 mutations. Interestingly, Pax2 is expressed in the cap mesenchyme during kidney development in the mouse. Although Pax2 has been widely recognized as one of the most important regulators for kidney development over two decades, defects in Pax2-null mutants prior to kidney development precludes examination of Pax2 functions in the developing kidney in vivo.
In this study, we further defined fate maps for kidney development and identified the Foxd1+ nephrogenic interstitium (cortical stroma) as a self-renewing progenitor population for stromal tissues of the kidney. Interestingly, Six2+ cap mesenchyme- and Foxd1+ nephrogenic interstitium-derived cells have distinct fate maps, indicating a lineage boundary between these nephron and stromal compartments during kidney organogenesis. To examine Pax2 functions for nephron progenitors, we inactivated Pax2 activity specifically in the cap mesenchyme. We found that the conditional Pax2 mutants fail to maintain the cap mesenchyme, a self-renewing nephron progenitor population. Surprisingly, fate map analysis in the mutants showed that cap mesenchyme-derived cells lacking Pax2 activity are not lost, but persist throughout kidney development. Detailed molecular marker analysis indicated that these Pax2-deficient cap mesenchyme-derived cells trans-differentiate

into stromal cells, through ectopic expression of stromal progenitor markers including Foxd1. Our mosaic analysis showed that Pax2 activity is cell autonomously required to maintain the cap mesenchyme. Taken together, our observations suggest that Pax2 maintains a self-renewing nephron progenitor population by repressing stromal cell fates. Thus, Pax2 activity establishes a lineage boundary between the nephron and stromal compartments during kidney organogenesis.

MIR-142-3P UNLOCKS THE HEMANGIOBLAST PROGRAM IN THE MESODERMAL PRECURSORS OF HEMATOPOIETIC STEM CELLS AND THE MAJOR VESSELS

Nimmo, Rachael[1], Ciau-Uitz, Aldo[2], Ruiz-Herguido, Cristina[3], Soneji, Shamit[1], Bigas, Anna[3], Patient, Roger[2], Enver, Tariq[1]
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Hematopoietic stem cells (HSCs) arise in a brief time window from hemogenic endothelial cells in the embryo. It is essential to understand how these cells are programmed during embryogenesis in order to successfully generate HSCs from pluripotent stem cells in vitro. Lineage tracing in mouse and Xenopus has shown that both HSCs and endothelium of the major vessels derive from lateral mesoderm, and this hemangioblastic precursor population has been well characterized in Xenopus. We investigated how miRNAs contribute to development of the HSC lineage using a Dgcr8 morpholino to globally inhibit miRNA processing in the Xenopus embryo. Most morphant embryos did not survive to tadpole stages and we observed major vascular and hematopoietic defects and a complete failure to form HSCs. These defects were traced back to a failure to correctly specify the hemangioblast precursors of both vascular and hemogenic endothelium in dorsal lateral plate (DLP) mesoderm. We identified miR-142-3p as the strongest candidate for regulating HSC development by ranking miRNAs based on their expression profile in mouse embryonic HSCs and ChIP-seq analysis of their combinatorial regulation by hematopoietic TFs. In addition, we detected expression of this highly conserved vertebrate miRNA in hemangioblast precursors in Xenopus DLP. Interestingly inhibition of miR-142-3p using a morpholino resulted in striking phenocopy of the hematopoietic and endothelial defects of Dgcr8 morphants with failure to form HSCs and reduced vasculogenesis. Previous work has shown that Fli1 is at the apex of a genetic cascade regulating hemangioblast specification via activation of Gata2, Flk1 and Etv2, that in turn trigger Scl expression and set in place the activation of a battery of genes required for endothelial and hematopoietic differentiation. Here we identify miR-142-3p as a master regulator of the adult hemangioblast acting upstream of Fli1 to instruct mesodermal cells in the DLP to acquire a hematoendothelial fate. Given the strong similarity of the defects in Dgcr8 and miR-142-3p morphants we asked whether reduction of miR-142-3p is responsible for the hemangioblast defect in Dgcr8 morphants. We demonstrated that a synthetic miR-142-3p mimic was sufficient to rescue hemangioblast formation in embryos globally depleted of miRNAs strongly suggesting that miR-142-3p is the major miRNA determining hematoendothelial specification of DLP mesoderm. We postulated that miR-142-3p represses an as yet unidentified inhibitor of hemangioblast programming in DLP mesoderm. Strikingly, Targetscan identified 3 core receptors of the TGFβ signaling pathway (Tgfbr1, Acvr2a and Acvr1b) as predicted targets of miR-142-3p. We therefore treated miR-142-3p morphants embryos with two different TGFβ receptor inhibitors and observed that hemangioblast specification is partially rescued in these embryos. In summary, we have demonstrated that miR-142-3p forms a double negative gate unlocking the regulatory network controlling hemangioblast specification of mesoderm, in part via modulation of TGFβ signalling.

DEVELOPMENTAL SEGREGATION OF BONE MARROW HSC NICHE FORMATION FROM ENDOCHONDRAL OSSIFICATION BY SPECIALIZED NEURAL CREST- AND MESODERM-DERIVED MSCS, RESPECTIVELY

Isern, Joan[1], Martín, Ana M.[1], García, Andrés[1], Martín-Pérez, Daniel[1], Arranz, Lorena[1], Aquino, Jorge B.[2], Torroja, Carlos[3], Sánchez-Cabo, Fátima[3], Jabs, Ethylin W.[4], Ernfors, Patrik[6], Sun, Li[5], Adameyko, Igor[6], Zaidi, Mone[5], Méndez-Ferrer, Simón[1]
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In the adult bone marrow (BM), multipotent mesenchymal stromal cells cells (MSCs) have been proposed to provide a niche for hematopoietic stem cells (HSCs), although their specialized functions and developmental origin are largely unknown. Also, it is unclear whether HSC niche-forming MSCs contribute to skeletogenesis and skeletal remodeling or whether they represent specialized cells that arise earlier in embryogenesis and persist in the adult.
Cells expressing GFP under the regulatory elements of the nestin promoter (Mignone et al., J Comp Neurol 2004; Nes-GFP+ cells) contain all MSC activity in the adult BM and have essential functions in the HSC niche (Mendez-Ferrer et al., Nature 2010). We found that Ki67-, slowly proliferating sub-endothelial Nes-GFP+ cells were associated with the invading arterioles in the developing BM, often near sympathetic nerve fibers. Fetal BM Nes-GFP+ cells were enriched in endogenous nestin expression and were distinct from Col2.3+ pre-osteoblasts and S100+ chondrocytes. As opposed to adult, both fetal Nes-GFP+ and Nes-GFP- BM stromal cells contained mesenchymal progenitor activity, as measured by colony forming units-fibroblastic (CFU-f). Strikingly, CFU-f efficiency dramatically dropped in stromal BM Nes-GFP-cells during the second week after birth and MSC activity was restricted to Nes-GFP+ cells in the BM afterwards. Fetal BM stromal Nes-GFP-cells were enriched in genes required for chondrogenesis while lineage tracing studies using Nes-CreERT2 mice have not shown contribution of nestin+ cells to chondrogenesis during E15.5-E18.5. We studied the role of nestin+ MSCs in adult physiological skeletal remodeling by intercrossing Nes-CreERT2 mice with two Cre-recombinase-inducible lines expressing either diphtheria toxin (DT) or its receptor. Histomorphometry analyses of the bones 6-13 months after tamoxifen and DT treatment of adult mice revealed increased number of osteoclasts and decreased mineralizing surface and mineral apposition rate following nestin+ cell deletion. This was paralleled by subtle cranio-facial abnormalities revealed by μCT, but overall bone mineral density and other skeletal parameters were not affected.
Selective cell deletion experiments showed that, while nestin+ MSCs scarcely contributed to skeletogenesis and bone remodeling, they were required for HSC migration to the developing BM. Indeed, depletion of nestin+ cells at E15.5 caused 48h later a ~4-fold reduction in fetal BM HSC activity that inversely correlated with an ~8-fold increase in HSC activity in the fetal liver (LTCIC). Compared to Nes-GFP- stromal cells, the expression of HSC maintenance genes (Cxcl12, Kitl, Vcam1) was 2-8-fold higher and progressively upregulated in Nes-GFP+ cells. Preliminary experiments suggest that Cxcl12 production by nestin+ MSCs is required to establish the HSC niche in the BM.
Finally, by combining conditional lineage tracing studies using neural crest drivers (Wnt1-Cre, Sox10-CreERT2 and PLP-CreERT2) with genome-wide comparison analyses we have identified two putative neural crest-derived Nes-GFP+ cells in the BM, a PDGFRα- Schwann cell progenitor and a PDGFRα+ MSC enriched in HSC maintenance genes.
In summary, these results suggest that HSC niche-forming MSCs share a common origin with sympathetic neurons and Schwann cells, previously shown to regulate HSCs. They also uncouple osteoprogenitor and stem cell niche functions respectively in mesoderm- and neural crest-derived MSCs

Poster Briefs

HEART FIELD ORIGIN OF GREAT VESSEL PRECURSORS RELIES ON NKX2.5-MEDIATED VASCULOGENESIS

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PROSTAGLANDIN E2 REGULATES CELL FATE DECISIONS AND ORGAN GROWTH DURING LIVER AND PANCREAS DEVELOPMENT

Nissim, Sahar[1], Sherwood, Richard[2], Wucherpfennig, Julia[2], Saunders, Diane[2], Frechette, Gregory[3], Harris, James[3], Cutting, Claire[2], North, Trista E.[4], Goessling, Wolfram[5]
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NKX2-5 SUPPRESSES THE FORMATION OF THE CONDUCTION CELLS AND THE PROLIFERATION OF ATRIAL CARDIOMYOCYTES

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VEGFR2/KDR/FLK-1 MARKS A NOVEL, FUNCTIONAL, AND CONSERVED LIVER PROGENITOR CELL

Goldman, Orit[1], Han, Songyan[1], Sourrisseau, Marion[1], Dziedzic, Noelle[1], Hamou, Wissam[1], Corneo, Barbara[2], D'Souza, Sunita[1], Kotton, Darrell[3], Sato, Thomas[4], Bissig, Karl-Dimiter[5], Evans, Matthew[1], **Gouon-Evans, Valerie[1]**
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Using the human embryonic stem cell differentiation system, we identified a novel human hepatic progenitor based on the unexpected expression of VEGFR2 (KDR/Flk-1). Indeed, KDR expression was thought to be restricted to mesodermal derivatives including endothelial, hematopoietic, cardiac and skeletal muscle precursors. However, our in vitro study provides evidence that KDR also marks an endoderm-derivative, the hepatic progenitor. Endoderm was induced with high doses of activin-A resulting at day 5 with an endoderm-enriched population based on CXCR4 and cKIT expression (85.3% +/- 10%, n=15 experiments). This population was devoid of mesendoderm cells, since the mesendodermal marker PDGFRa was virtually absent. In addition, to exclude mesodermal derivatives, KDR-expressing cells detected in day 5 cultures (about 2%) were removed from the purified CXCR4+cKIT+ endoderm-enriched cell fraction. Following further culture of the purified endoderm cells, a large fraction of cells (54.2% +/- 10%, n=16 experiments) expressing KDR consistently developed in hepatic culture conditions, concomitantly with KDR^{neg} hepatic cells. We confirmed that KDR^{pos} cells were not of the endothelial lineage, as they did not express the endothelial marker CD31 (PECAM-1). Both KDR^{pos} and KDR^{neg} populations express at similar levels the endoderm marker GATA4 and the hepatic epithelial marker CK18, while alpha fetoprotein (AFP) and albumin (ALB) proteins were exclusively found in KDR^{neg} hepatic cells. Coculture experiments with purified KDR^{pos} cells and KDR^{neg} cells revealed that the KDR^{pos} cells are supportive for the maturation of the KDR^{neg} hepatic cells, as inhibition of KDR function (using a blocking antibody or small molecules) abolished hepatic maturation. We demonstrated that purified KDR^{pos} cells grown in three-dimensional structures differentiate into KDR^{neg} hepatic cells expressing normal levels of AFP and ALB. Hepatic cells derived from the KDR^{pos} progenitors are functional cells, as they support hepatitis C virus (HCV) replication as efficiently as the KDR^{neg} hepatic cells and even the best case Huh-7.5 hepatoma cell line. Thus, KDR^{pos} cells are not only supportive cells for hepatic cell maturation, but are also progenitors for the generation of KDR^{neg} bona fide hepatic cells.
Examination of early embryos revealed that the KDR/Flk-1^{pos} hepatic progenitor is also conserved in the mouse. We identified Flk-1^{pos} cells in endoderm expressing Foxa2 of E8.0 embryos prior to hepatic specification. We provide definitive in vivo proof for the contribution of the Flk-1^{pos} progenitors to liver development by a lineage tracing study, marking all cells expressing Flk-1 as well as their progeny (Cre-Flk-1 × Rosa26-^{Loxp}STOP^{Loxp}-YFP mice). Analyses of fetal and adult livers identified a substantial subset of YFP^{pos} hepatoblasts (30% to 50% in all 36 E13.5 fetal livers analyzed) that were generated from Flk-1^{pos} progenitors. Subsequently, the YFP^{pos} hepatoblasts gave rise to a subset of adult YFP^{pos} hepatocytes and YFP^{pos} cholangiocytes.
In summary, analyses of a human ESC in vitro model system together with an in vivo lineage tracing mouse model, reveal the previously unknown existence of a conserved murine and human KDR/Flk-1^{pos} liver progenitor.

Friday, June 14, 2013 4:00 PM - 5:50 PM

Room 258

Concurrent IVB: Self Renewal Mechanisms

DYNAMIC INTERACTIONS BETWEEN STEM CELLS AND THEIR MICROENVIRONMENT DURING INTESTINAL HOMEOSTASIS AND REGENERATION IN DROSOPHILA

Xi, Rongwen
National Institute of Biological Sciences, Beijing, China

Intestinal stem cells (ISCs) in the Drosophila midgut are scattered distributed basally in the epithelium on a thin-layer of basement membrane that separates the epithelium from the surrounding visceral muscle cells. ISCs are capable of self-renewal and multiple lineage differentiation through which they are maintained life-long while continuously generate short-lived absorptive and secretory progenies. ISC's mitotic activity is readily adjusted to needs such that they have a slow division rate to maintain normal epithelial homeostasis, but their activity can be promptly increased in response to tissue damage to accelerate epithelial regeneration and tissue repair. We are interested in understanding how these ISCs are long-term maintained and how their activities are adjusted to meet needs. We find that the surrounding visceral muscle cells produce multiple secreted signals that promote ISC maintenance and proliferation, and therefore function as regulatory niche cells for ISCs. Here I will present new data suggesting that the niche cells and the stem cells are

intimately associated with each other by a positive feedback loop via signaling pathways. This feedback loop is important not only for normal tissue homeostasis, but also for stem cell activation and consequently effective regeneration following damage. Our findings may provide new insights into the intimate relationships between the stem cells and the niches that are critical for maintaining tissue homeostasis and regeneration.

TRANSCRIPTIONAL REGULATION MEDIATED BY THE DNA REPAIR COMPLEX XPC-RAD23B-CETN2 IN EMBRYONIC STEM CELLS

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Embryonic stem cell (ESC) self-renewal and pluripotency are transcriptionally controlled by the core transcription factors OCT4, SOX2 and NANOG with cofactors, chromatin regulators, non-coding RNAs and other terminal effectors of developmental signalling pathways. Identifying components of these circuitries and their interplay provides the knowledge base to promote efficacious deployment of ESCs and improves methods for induction and differentiation of pluripotent stem cells. We recently identified a new stem cell transcriptional coactivator (SCC) required for in vitro, synergistic activation of the Nanog promoter by OCT4 and SOX2. Biochemical characterization of SCC revealed it to be the nucleotide excision repair complex XPC-RAD23B-CETN2, although the DNA-repair activity is not required for its transcription function. SCC depletion compromises ESC pluripotency and somatic cell reprogramming. To reveal to what extent the complex functions as an OCT4/SOX2 cofactor in vivo, we used chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq) and mapped regions bound by RAD23B/SCC in murine ESCs. SCC peaks are enriched around the transcription start site (TSS) of active and inactive genes, suggesting a role in both transcriptional activation and repression. Around 60% of the high-confidence SCC binding sites co-localize with OCT4/SOX2-occupied regions, predominantly at distal enhancers of active genes. Sequence analysis of SCC peaks confirms that OCT4/SOX2 recognition motifs are the most significantly enriched at these sites. The majority of genes bound by SCC at their TSS are also marked by distal peaks, suggesting that SCC might participate in the crosstalk between distal enhancers and the basal transcription machinery. In fact, Mediator and cohesin, which are thought to mediate gene activation by DNA-looping, co-occupy a significant proportion of SCC binding sites. SCC loading onto DNA follows OCT4/SOX2 binding, as shown by progressive reduction of RAD23B ChIP-enrichment on OCT4/SOX2 target genes upon OCT4 knock-down. Within SCC, XPC is essential for interaction with OCT4 and/or SOX2, as demonstrated by co-immunoprecipitation experiments performed in the absence of RAD23B and CETN2. We further confirmed this result through ChIP and ChIP-seq of RAD23B in Xpc -/- ESCs, where RAD23B signal on OCT4/SOX2 target genes drops to background levels. Overlap of SCC binding sites with other key ESC transcription factors (STAT3, cMYC, nMYC, KLF4, ZFX, E2F1, TCFCP2l1, ESSRB) shows variable degrees of co-occupancy, hinting at OCT4/SOX2-independent functions for SCC/RAD23B. A gene ontology classification of SCC targets reveals a weak over-representation of transcription regulators among the active genes, but a strong bias towards Polycomb-repressed developmental genes. We are now identifying the bona fide transcriptional targets of SCC in ESCs via a combinatorial ChIP-seq/RNA-seq approach, comparing wild-type versus SCC-null ESCs. In conclusion, our genome-wide analysis confirms in vivo that SCC is extensively recruited by OCT4/SOX2 to activate their target genes, and that recruitment relies on XPC. Overlap of SCC binding sites with other ESC transcription factors uncovers previously unrecognized and potentially widespread roles for SCC in coordinating ESC-specific transcriptional programs, while laying the basis for a possible connection between DNA-repair and pluripotency.

THE THO COMPLEX REGULATES PLURIPOTENCY GENE MRNA EXPORT AND MAINTAINS ESC SELF-RENEWAL

Wang, Li, **Hu, Guang**
National Institute of Environmental Health Sciences/National Institute of Health, RTP, NC, USA

To systematically study the molecular basis of embryonic stem cell (ESC) self-renewal and pluripotency, we have previously carried out a genome-wide RNAi screen in mouse ESCs. We identified over 100 genes whose down-regulation caused differentiation, including genes encoding members of the THO complex. The THO complex is a nuclear protein complex that plays an important role in mRNP biogenesis connecting transcription elongation, 3'-end formation, transcript release, and mRNA export, and it is required for mouse pre-implantation development. Here we show that components of the THO complex, Thoc2 and Thoc5, are highly expressed in mouse ESCs compared to somatic cells, and are down-regulated during differentiation. They are required for ESC maintenance and their silencing leads to differentiation. Interestingly, Thoc2 or Thoc5 silencing results in down-regulation of the pluripotency genes such as Nanog and Sox2 at the protein but not mRNA level, suggesting that the THO complex regulates pluripotency gene expression post-transcriptionally. Indeed, RNA-immunoprecipitation showed that Thoc2 directly interacts with many pluripotency gene mRNAs under self-renewing but not differentiation conditions, and the interaction is mediated by Thoc5. Furthermore, Thoc2 or Thoc5 silencing causes nuclear accumulation of those pluripotency gene mRNAs, indicating that the THO complex is important for their export. Functionally, Thoc5 overexpression delays ESC differentiation during LIF withdrawal and its silencing inhibits the reprogramming of somatic cells into iPS cells. Together, our results support a model in which the THO complex maintains ESC self-renewal by controlling the proper export of pluripotency gene mRNAs. We propose that this post-transcriptional regulation of pluripotency gene expression serves as an important regulatory step between self-renewal and differentiation by allowing ESCs to quickly respond to differentiation cues without full commitment.

THE MECHANISM UNDERLYING EPIDERMAL TISSUE SELF-RENEWAL BY THE RNA DEGRADING EXOSOME COMPLEX

Mistry, Devendra S., Chen, Yifang, Sen, George L.
University of California-San Diego, La Jolla, CA, USA

Epidermal tissue homeostasis is maintained by a balance between self-renewal and differentiation of the basal progenitor and stem cells. A majority of the factors identified so far for the cell fate determination of the epidermal progenitor cells act at the transcriptional level. We recently proposed a novel post-transcriptional mechanism in which the RNA degrading exosome complex promotes epidermal tissue self-renewal by destabilizing the mRNA of the differentiation promoting transcription factor GRHL3. Here, we identify a new target for the exosome complex and further elucidate the mechanism by which it promotes progenitor cell self-renewal. Knockdown of the exosome component Exosc9 (Exosc9i) increases the half-life of differentiation promoting transcription factor OVOL1. Simultaneous knockdowns of both Exosc9 and OVOL1 revert back the Exosc9i induced changes in the mRNA levels of differentiation related factors. Knockdown of Nuclear Exosome Targeting (NEXT) component MTR4 increases the mRNA levels of differentiation related factors that are also increased by Exosc9i. Exosc9 binds to the genomic regions of differentiation promoting transcription factor GRHL3. Together our results suggest that the exosome complex promotes epidermal tissue self-renewal by utilizing the NEXT adaptor complex and targeting the pre-mRNA transcripts of the differentiation promoting factors as they are being synthesized.

Poster Briefs

HMGA2 EXPRESSION IS REQUIRED FOR THE HIGH SELF-RENEWAL ACTIVITY OF FETAL HEMATOPOIETIC STEM CELLS

Copley, Michael R., Kent, David G., Benz, Claudia, Babovic, Sonja, Wohrer, Stefan, Treloar, David Q., Day, Chris W., Rowe, Keegan M., Eaves, Connie J.
Terry Fox Lab, BC Cancer Agency, Vancouver, BC, Canada

MUC1* LIGAND, NM23, IS A NOVEL GROWTH FACTOR THAT REVERTS UNMODIFIED HUMAN STEM CELLS TO THE NAÏVE STATE IN A BFGF-FREE, FEEDER-FREE DEFINED SYSTEM

Bamdad, Cynthia C.[1], Smagghe, Benoit J.[1], Stewart, Andrew K.[1], Carter, Mark G.[1], Shelton, Laura M.[1], Bernier, Klyle J.[1], Hartman, Eric J.[1], Calhoun, Amy K.[1], Hatziioannou, Vasilios M.[1], Gabriele Lillacci, Gabriele[2], Kirk, Brian A.[1], Kosik, Kenneth S.[1]
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RUNX1 CONTROLS A REVERSIBLE TRANSITORY STATE BETWEEN STEM AND PROGENITOR CELLS IN MOUSE HAIR FOLLICLE THAT IS AT THE ORIGIN OF SKIN TUMORS

Tumbar, Tudorita
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THE RATE OF PROTEIN TRANSLATION REGULATES HEMATOPOIETIC STEM CELL SELF-RENEWAL

Signer, Robert A.J., Magee, Jeffrey A., Morrison, Sean J.
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Protein translation is an essential process that is dynamically regulated to control various cellular functions including proliferation, metabolism and proteostasis. However, little is known about how translation is regulated in stem cells and whether the rate of protein synthesis affects stem cell function. We quantified protein synthesis in hematopoietic stem, progenitor and differentiated cells in the bone marrow in vivo, and found that protein translation is significantly lower in hematopoietic stem cells (HSCs) compared to other hematopoietic cell types. This raised the possibility that precise regulation of protein synthesis is essential for maintaining stem cell function. To test this, we examined the consequences of either decreasing or increasing protein synthesis in HSCs using genetic mouse models. We found that HSCs heterozygous for the gene encoding the ribosomal protein L24 ($Rpl24^{+/-}$), have a reduced rate of protein synthesis and a severe reduction in long-term multilineage reconstituting activity. We also found that conditional deletion of the Pten tumor suppressor significantly increases translation in HSCs, and that increased translation leads to HSC depletion and leukemogenesis following Pten deletion. In $Pten^{-/-}$;$Rpl24^{+/-}$ compound mutant mice, translation rates in HSCs are restored to near wild-type levels, and the functional HSC deficits observed in both the $Pten^{-/-}$ and $Rpl24^{+/-}$ mice are completely rescued. In addition, heterozygosity for Rpl24 impedes the development of hematopoietic neoplasms after Pten deletion. These data demonstrate that protein translation is tightly regulated in HSCs, and both decreases and increases in translation can lead to stem cell dysfunction and/or depletion. Regulation of protein translation is thus a crucial mechanism underlying stem cell self-renewal and cancer development.

Friday, June 14, 2013 4:00 PM - 5:50 PM

Room 253

Concurrent IVC: Immunology and Stem Cells

ASSESSING THE IMMUNE RESPONSE TO TRANSPLANTATION OF PLURIPOTENT STEM CELL DERIVATIVES

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The differentiated progeny of pluripotent stem cells may eventually be used in cell replacement therapy (CRT) to reverse organ damage and/or failure. However, in this setting transplanted tissue may be subjected to a destructive immune response that ultimately leads to their rejection. In this session, I discuss the current consensus regarding immunogenicity toward pluripotent stem cell derived tissues and advance potential approaches to engender their acceptance in clinical CRT.

DIRECTED DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS INTO THYMIC EPITHELIAL PROGENITORS

Parent, Audrey, Russ, Holger A., Khan, Imran, LaFlam, Taylor N., Metzger, Todd C., Anderson, Mark S., Hebrok, Matthias
Diabetes Center, University of California, San Francisco, San Francisco, CA, USA

The thymus plays a key role in the immune system by providing the necessary environment for the development of T lymphocytes and for the establishment of self-tolerance. Thymic epithelial cells (TECs) are a critical cell population necessary for the formation of the thymic anlage and are essential for T cell maturation. Since the thymus undergoes profound degeneration with age as well as when exposed to stresses such as irradiation and chemotherapy, the use of stem cells as a potential source of TECs to enhance or restore thymic function is of great therapeutic interest. However, directed differentiation of human pluripotent stem cells into TECs has not been successfully accomplished and therefore remains an important challenge. Here, we describe a novel in vitro method to direct the differentiation of human embryonic stem cells (hESCs) into thymic epithelial progenitors (TEPs) using a combination of factors mimicking thymic organogenesis. Gene expression and immunofluorescence analysis showed that cells differentiated using this protocol expressed HOXA3 and EYA1, two critical regulators of thymus development. Importantly, these culture conditions also induced a significant upregulation of FOXN1, a key transcription factor required for the maturation of TEPs into mature TECs. The efficiency of the differentiation protocol was confirmed on multiple hESC lines, demonstrating that this method can be applied to other human pluripotent cell lines. Additional gene expression analysis for markers of other endoderm derivatives revealed that, while markers of thyroid (NKX2.1, PAX8), lung (NKX2.1, FOXP2), parathyroid (PTH), and pancreas (PDX1) were not induced in our cultures, liver markers (AAT, ALB, CYP3A4, CYP3A7) could be detected. Furthermore, we are currently developing assays to investigate if TEPs derived using this method can support the development of functional T cells. Given the importance of TECs in establishing self-tolerance, our results represent a critical step in the development of strategies to manipulate immune tolerance in the context of autoimmune disorders and allogeneic donor transplants.

PROGRAM AND ABSTRACTS

INDUCED TOLERANCE TO EMBRYONIC STEM CELL TRANSPLANTATION BY HOST CONDITIONING WITH TOTAL LYMPHOID IRRADIATION, ANTITHYMOCYTE SERUM, AND REGULATORY T CELL

Pan, Yuqiong[1], Leveson-Gower, Dennis B.[1], de Almeida, Patricia E.[2], Pierini, Antonio[1], Florek, Mareike[1], Wu, Joseph C.[3], Negrin, Robert S.[1]
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Embryonic stem cells (ESCs) hold tremendous promise for the treatment of a broad range of serious medical conditions. However, their utility is limited by the possibility that upon transplantation the ESCs will be rejected by the immune system of the allogeneic recipients, which has been observed in animal modeling to date. One alternative is to use autologous induced pluripotent stem (iPS) cells, however, there still remains significant concern about the utility and immunogenicity of this cell population, especially due to the recent findings that iPS cells can also provoke immune response due to partial reprogramming and genetic instabilities. Therefore, overcoming the immunogenicity or inducing tolerance to pluripotent stem cell transplantation remains a critical issue before the enormous promise of ESCs for the treatment of diseases can be realized. Therefore, the objective of our study is to establish tolerance to transplanted ESCs as well as mature cell populations derived from ESCs by utilizing immune regulatory strategies.
We have explored highly purified CD4+CD25+FoxP3+ regulatory T (Treg) and natural killer T cell populations to induce tolerance to transplanted ESC across a major mismatched barrier. With either luciferase (luc+) ESC or luc+ Treg populations, we utilized Bioluminescent Imaging (BLI) to non-invasively track the fate of ESCs to explore both the survival of ESC populations in allogeneic hosts as compared to syngeneic and severe combined immunodeficiency (SCID) hosts, and also the impact of regulatory T cells on their survival. We have found that allogeneic recipients conditioned with total lymphoid irradiation (TLI) and antithymocyte serum (ATS) or TLI plus Treg infusion can prolong the survival of ESC allografts after transplantation. We have also examined the impact of these clinically translatable treatments on inducing the tolerance of transplanted differentiated ESC populations.
Our findings provide insights into the mechanisms of the immune rejection of ESCs, and also develop clinically translatable strategies of inducing tolerance to adoptively transferred ESCs for the cellular replacement therapy (CRT) treatment of medical disorders.

BLOCKADE OF COSTIMULATORY MOLECULE SIGNALING PROMOTES SURVIVAL OF HUMAN EMBRYONIC STEM CELL-DERIVED ENDOTHELIAL CELLS BY INDUCING T CELL IMMUNOGLOBULIN 3 UPREGULATION AND IMPROVES CARDIAC FUNCTION

Ransohoff, Julia D.[1], Huber, Bruno C.[1], Ransohoff, Katherine J.[1], de Almeida, Patricia E.[1], Riegler, Johannes[1], Gong, Yongquan[1], Sanchez-Freire, Veronica[1], Dey, Devaveena[1], Kooreman, Nigel G.[1], Diecke, Sebastian[1], Robbins, Robert C.[2], Gold, Joseph D.[1], Wu, Joseph C.[1]
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Human embryonic stem cell (hESC) derivatives are attractive candidates for therapeutic use. The therapeutic potential of these cells, however, may depend on their long-term survival following transplantation. The immunogenicity of stem cell-based grafts presents a significant barrier to their clinical translation and the requirement to demonstrate efficacy and safety of human stem cell-derived therapies in animal models adds an additional level of complexity, as xenograft rejection is difficult to overcome. Here we test the hypothesis that a short-course, dual-agent regimen of two costimulatory blockade agents, CTLA4-Ig and anti-LFA-1 (costim), can induce the superior engraftment of hESC derivatives compared to current immunosuppressive agents.
We transduced hESCs with a double fusion reporter gene construct expressing GFP and firefly luciferase and differentiated these cells to endothelial cells (hESC-ECs). Reporter gene expression enabled longitudinal assessment of cell engraftment by bioluminescence imaging (BLI). Costim therapy resulted in superior mouse and human EC engraftment compared to cyclosporine therapy in a murine hindlimb model ($p<0.05$), and mitigated immunological rejection of hESC-ECs after transplantation into the ischemic myocardium ($p<0.05$). This robust hESC-EC engraftment had a cardioprotective effect after myocardial injury, as assessed by MRI; we observed increased left ventricular ejection fraction ($p<0.05$), decreased end diastolic/systolic volumes ($p<0.05$), and attenuated cardiac remodeling in animals receiving hESC-ECs with costim therapy compared to animals receiving cells only as well as animals receiving costim therapy but no hESC-ECs. In investigating the mechanism by which costim therapy promotes cell engraftment, we found that costim treatment induces the marked upregulation of T cell immunoglobulin and mucin domain 3 (TIM3) on splenocytes ($p<0.05$), hESC-EC-implanted muscle cells ($p<0.05$), and lymph node cells ($p<0.05$) of treated hosts. Our data show costim therapy reduces a pro-inflammatory cytokine profile, inducing decreased interleukin-2 and interferon-, and increased interleukin-4 and macrophage inflammatory protein-1 levels.
This study demonstrates that short course costim therapy is a superior alternative to current clinical immunosuppressive strategies to promote the survival of hESC derivatives. We also make the important observation that significantly increased myocardial function, as assessed by longitudinal MRI, requires both cell transplantation and effective immunosuppression to extend the window for functional preservation following injury. This regimen may function through a TIM3-dependent mechanism involving the induction of inhibitory molecules associated with T cell exhaustion. This study represents an important step forward in overcoming the immunologic barriers that have continued to hamper the full realization of highly promising cell-based therapies.

Poster Briefs

BONE CELLS GOVERN T LYMPHOPOIESIS BY REGULATING THYMIC EMIGRANTS FROM BONE MARROW

Yu, Vionnie W. C.[1], Lotinun, Sutada[2], Saez, Borja[1], Cook, Colleen[1], Yusuf, Rushdia Z.[1], Ferraro, Francesca[1], Lymperi, Stefania[1], Raaijmakers, Marc[3], Wu, Joy[4], Kronenberg, Henry M.[4], Baron, Roland[2], Scadden, David T.[1]
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HOST MACROPHAGES ARE A BARRIER TO THE ENGRAFTMENT OF EMBRYONIC STEM CELL-DERIVED HEMATOPOIETIC PROGENITORS

Thompson, Heather L.[1], McLelland, Bryce T.[1], van Rooijen, Nico[2], Manilay, Jennifer O.[1]
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MESENCHYMAL STEM CELLS STIMULATE AN IMMUNE RESPONSE BY PROVIDING IMMUNE CELLS WITH TOLL-LIKE RECEPTOR 2 LIGAND

Weinstock, Ada, Pevsner-Fischer, Meirav, Zipori, Dov
Molecular cell biology, Weizmann Institute of Science, Rehovot, Israel

HUMAN IPS CELL-DERIVED ANTIGEN-TARGETED T LYMPHOCYTES ERADICATE CD19-POSITIVE TUMOR CELLS

Themeli, Maria, Kloss, Christopher C., Sadelain, Michel
Center for Cell Engineering, Molecular Pharmacology and Chemistry Program, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Pluripotent stem cells may be harnessed to generate tumor-targeted T lymphocytes for cancer immunotherapy. We previously demonstrated that human T cells targeted to the CD19 antigen, which is expressed on the vast majority of leukemias and lymphomas, can eradicate B cell malignancies in mice, using chimeric antigen receptors that redirect T cell specificity in HLA-independent fashion. We also showed that "second generation" CARs that provide combined activation and co-stimulatory signals enhance T cell expansion upon repeated antigen exposure to antigen and in vivo T cell persistence. The generation of optimal T cells may be further enhanced by engineering naïve or memory T cells, which possess ideal intrinsic features for directing effective anti-tumor responses. Merging induced pluripotent stem cells (iPS) and CAR technology, we characterize here the phenotype and anti-tumor function of human iPS-derived T cells that are genetically targeted to a predetermined tumor-associated antigen.
To this end, we generated iPS cells from peripheral blood lymphocytes (T-iPS) and we consecutively genetically engineered them to express 1928z, a second-generation CAR currently used in clinical trials. Stably transduced 1928z-T-iPS lines were then directed to differentiate in vitro towards the hematopoietic lineage by EB formation in serum-free conditions and next towards the T lymphoid lineage in a OP9-DL1 co-culture system. By day 20 of differentiation around 80% of the cells were CD3+TCR$\alpha\beta$+, with some expressing CD8 (13%) and/or CD56 (20-30%). Most of the generated T lymphocytes had a CD45RA+CD62L- effector memory phenotype while 6% had a CD45RA+CD62L+ naïve phenotype. The generated T cells all expressed the 1928z CAR on the surface (1928z-TiPS-T). We next examined if 1928z-TiPS-T cells show basic T cell function after exposure to cell bound CD19. When cultured in the presence of artificial antigen-presenting cells (AAPCs) expressing CD19, 1928z-TiPS-T cells rapidly responded by engaging the antigen, forming clusters and eradicating the AAPC layer. Twenty-four hours after CD19 antigen engagement by the CAR, the expression of activation markers such as CD69 and CD25 was markedly increased. Furthermore, 1928z-TiPS-T cells secreted type 1 cytokines such TNF-α, IFN-γ and IL-2. We next sought to investigate if 1928z-TiPS-T cells are expandable and show clinically relevant cytotoxic function. We were able to expand 1928z-TiPS-T cells 45-fold after the first stimulation on AAPCs and around 1000-fold after 3 weekly stimulations. The expanded cells still had an effector memory phenotype and up-regulated the expression of natural cytotoxicity receptors. The cytotoxic potency of expanded 1928z-TiPS-T cells was tested in CTL assays. Importantly, we found that 1928z-TiPS-T cells displayed highly specific lytic activity against CD19+ tumor cells lines in vitro.
In conclusion, we demonstrate that TiPS-derived CAR+ T cells display key aspects of T lymphocyte function and can specifically eradicate target tumor cells. The genetic modification of human pluripotent stem cells with CARs is, thus an effective approach to rapidly and efficiently generate antigen-specific T cells with a clinically relevant function,. The iPS cell technology not only offers new perspectives to generate naïve T cells of a predetermined specificity, but is also an excellent platform for safe genetic modifications to augment the potency of immune effectors.

Friday, June 14, 2013 4:00 PM - 5:50 PM

Ballroom East

Concurrent IVD: Stem Cells and Cancer

EXAMINING THE 'CANCER STEM CELL' HYPOTHESIS AS IT RELATES TO BREAST CANCER

*Ole William Petersen[1], **Mina J. Bissell[2],** +Jiyoung Kim[2], +René Villadsen[2] and William C. Hines[1]
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The two most frequent subtypes of human breast cancer are the luminal and the basal-like, named after their resemblance to the two major lineages in the normal human breast. The majority of human breast cancers exhibit luminal epithelial differentiation, but most aggressive behavior, including invasion and purported 'cancer stem cell' (CSCs) properties, are considered characteristics of basal-like cells. The existence and the behavior of breast tumor CSC's are matters of much controversy. Even in normal primary breast tissue, we now have evidence to show that there are at least 11 distinct subpopulations; whether these are distinct and stable progenies or exhibit phenotypic variation due to their positions in the breast microenvironment is not clear and is under investigation.
We have succeeded answering the following questions about the cancer cells: Must luminal-like breast cancer cells become basal-like to initiate tumors or to invade? Could luminally differentiated cells within a basally initiated hierarchy also be tumorigenic? To answer these questions, we used rare and mutually exclusive lineage markers to isolate subsets of luminal-like, and basal-like cells from human breast tumors. Among more than 150 antibodies tested, we chose MM, a milk mucin, and CD271. MM was detected by the M18 antibody, which recognizes branched glycans (15) and CD271/p75NTR was detected by the ME20.4 antibody. We enriched for populations with or without prominent basal-like traits from individual tumors or single cell cloning from cell lines and recovered cells with a luminal-like phenotype. Tumor cells with basal-like traits mimicked phenotypic and functional behavior associated with 'stem cells' assessed by expression of selected genes, mammosphere formation and lineage markers. Surprisingly, however, luminal- like cells without basal-like traits, were fully capable of initiating invasive tumors in NOD SCID gamma (NSG) mice. Indeed, these phenotypically pure luminal-like cells generated larger and more invasive tumors than their basal-like counterparts. The tumorigenicity and invasive potential of the luminal-like cancer cells relied strongly on the expression of the gene GCNT1, which encodes a key glycosyltransferase controlling O-glycan branching. These findings demonstrate that basal-like cells, as defined currently, are not a requirement for breast tumor aggressiveness. Also whereas there appears to be a differentiation hierarchy in freshly isolated cells, within a single tumor, there are multiple 'stem-like' cells with tumorigenic potential casting doubt on the hypothesis of hierarchical loss of tumorigenicity.
**Both senior authors contributed equally.*

A CHROMATIN FACTOR SCREEN IN ZEBRAFISH IDENTIFIES NEURAL CREST REGULATOR SATB2 AS A NOVEL DRIVER OF MELANOMA

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Migratory neural crest progenitors give rise to diverse cell lineages, including melanocytes. Genetic alterations and epigenetic changes in melanocytes promote the development of malignant melanoma. To interrogate the role of chromatin factors that stimulate melanoma in vivo, we used a zebrafish model that develops invasive melanoma when melanocytes are induced to express human oncogenic BRAFV600E in a p53-deficient background. When mitfa loss-of-function alleles are introduced, neural crest progenitors cannot differentiate into melanocytes, and melanoma cannot develop. Genomic integration of the transposon-based expression vector MiniCoopR into Tg(mitfa:BRAFV600E;p53-/-;mitfa-/-) zebrafish, rescues melanocytes and melanoma, and drives the expression of a candidate gene in rescued pigment lineages. We developed an efficient MiniCoopR-based pooling approach to functionally test 83 chromatin factors, and identified 7 pools that strongly accelerated melanoma onset. Single factor validation revealed several new drivers of melanoma. One of the factors is SATB2. This gene represents a class of transcriptional regulators that control gene expression by orchestrating higher-order chromatin structure, and function as transcriptional co-factor. SATB2 is expressed in distinct neural crest progenitors, and inactivating mutations induce defects in neural crest lineages in fish and mammals alike. In humans, these include craniofacial dysmorphisms like cleft palate and mandibular hypoplasia, generalized osteoporosis, and mental retardation. SATB2 overexpression is linked to colorectal cancer, and head and neck squamous cell carcinoma. Interestingly, overexpression of its closely related ortholog SATB1 correlates with metastatic melanoma, and poor patient survival. Expression profiling by RNA-SEQ on zebrafish SATB2-overexpressing tumors versus EGFP-overexpressing control tumors, in combination with ChIP-SEQ using an anti-human SATB2 antibody to identify SATB2-bound genes, revealed that the neural crest program is stimulated by SATB2 overexpression. Our data suggests that SATB2 misexpression induces a fate switch that contributes to melanoma formation, which could lead to important insights into the epigenetic regulation of melanoma, and the identification of therapeutic targets.

BMP2 DETERMINES LUMINAL SWITCH AND PROMOTES RELATED BREAST TUMORS THROUGH THE DISRUPTION OF THE FOXC1/FOXA1 BALANCE

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Abnormalities of the tumor niche are suspected to play a role in tumorigenesis but the underlying mechanism remains unknown. We demonstrate here that a soluble factor provided by the microenvironment, BMP2, promotes the malignant transformation of predisposed mammary epithelial cells. First, we demonstrate that, despite their close similarity, BMP2 and BMP4 have very different functions in the human mammary gland. BMP4 acts mainly on myoepithelial progenitors and on the stem cell/early progenitor pool whereas BMP2 regulates the conversion of these immature cells to luminal progenitors. While available data implicate BMPs only in late stages of tumorigenesis and cancer spreading, we demonstrate here that BMPs also promote the transformation of immature normal cells. Indeed, we show that long term exposure to BMP2, initiates the formation of luminal tumors in an inflammatory context that is mimicked by exogenous IL6 treatment in our model. Analysis of gene expression during transformation revealed that BMP2 promotes luminal tumor formation by controlling expression of SMAD5, the FOXC1/FOXA1 balance in favor of FOXA1 and simultaneously with the up-regulation of GATA3. Our data provide insight into the etiology of breast cancer by revealing a new mechanism through which the stem cell microenvironment can promote the malignant transformation of epithelial cells. We argue that these events, on one side on the niche and on the other side on (stem) epithelial cells fuel cell transformation mediated by BMP2 and illustrate the power of the stem cell niche to deliver exogenous cues that promote transformation and dictate the ultimate breast tumor type.

TARGETING CANCER STEM CELLS IN MEDULLOBLASTOMA IS NECESSARY BUT NOT SUFFICIENT FOR TUMOUR CONTROL

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Medulloblastoma (MB) is a paediatric brain tumour thought to arise from undifferentiated neural precursors of the cerebellum. Patched1 (Ptch1), a negative regulator of the Sonic Hedgehog (SHH) pathway, is mutated in about 25% of MB cases. In mice, heterozygous deletion of Ptch1 leads to downstream activation of the SHH transducer Smoothened (Smo) and to MB generation. Data from clinical trials indicate that MBs patients treated with Smo inhibitors go into remission but subsequently succumb to tumours harbouring Smo mutants resistant to Smo antagonists. We provide here a complementary interpretation of these data based on the cancer stem cell (CSC) hypothesis. Previously, we identified a small population of neural stem-cell like cells in human MBs and in MBs generated in the Ptch1 mice model. We surveyed databases of MB samples for expression of the neural stem cell markers Sox2 and EGFR and found significant association of these factors with the SHH MB subgroup, implicating putative CSC in this MB subgroup. We discovered that the proliferation of Sox2+ cells derived from Ptch1 MBs is Smo independent, suggesting that small population of cells within MB tumours are resistant to treatment with SHH inhibitors and can provide a reservoir of cells for tumour recurrence. In the absence of drugs with specific activity against CSC we screened libraries of small molecules and identified an uncharacterized anti-inflammatory drug, as an inhibitor of Sox2+ cells. We found that degradation of the EGFR plays an important role in this activity. Spontaneous MBs in Ptch1+/- mice injected with the drug displayed significant reduction in the frequency of Sox2+ cells, a decline in colony formation and decreased engraftment potential, collectively indicating that this agent targets CSC in SHH MBs. While the survival of primary Ptch1+/- mice was not enhanced by this agent, combination treatment with the Smo antagonist GDC0449 decrease secondary tumour formation as readout for MB recurrence and self-renewal. Our work identifies novel anti CSC activity for a clinically used compound, moreover, we found that pharmacological targeting of CSC in a preclinical model of MBs is not sufficient to block tumour growth. However, targeting both stem-cells and non stem-cells within a tumour can potentially prevent recurrence in SHH MB.

Poster Briefs

BCL11A: A NOVEL BREAST CANCER GENE WHICH PLAYS A CRITICAL ROLE IN MAMMARY STEM CELLS AND SUPPRESSES P53

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E2F1 IS CRITICAL FOR SURVIVAL OF CHRONIC MYELOID LEUKAEMIA (CML) STEM CELLS

Pellicano, Francesca[1], Hopcroft, Lisa[1], Park, Laura[1], Sinclair, Amy[1], Sinclair, Amy[1], Leone, Gustavo[2], Girolami, Mark[3], Whetton, Anthony[4], Kranc, Kamil[1], Holyoake, Tessa[1]
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IMPLICATING THE PLURIPOTENCY FACTOR LIN28 IN KIDNEY DEVELOPMENT AND WILMS TUMOR

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OVEREXPRESSION OF THE EMT REGULATOR ZEB2/SIP1 RESULTS IN A BLOCK IN T CELL DEVELOPMENT AND IDENTIFIES THIS GENE AS A NEW DRIVER FOR T CELL LYMPHOBLASTIC LEUKEMIA

Haigh, Jody
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Zeb2 is a member of the ZEB family of transcriptional regulators. Its expression was correlated with the formation and/or function of cancer stem cells in solid tumors. We have previously demonstrated that Zeb2 is highly expressed in the hematopoietic system and evidence from mouse retroviral mutagenesis studies points to a role for Zeb2 in initiation and/or progression of leukemia/lymphoma. In this study we examined the roles of Zeb2 in the stem cells of the hematopoietic system and in leukemia formation through a conditional gain-of-function approach in the mouse. For this, we inserted a Zeb2 encoding cDNA preceded by a floxed transcriptional stop cassette within the Rosa26 locus. Using the Tie2-cre line (that is active in the endothelium and entire hematopoietic system), we were able investigate the effects of Rosa26 locus based overexpression of Zeb2 in HSC biology and during leukemic transformation. Bi-allelic overexpression of Zeb2 from the Rosa26 locus resulted in altered T cell development. A partial block in differentiation was observed both in vivo as in vitro, using an OP9-DL1 coculture system, at the DN3 pre-T cell stage. In addition, Zeb2 overexpressing mice spontaneously develop thymic lymphomas starting at 6 months of age, indicating that Zeb2 acts as a driver in T cell malignancies, most likely due to the disruption of normal thymocyte differentiation. To further investigate the role of Zeb2 in T-ALL progression, we have bred these mice onto a tumor-prone background ($p53^{flox/flox}$) and observed a significant decrease in T-cell lymphoma/leukemia latency and an increase of the stem/progenitor markers c-Kit and CD44, suggesting an increase in leukemic stem cells. Indeed, using a minimal dilution series of tumour cells into NOD/SCID mice we could demonstrate a 100-fold increase in leukemia initiating cells in the Zeb2 overexpressing tumors.

To assess the relevance of these findings with human disease, we screened a cohort of T-ALL patients and found increased expression of ZEB2 predominantly associated within immature/ETP-ALL patients. Compared to other T-ALL subgroups (cortical or mature), ETP-ALLs are defined by a gene-expression profile similar to Early Thymic Precursor (ETP) cells, a subset of thymocytes with stem cell-like features, and a striking feature is that these patients have poor response to conventional therapies with a high risk of remission failure or hematological relapse. The basis of the poor prognosis in ETP-ALL patients has yet to be identified, although one potential contributing factor is the stem cell properties of this aggressive leukemia. Importantly, we could identify two ETP-ALL patients who presented a unique chromosomal translocation t(2;14)(q22;q32) on karyotype. This new translocation involves the ZEB2 locus and the BCL11B locus as confirmed by FISH analysis. Such BCL11B-associated translocations lead to the overexpression of the partner gene, thereby identifying ZEB2 deregulation as a molecular driving force for the development of ETP-ALL in these two patients. In conclusion, we have shown that Zeb2 overexpression affects early T cell development and predisposes mice to develop an aggressive form of T-ALL with increased stem cell properties. This mimics patients with aggressive ETP-ALL driven by ZEB2 translocations, demonstrating that the EMT regulator ZEB2 is an oncogene for T-ALL.

Friday, June 14, 2013 4:00 PM - 5:50 PM

Room 205

Concurrent IVE: Chromatin Regulation in Stem Cells

CHEMICAL CONTROL OF GENE EXPRESSION

Bradner, Jay
Dana-Farber Cancer Institute, USA

Epigenetics classically defines the study of heritable phenotypes not genetically encoded in DNA. In cancer, epigenetic proteins are among the most promising and intently pursued targets in drug discovery. Already, inhibitors of DNA methyltransferases and histone deacetylases have demonstrated substantial clinical efficacy leading to regulatory approval for use in hematological malignancies. These events have triggered intense competition to develop inhibitors of chromatin-associated, gene regulatory complexes. Beyond drug development, chemical probes of gene regulatory proteins can provide insights into mechanisms of development and disease. We have approached the complex biochemistry of gene regulatory complexes with discovery chemistry, toward the goal of developing a chemical toolbox of novel, small-molecule modulators of chromatin structure and function. This presentation will describe recent research toward the development of drug-like inhibitors of epigenetic "readers" (BET bromodomains), "writers" (DOT1L and EZH2 lysine methyltransferases), and "erasers" (soft and selective HDAC inhibitors). In addition, research on the effect of unrestricted availability of chemical probes will be described, so as to describe the consequence of pursuing a more open-source model of drug discovery.

A FUNCTIONAL MAP OF NUCLEAR PROTEOLYSIS

Catic, Andre, Suh, Carol Y., Hill, Cedric T., Daheron, Laurence, Henkel, Theresa, Scadden, David T.
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Technological advances allow us to draw an ever-finer map of DNA and of its occupying transcriptional regulators and chromatin. This map is static by default and only describes the constellation of proteins and nucleic acids at a given time. However, many transcription factors are short-lived and their activity and turnover are functionally linked,

allowing for a tight regulation of gene expression. Still, the global patterns by which DNA-bound regulators are eliminated and the quantitative contribution of localized proteolysis on individual gene expression remained poorly understood.
We established an assay to identify DNA-associated proteins that are slated for degradation by the ubiquitin-proteasome system. Specifically, our study had three goals. First, assess degradation of DNA-bound factors on a genome-wide scale. Second, define sites of proteolysis in the context of gene expression and chromatin architecture. Third, identify transcriptional regulators with high turnover dynamics and determine the impact of their degradation on relevant gene transcription. Our investigation of mouse preadipocytes links degradation of DNA-bound proteins to active gene promoters and enhancer sites. Proteolysis is associated with distinct gene ontologies and can either promote or suppress expression. Nuclear encoded mitochondrial genes in particular show signs of rapid protein turnover. Utilizing integrative genomics, we discovered a dynamic interaction between the nuclear receptor corepressor NCoR1 and the transcription factor CREB at these degradation sites. Continuous elimination of NCoR1 is required to maintain transcript levels and inhibiting its turnover impedes mitochondrial biogenesis. By charting the nuclear locations of protein degradation and functionally linking proteasome activity with gene expression, we provide the first genome-wide map of DNA-associated proteolysis in mammalian cells.

A LIBRARY OF ENDOGENOUSLY TAGGED FLUORESCENT PROTEINS IN EMBRYONIC STEM CELLS REVEALS A LINKER HISTONE CHAPERONE INVOLVED IN PLURIPOTENCY AND DIFFERENTIATION

Raghu Ram, Edupuganti, Aaronson, Yair, Sailaja, Badi Sri, Nissim-Rafinia, Malka, **Meshorer, Eran**
Genetics, The Hebrew University of Jerusalem, Jerusalem, Israel

Chromatin regulators are essential for mouse embryonic stem cell (ESC) maintenance and differentiation. ESCs are unique in their ability to self-renew and differentiate into all cell types of the organism. A core set of transcription factors in concert with chromatin regulators maintains the "stem cell state". To identify novel regulators involved in stem cell maintenance and differentiation, we created a library of mouse ESC clones using a gene-tagging approach; with each clone expressing a fluorescent tagged protein (YFP or Cherry) under the control of its own endogenous promoter. Using this library, we performed a screen for proteins that are specifically down-regulated during early stages of ESC differentiation. Using time-lapse live imaging of differentiating cells, we identified candidate genes and systematically investigated their role in ESC function. One of the identified proteins is SET nuclear oncogene (SET), a multifunctional linker histone chaperone, which remarkably, shifts from the SETα isoform to the SETβ isoform by alternative promoter usage during early ESC differentiation. Using various biochemical and genetic methods, we show that SET is essential for active proliferation and differentiation of ESCs. SET depletion caused perturbed neuronal differentiation, with aberrant silencing of pluripotency genes and failed induction of neuronal genes. Mapping SET-bound genomic regions by ChIP-seq, we identified the neuronal targets of SET, suggesting a role in facilitating neuronal differentiation. Importantly, SET function is isoform specific. SETβ, but not SETα decreased reprogramming efficiency, and photobleaching methods demonstrated a selective role for SETα in maintaining a hyper-dynamic chromatin state in ESCs. Taken together, this work provides the first endogenously labeled fluorescent tag library in ESCs, and identify a novel chromatin regulator of proliferation and differentiation in ESCs.

HISTONE VARIANT H2A.X-MEDIATED EPIGENETIC MECHANISMS ARE CRITICAL FOR MAINTAINING GENOME STABILITY AND PLURIPOTENCY IN ES AND IPS CELLS

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Advance in cellular reprogramming (induced pluripotent stem cells, iPSc) presents a promising venue for regenerative medicine. Successfully reprogrammed cells should regain pluripotency and unlimited self-renewal capacity while maintaining genome integrity. However, how to screen the clones that fully recapitulate these fundamental features of the ESC is still a challenge. Recent studies reveal that iPS clones are quite different in support embryonic development in tetraploid complementation assays and germline transmission despite similar expressions of pluripotent genes, such as Oct4. In addition, several recent studies showed that many iPSC clones are prone to genomic instability, such as copy number variation (CNV). How to identify iPS clones that faithfully recapitulate ES cells in pluripotency and genome integrity is imperative for potential clinical applications. In addition, it is intriguing whether the compromised pluripotency and CNV reflects potential dysregulation of yet unknown cellular mechanisms.
Herein, we show that a specialized histone variant, H2A.X, plays unexpected roles in maintaining genome integrity in pluripotent ESC and iPSC. In ESC, H2A.X is specifically enriched at genomic regions sensitive to replication stress; H2A.X deficiency leads to frequent genomic instability at these regions (CNV), defective replication stress response and impaired DNA repair. We also demonstrated that H2A.X plays a novel role in the transcriptional repression of critical developmental genes via regulating the chromatin structures at poised developmental enhancers. In iPSC, faithful H2A.X deposition plays unexpected roles in genome integrity and pluripotency. In iPSC clones which support the development of "all-iPS" animals in tetraploid complementation assays, H2A.X depositions faithfully recapitulate the ESC pattern and therefore, prevent genome instability and other defects. On the other hand, in iPSC lines that fail to produce whole animals in tetraploid complementation assays, H2A.X depositions are greatly reduced at replication stress domains, which lead to frequent genome instability (CNV) thereat, reminiscent of the H2A.X deficient ESC. In the same clones, H2A.X is also aberrantly enriched at the genes critical for iPSC pluripotency and silences their expression. Thus, our work demonstrated that genome integrity and pluripotency are unexpected linked by H2A.X-mediated novel epigenetic mechanisms. In summary, H2A.X deposition serves as a novel epigenetic mark for pluripotent stem cells, which can be used to distinguish the developmental potentials of the iPSC lines.

Poster Briefs

A DEDICATED ROLE FOR TRANSCRIPTION FACTOR CDX2 IN INTESTINAL STEM CELL FUNCTIONS

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ENHANCER TRANSCRIBED RNAS IN EMBRYONIC STEM CELLS ARE REGULATED BY THE TET FAMILY OF PROTEINS

Rao, Sridhar[1], Pulakanti, Kirthi[2], Pinello, Luca[3], Stelloh, Cary[4], Blinka, Steven[5], Allred, Jeremy[5], Milanovich, Samuel[6], Kiblawi, Sid[4], Peterson, Jonathan[4], Yuan, Guo-Chen[7]
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PREDICTION OF CHROMATIN STATE VARIABILITY

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OCT4 SWITCHES PARTNERING FROM SOX2 TO SOX17 TO REINTERPRET THE ENHANCER CODE AND SPECIFY ENDODERM

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How regulatory information is encoded in the genome is poorly understood and poses a challenge when studying biological processes. We demonstrate here that genomic redistribution of Oct4 by alternative partnering with Sox2 and Sox17 is a fundamental regulatory event of endodermal specification. We show that Sox17 partners with Oct4 and binds to a unique 'compressed' Sox/Oct motif which earmarks endodermal genes. This is in contrast to the pluripotent state where Oct4 selectively partners with Sox2 at 'canonical' binding sites. The distinct selection of binding sites by alternative Sox/Oct partnering is underscored by our demonstration that rationally point-mutated Sox17 partners with Oct4 on pluripotency genes earmarked by the canonical Sox/Oct motif. In an assay of endodermal differentiation we demonstrate that the compressed motif is required for proper expression of endodermal genes. Evidently, Oct4 drives alternative developmental programs by switching Sox partners that affects enhancer selection, leading to either an endodermal or pluripotent cell fate. This work provides a rationale for the 'enhancer code', that highlights a direct link between the DNA sequence and a developmental outcome.

Saturday, June 15, 2013 9:00 AM - 11:10 AM

Plenary Hall B1

Plenary V: Stem Cells and Fate Control

Supported by Development

TRANSCRIPTION FACTOR CONTROL OF TRANSITIONS IN PLURIPOTENT CELL STATES

Chambers, Ian, Karwacki, Violetta
Institute for Stem Cell Research, MRC Centre for Regenerative Medicine, 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 gene regulatory network centred on Oct4, Nanog and Sox2. ES cells fluctuate between states of high and low Nanog expression that direct efficient or inefficient self-renewal. Recently, we and others have shown that Nanog is under autorepressive control. To date, robust self-renewing states have only been reported by chemical inhibition of signalling pathways or enforced transgene expression. Here we identify an ES cell state in which Nanog is expressed homogeneously and which exhibits stable robust pluripotency. Consistent with a model in which differentiation proceeds through Nanog-negative cells, these ES cells show an attenuated ability to commit to differentiation with a major block in the movement from an ES cell state to an EpiSC state. Both heterogeneous Nanog expression and rapid differentiation kinetics can be restored by simple modulation of the pluripotency regulatory network within the range of concentrations found in wild-type cells. Our findings suggest that robust pluripotency originates from a subset of cells present within wild type cultures and that the full range of parameters present in wild-type cultures enables effective differentiation.

PLURIPOTENT STEM CELLS AND CELL FATE PROGRAMMING FOR MODELING NEUROLOGICAL DISEASE

Brüstle, Oliver
Institute of Reconstructive Neurobiology, University of Bonn Medical Center, Bonn, Germany

A major challenge for studying the molecular pathomechanisms underlying neurodegenerative disorders is the limited experimental access to disease-affected human nervous system tissue. This problem has been partially overcome with the availability of human pluripotent stem cells (PSC). We have established protocols for the derivation of long-term self-renewing neuroepithelial stem (lt-NES) cells from human embryonic stem cells (hESC). Lt-NES cell-derived neurons express the entire array of Alzheimer's disease (AD) associated human proteins and can be used to explore mutants associated with aberrant APP and tau processing - two key pathogenic routes associated with this disorder. Derivation of lt-NES cells from induced pluripotent stem cells (iPSC) extends this approach to a patient-specific level. Using this strategy we detected an unexpectedly low responsiveness of human neurons to gamma-secretase modulators. These data are concordant with the observed failure of these compounds in clinical trials and underline the importance of assessing compound efficacy in the appropriate human target cell type. Interestingly, lt-NES cell-derived neurons recapitulate pathological protein aggregation already a few weeks after in vitro differentiation, which makes them a formidable tool for studying the earliest cytopathological events in proteinopathies such as polyglutamine disorders. While cell reprogramming and iPSC-derived lt-NES cells represent robust tools for studying monogenic diseases in the context of selected patient families, there is a strong interest in extending this methodology to complex disorders. Requiring samples from large numbers of patients, such studies call for even more efficient methods for establishing disease-specific cells. We found that overexpression of two neurogenic transcription factors together with small molecule-based inhibition of GSK-3ß and SMAD signaling permits direct conversion of human fibroblasts into induced neurons (iNs) with yields of up to >200% - efficiencies, which should be suitable for the direct generation of bulk quantities of human iNs for biomedical applications.

MECHANISM AND RECONSTITUTION IN VITRO OF GERM CELL DEVELOPMENT IN MICE

Saitou, Mitinori
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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 development in mice at a single-cell resolution, 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 (ESCs) and

induced pluripotent stem cells (iPSCs)], we have succeeded in precisely reconstituting the specification and subsequent development of germ cells in culture both in males and females: ESCs/iPSCs are induced into epiblast-like cells (EpiLCs) and then into primordial germ cell-like cells (PGCLCs), which contribute to spermatogenesis and oogenesis and to fertile offspring. Our work will serve as a foundation for systems analysis of germ cell development, including the elucidation of key transcriptional network for germ cell specification, the mechanism of genome-wide epigenetic reprogramming, and the mechanism for meiosis, as well as for the reconstitution of the entire germ-cell development process in vitro, not only in mice but also in other mammals, including humans.

TRANSLATING THE NATURAL HISTORY OF HUMAN STRIATAL DEVELOPMENT INTO PLURIPOTENT STEM CELL DIFFERENTIATION

Cattaneo, Elena
University of Milano, Milano, Italy

Our laboratory works on Huntington's Disease, an inherited neurodegenerative disorder. On the one end we are studying the physiology and the evolution of the causative gene (Lo Sardo & Zuccato et al., Nat Neurosci., 2012) as well as the toxicity elicited by its mutant version (Zuccato et al., Physiol. Review, 2010). On the other end we aim at harnessing the potential of human pluripotent stem cells for the generation of the medium-sized spiny and cortical neurons that are affected in the disease. However, to achieve such a goal requires a more detailed understanding of the biology of human fetal striatal and cortical development.
Here we will present unpublished data on the spatio-temporal pattern of transcription factors that mark human fetal striatal development. We will reveal the antigenic attributes that qualify the striatal progenitors as they transition towards terminal neuronal differentiation (also see Castiglioni et al., ISSCR 2013 abstract). By exploiting gene expression profiling we also expect to benchmark the human transcriptome architecture associated with neuronal specification within the striatum (also see Onorati et al., ISSCR 2013 abstract; and Biella et al., ISSCR 2013 abstract). Incorporation of some of these informations into human pluripotent stem cell differentiation has already allowed, for the first time, the generation of authentic and functionally active DARPP32+/CTIP2+ medium-sized spiny neurons (Delli Carri & Onorati et al., Development, 2013).

EPIGENOMIC REGULATION OF CARDIAC LINEAGE DEVELOPMENT

Bruneau, Benoit G.
Gladstone Institute of Cardiovascular Disease, UCSF, San Francisco, CA, USA

Regulation of chromatin states is essential for transcriptional change during development and cellular differentiation. Histone modification and chromatin remodeling are well-characterized modes of chromatin regulation. Despite this, how these disparate modes of regulation cooperate to modify chromatin and gene expression during transitions in cell state is not well understood. We investigated the function of the chromatin remodeling factor Brg1 in the directed differentiation of embryonic stem cells to cardiomyocytes. We demonstrate that Brg1 is required for the differentiation of cardiomyocytes and the induction of essential genes during the emergence of mesodermal precursors. We find a global requirement for Brg1 in transcriptional activation, and a surprising function in maintaining Polycomb-mediated repression of key developmental regulators. BRG1 associates predominately with H3K27ac genome-wide and loss of Brg1 during mesoderm differentiation causes reduced levels of H3K27ac at dynamic enhancers, suggesting a role for Brg1 in enhancer activation. Moreover, we observe reduced H3K27me3 at derepressed developmental regulators in Brg1-deleted mesodermal cultures, which suggests cooperative function between Brg1 and Polycomb complexes during differentiation. Taken together, our findings support a broad role for Brg1 in modulating both active and repressive chromatin states during the early stages of cardiac differentiation. Together our data reveal that epigenomic regulation of cell type specification is highly dynamic and coordinated.

Saturday, June 15, 2013 1:00 PM - 2:55 PM

Plenary Hall B1

Plenary VI: Genomics and Epigenomics of Stem Cells

Supported By Lieber Institute for Brain Development

PROGRAMMING AND REPROGRAMMING CELL FATE

Zaret, Kenneth S.
Institute for Regenerative Medicine, Epigenetics Program, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

We are interested in the genetic regulatory mechanisms that underlie cell fate control in mammalian development and during the directed conversion of one cell type into another. A mechanistic understanding of cell fate control is central to our ability to modulate intrinsic mechanisms by which stem cells generate biomedically relevant cell types as well as for converting cells for therapeutic purposes. Our focus is on the early events in cell type control, unveiling the means by which transcription factors interact with chromatin templates. Understanding how transcription factors can find their gene targets in "naïve" chromatin that has not yet been programmed for activity explains much about the potential and limitations by which we can currently modulate cell fate at will. Our laboratory discovered pioneer transcription factors, such as FoxA, that have the inherent property of binding to their target sites on nucleosomal DNA and thereby enabling or initiating cell type-inducing events in embryonic development. Recent studies of ours and others have revealed pioneer activity for various transcription factors that elicit cell type reprogramming. Yet we have also found chromatin modification states that are refractory to binding by pioneer factors and that impede cellular reprogramming. Diminishing such refractory chromatin states dramatically enhances reprogramming. Current efforts are to determine DNA and chromatin binding features that predict how to control cell fate and to apply those principles to generate cells that will be useful therapeutically, particularly as they relate to pluripotent cells and the liver and pancreatic beta cell lineages.

PROBING THE SOMATIC MUTATIONAL SPECTRA AND EPIGENETIC PLASTICITY OF NEURONS AND FIBROBLASTS USING REPROGRAMMING AND WHOLE GENOME SEQUENCING

Baldwin, Kristin
Scripps Research Institute, La Jolla, CA, USA

Reprogramming amplifies the genome of an individual somatic cell as they are converted into induced pluripotent stem cells (iPSCs) or somatic cell nuclear transfer (SCNT) derived stem cells. Functionally relevant genomic changes in individual cells can impact the safety and utility of iPSCs. Therefore, the promise of personalized regenerative medicine depends to some extent on gaining an understanding of the extent and origin of somatic mutation. However, outside of the immune system, very little is known about how different cell lineages accumulate unique genomes via somatic mutational processes. Intriguingly, recent single-cell genomic studies indicate that neurons (and perhaps other cell types) may accumulate functionally relevant somatic mutations via transposon insertion or other mechanisms. Transposon activation has also been a concern for iPSCs. However, by applying genome-wide structural variant detection to fibroblast derived iPSCs, we observe few structural changes and no de novo transposition events, despite detection of exogenous retroelement insertions. To address the impact of somatic mutation on neurons, we have amplified the genomes of individual adult neurons by cloning them using SCNT. We demonstrate that adult post mitotic cortical neurons can be reprogrammed to pluripotency and that a subset of neuronal SCNT stem cell lines can produce fertile adult mice. Whole genome sequencing of these neurons reveals a complex mutational spectrum of structural variants, single nucleotide variants and indels, but no de novo transposition events or recurrent DNA rearrangements. Surprisingly, point mutations were enriched in neuronal genes and we observed highly complex chromosomal rearrangements resembling chromothripsis. Results of these studies supply the first genome-wide analysis of an adult post mitotic neuron and concur with the mutational rates seen in recent studies of germ lineages. Additional studies of iPSCs generated from fibroblasts will help to establish the relative impact of cell division, age and lineage on the mutational spectrum of different somatic cells.

REPROGRAMMING OF HUMAN SOMATIC CELLS TO PLURIPOTENCY BY SOMATIC CELL NUCLEAR TRANSFER

Mitalipov, Shoukhrat
Oregon Health and Science University, USA

Cytoplasmic factors present in mature, metaphase II (MII)-arrested oocytes have a unique ability to reset the identity of transplanted somatic cell nuclei to the embryonic state. We recently demonstrated the successful reprogramming of human skin fibroblasts into ESCs following somatic cell nuclear transfer (NT-ESCs). A battery of pluripotency tests performed on human NT-ESCs confirmed their similarities to genuine IVF-derived ESCs. Transcriptional interrogation indicated that NT-ESCs departed from their parental somatic cell gene expression pattern with upregulation of pluripotency associated genes. In addition, NT-ESCs demonstrated the ability to differentiate into a variety of other cell types in teratoma tumors or by in vitro-directed differentiation.

OUTSTANDING YOUNG INVESTIGATOR AWARD (OYIA) LECTURE
DIRECT LINEAGE REPROGRAMMING TOWARDS THE NEURAL LINEAGE

Wernig, Marius
Stanford Univ, Palo Alto, CA, USA

Cellular differentiation and lineage commitment are considered robust and irreversible processes during development. Challenging this view, we found that expression of only three neural lineage-specific transcription factors could directly convert fibroblasts into functional in vitro. These induced neuronal (iN) cells expressed multiple neuron-specific proteins, generated action potentials, and formed functional synapses. Thus, iN cells are bona fide functional neurons.
The question arose whether a defined non-ectodermal cell can be converted into iN cells given the heterogeneity of fibroblast cultures. We therefore tested whether (endoderm-derived) hepatocytes can be reprogrammed to iN cells. Surprisingly, using the same 3 transcription factors primary mouse hepatocytes could be converted very efficiently into fully functional iN cells. Moreover, gene expression studies on the global and single cell level confirmed not only the induction of a neuronal transcriptional program but also the efficient silencing of the hepatocyte-specific expression pattern. We therefore concluded that iN cells are not hybrid cells with equal identities of the starting cell and neurons, but cells with a predominant neuronal identity with an epigenetic or transcriptional memory of the starting cell population.
Completely unexpectedly, the iN cell reprogramming process is substantially more efficient and faster than reprogramming to pluripotent stem cells. We assume that this may be explained by different properties of the transcription factors used for reprogramming. Indeed we found that Ascl1, one of the key driving iN cell transcription factors, is not only a pioneering factor in the sense that it can bind nucleosomal DNA, but it also binds its physiological targets two days after infection in mouse fibroblasts. Intriguingly, this property is different from Oct4, Sox2, Klf4, which have been shown to bind nucleosomal DNA but mostly bind ectopic genomic sites. This may be a molecular explanation for the relatively high iN cell conversion efficiencies from mouse fibroblasts.

Saturday, June 15, 2013 3:40 PM - 6:00 PM

Plenary Hall B1

Plenary VII: Making Tissues and Organs

Supported by GlaxoSmithKline

GENERATION OF ANTERIOR FOREGUT ENDODERM DERIVATIVES FROM HUMAN PLURIPOTENT STEM CELLS

Snoeck, Hans
Columbia University Medical Center, New York, NY, USA

Directed differentiation of human pluripotent stem cells (hPSCs) is a promising avenue to generate mature cell types for disease modeling, drug screening and application in regenerative medicine. While efforts at specification and induction of several endodermal, mesodermal and ectodermal lineages have resulted in significant progress, the derivation of anterior foregut endoderm (AFE), the most proximal section of the endoderm, has been challenging until recently. Several clinically important organs, including lung and airway, thymus, parathyroids and thyroid are derived from the AFE, however. In particular, the ability to generate lung and airway epithelial cells from hPSCs has applications in regenerative medicine for lung diseases, drug screening and disease modeling, and provides a model to study human lung development, while generation of thymic epithelial cells would have important applications for immunomodulation and the generation of patient-specific mouse models of the human immune system. To generate AFE derivatives, primitive streak and DE have to be induced first. Subsequently, anteroposterior patterning of the DE has to be achieved to generate AFE. Strategies to generate AFE have recently been developed. Preliminary studies on iPSCs from patients with DiGeorge Syndrome, a disease associated with defects, among others, in the development of derivatives of the pharyngeal endoderm, revealed a specific defect during the induction of AFE from DE. For further organ domain specification, dorsoventral patterning of AFE is required. Using this approach, a highly efficient method for directed differentiation of hPSCs into functional lung and airway epithelial cells was established. In this strategy, blocking of signaling pathways that are critical for lung development in the mouse, retinoic acid, Wnt and BMP signaling, modeled defects in early lung development observed in corresponding genetic mouse knockouts, thus validating this approach.

REGENERATION OF SQUAMOUS EPITHELIA FROM STEM CELLS OF CULTURED GRAFTS

Pellegrini, Graziella
University of Modena E Reggio Emilia, Modena, Italy

PURPOSE: The only cultured cell types extensively used for tissue regeneration are the keratinocyte and the chondrocyte. Cultured autologous keratinocytes derived from the epidermis have been used for many years to produce grafts that generete an epidermis over a full-thickness wound, such as a third-degree burn. But there have been many failures of engraftment, and in the absence of criteria for the quality of the cultures, the causes of failure cannot be analyzed.
METHODS: It has become clear that the essential feature of the grafts is the presence of an adequate number of stem cells. This article describes the criteria for estimating that number. Advances in graft preparation, combining better preservation of stem cells with ease of application of the graft, are also described. These improvements have been applied to

PROGRAM AND ABSTRACTS

cultures of ocular limbal cells, which contain the keratinocyte stem cells of the corneal epithelium.
RESULTS: Cultures meeting the criteria of stem cell number have been grafted to 116 patients suffering from chemical destruction of the limbus. The procedure has been highly successful in the alleviation of suffering and the restoration of vision.

REGENERATING KIDNEY AND HOLLOW ORGANS

Bertram, Timothy
Tengion Inc., Winston Salem, NC, USA

Regeneration of three-dimensional tissues and organs is a medical frontier. Such advances offer substantial improvements to standards-of-care for organ replacement. Applying cell/biomaterial composite technologies to promote regeneration of hollow and solid organs of the urinary system has immediate application for patients suffering from chronic kidney disease or bladder replacement associated with cancer therapy. The incidence and prevalence of CKD is rising worldwide. There are approximately 85,000 patients awaiting kidney transplant in America; nearly the same number of patients die each year as they undergo end stage renal disease (ESRD) renal replacement therapy (dialysis or kidney transplantation). Bladder cancer represents the 4th most common cause of cancer with bladder removal the primary recourse for those patients failing to respond to chemotherapeutic intervention. New treatment approaches are required to improve quality of life among CKD and ESRD populations and refractory patients with bladder cancer who currently have very limited healthcare options. Regenerative technologies now hold the promise of delaying dialysis and renal transplantation as well as a platform for reconstructing viscous organs of the lower urinary tract. Understanding the cellular and tissue regeneration mechanisms of urinary and renal tissues provides insights into the potential design of novel molecular and cell-based therapeutic approaches to treat such maladies. This presentation will focus on applications of regenerative medicine for reconstruction of the lower urinary tract after cancer related cystectomy and regeneration of functional renal tissue within the context of chronic kidney disease. Comparisons and contrasts with current clinical management approaches and the opportunities provided by regenerative medical technology will be discussed.

CLOSING KEYNOTE ADDRESS

SECRETS OF THE HUMAN GENOME

Lander, Eric
Broad Institute of MIT and Harvard University, USA

No abstract submitted

INNOVATION SHOWCASES

THURSDAY, JUNE 13, 11:45 AM - 12:15 PM

MESENCHYMAL STROMAL/STEM CELLS EXPANSION

Jessie H.-T. Ni, Ph.D.
Irvine Scientific
ROOM 255

Mesenchymal stromal/stem cells (MSCs) are one of the most clinically advanced primitive cells due to their capabilities to support hematopoiesis, to differentiate along mesenchymal and non-mesenchymal lineages, and their immunomodulating properties. This workshop will illustrate how we optimize and validate critical nutrients and growth factors to develop media which achieves specific expansion of MSCs derived from bone marrow, cord blood, amniotic fluid and adipose tissue. Media performance on primary MSCs growth will also be reviewed.

THURSDAY, JUNE 13, 11:45 AM - 12:15 PM

DEFINING STEM CELL POPULATIONS FOR EXPERIMENTAL SUCCESS

Joy Aho, PhD
R & D Systems, Inc.
ROOM 254 A

Stem cell research studies most often require weeks of cell culture before an experimental hypothesis can be tested. Common issues that hinder research progress include the presence of unknown variables within the starting cell population and the use of lower quality or inconsistent reagents. Here we present simple solutions to reduce experimental variability, improve data consistency, and facilitate the transition from the laboratory to a preclinical setting.

THURSDAY, JUNE 13, 11:45 AM - 12:15 PM

ADVANCEMENTS IN HUMAN IPS CELL DERIVATION TECHNIQUES FROM BLOOD DERIVED CELL TYPES

Brad Hamilton, Director of Research & Development
Stemgent
ROOM 257 A-B

Stemgent is a leading provider of innovative research tools and reagents for the generation and manipulation of induced pluripotent stem (iPS) cells. Stemgent's non-integrating mRNA reprogramming platform incorporates a miRNA cocktail to enable fast and efficient iPS cell line establishment from diseased human fibroblasts that are typically refractory to other reprogramming methods. Here we present data highlighting internal advancements made in applying various reprogramming factor delivery systems to human blood derived cell types.

THURSDAY, JUNE 13, 11:45 AM - 12:15 PM

PLURISTEM MEDIUM: WEEKEND & HOLIDAY FREE CULTURE OF PLURIPOTENT HUMAN ES/IPS CELLS

Vi Chu
EMD Millipore Corporation
ROOM 253 A-C

We describe a serum-free and feeder-free medium called PluriSTEM that enables weekend-free culture of human pluripotent stem cells and allows for media exchanges every other day without compromising the morphology or long term functionality of the cells. Pluripotent cells could also be maintained as suspension cultures with minimal media changes. Pluripotent cells maintained in PluriSTEM medium could be transitioned into expandable neural progenitor cells and further directed towards specific neural subtypes.

THURSDAY, JUNE 13, 11:45 AM - 12:15 PM

EFFICIENT DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO SPECIFIC CELL TYPES USING FULLY DEFINED STEMDIFF™ REAGENTS

Michael Riedel
STEMCELL Technologies
ROOM 258 A-C

This tutorial will describe the efficient generation of specific cell types from human pluripotent stem cells (hPSCs) with a particular focus on the STEMdiff™ Definitive Endoderm Kit. Integration of STEMdiff™ differentiation systems with the TeSR™ line of pluripotent stem cell maintenance products, including TeSR™-E8™, and with downstream products for more specified cell lineages will also be discussed. Consistent performance and integration with existing STEMCELL products allows STEMdiff™ reagents to provide improved standardization of hPSC differentiation.

THURSDAY, JUNE 13, 12:30 PM - 1:00 PM

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

Oksana Sirenko, PhD
Molecular Devices, LLC.
ROOM 255

iPSC-derived cells show great promise for toxicity evaluation and disease modeling. We developed multi-parametric methods for toxicity assessment of pharmaceutical drugs and environmental agents. We will present phenotypic high-throughput assays using fast kinetic fluorescence, plate readers and high content analysis measuring the impact of pharmacological compounds on cardiac physiology, general and mechanism-specific hepatotoxicity, and neuronal development. We will describe methods for quantitative analysis of disease phenotypes and summarize results from testing compound libraries.

THURSDAY, JUNE 13, 12:30 PM - 1:00 PM

LARGE PARTICLE FLOW CYTOMETRY PROVIDES HIGH THROUGHPUT ANALYSIS AND AUTOMATION FOR CELL CLUSTERS (EBS, SPHEROIDS) AND ENCAPSULATED 3D CELL CULTURES.

Rock Pulak, PhD
Union Biometrica, Inc.
ROOM 254 A

Some cell types will naturally form cell clusters when grown in culture. This is true for embryonic stem cells (embryoid bodies), neural stem cells (neurospheres), certain solid tumors (tumorspheres) and others. This characteristic reflects programs of development that require cell-cell interactions and cell-substrate contacts. Union Biometrica discusses the use of their Large Particle Flow Cytometers for automating the multiparametric analysis and gentle intact dispensing of these types of cell clusters to wells of multi-well plates.

INNOVATION SHOWCASES (CONT.)

THURSDAY, JUNE 13, 12:30 PM - 1:00 PM

BUILDING BRIDGES FROM RESEARCH TO THERAPY: DEVELOPMENT OF A NOVEL REPROGRAMMING AND CULTURE SYSTEM FOR THE GENERATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS (HIPSCS) UNDER DEFINED CONDITIONS.

Thomas Fellner, PhD, MBA
Lonza
ROOM 257 A-B

Generation of hiPSCs in defined conditions is not only a prerequisite for the development of hiPSC-based therapies; it also has advantages in basic research applications. Here we describe a novel reprogramming and culture system that combines a modified episomal plasmid-based technology with a newly developed medium, matrix and passaging solution. This cGMP-compliant system enables the efficient and robust generation of hiPSCs under defined conditions supporting the utilization of these cells for clinical applications.

THURSDAY, JUNE 13, 12:30 PM - 1:00 PM

FLOW CYTOMETRY APPLICATIONS FOR ISOLATING AND ANALYZING COMPLEX HETEROGENOUS STEM CELL CULTURES

Christian Carson, PhD, Associate Director Stem Cell & Phosflow
BD Biosciences
ROOM 253 A-C

There is a need for robust and standardized flow cytometry tools and methods for stem cell research. Applications discussed in this tutorial will include:

- Cell surface marker screening identifies CD200 as marker for the isolation of hESC-derived neurons by FACS
- Tips and tricks for efficient cell sorting of hESC, hIPSC, NSC and neurons
- Quantification of cell proliferation, apoptosis and differentiation status of endoderm and ectoderm cell cultures by flow cytometry

THURSDAY, JUNE 13, 12:30 PM - 1:00 PM

COMPLETE AND FLEXIBLE CULTURE SYSTEMS TO SUPPORT HUMAN PLURIPOTENT STEM CELL RESEARCH

Erik Hadley
STEMCELL Technologies
ROOM 258 A-C

This tutorial will provide an overview of feeder-free culture systems commonly used to maintain human pluripotent stem cells (hPSCs). Simplified TeSR™-E8™ medium complements the established mTeSR™1-based system to allow flexibility in experimental design according to specific research needs, while maintaining consistent, high quality cell cultures. We will discuss convenient passaging protocols for these media as well as their suitability for specific applications including cellular reprogramming and downstream differentiation.

FRIDAY, JUNE 14, 11:45 AM - 12:15 PM

TOWARD A CLINICAL GRADE EXPANSION OF HUMAN MESENCHYMAL STEM CELLS: A COMPLETE SERUM-FREE, XENO-FREE CULTURE SYSTEM

Mark Weiss and David Fiorentini
Biological Industries Israel Beit Haemek Ltd.
ROOM 255

Human mesenchymal stem cells (hMSCs) serve as new promising tool for regenerative medicine and cell therapy with the advantageous over other stem cells types, mainly due to their safety record, multipotent characteristic, the broad variety of their tissue sources and for being immuno-privileged. The presentation addressed the ability of a developed xeno-free culture medium as well as solutions for attachment, dissociation, and freezing to support long-term expansion of multipotent hMSC suitable for clinical applications.

FRIDAY, JUNE 14, 11:45 AM - 12:15 PM

CLEANING UP THE MESS: ISOLATION OF PURE GERM LAYER DERIVATIVES

Sebastian Knoebel, Senior R&D Project Manager Stem Cells
Miltenyi Biotec GmbH
ROOM 254 A

Standardized differentiation of pluripotent stem cells requires careful monitoring and selection of the desired cell progeny. Surface biomarkers help defining the phenotypic status and enable straightforward magnetic selection of distinct cellular subsets. Here, we discuss tools that facilitate efficient differentiation into each of the three developmental germ layers. The use of purified cell populations guarantees predictable and reproducible results and makes the stem cell workflow amenable to automation and clinical translation.

FRIDAY, JUNE 14, 11:45 AM - 12:15 PM

GENERATING PARKINSON'S DISEASE MODELS USING FOOTPRINT-FREE REPROGRAMMING AND A NOVEL METHOD FOR NSC DIFFERENTIATION

Dr. Birgitt Schuele, MD, Assistant Professor, Clinical Molecular Geneticist, The Parkinson's Institute, Sunnyvale CA
Life Technologies
ROOM 257 A-B

The ability to generate iPSC-derived models that recapitulate phenotypes associated with Parkinson's Disease holds promise to accelerate understanding of the disease with a goal of finding a cure. By using non-integrating Sendai-virus (SeV) based CytoTune® reprogramming technologies, we have created patient-derived iPSCs of known genetic backgrounds, and used a novel method to create neural stem cells (NSCs) without going through the laborious process of EB formation followed by rosette isolation.

FRIDAY, JUNE 14, 11:45 AM - 12:15 PM

GAINING INSIGHTS INTO TUMOR HETEROGENEITY AND CANCER STEM CELL PATHWAYS BY USING HIGH-THROUGHPUT FLOW CYTOMETRY

Justin D. Lathia, PhD, Department of Cellular and Molecular Medicine, Lerner Research Institute, Cleveland Clinic
BD Biosciences
ROOM 253 A-C

Advanced cancers have a high degree of cellular heterogeneity and are often organized in a hierarchy with a self-renewing cancer stem cell (CSC) at the apex. A key issue for CSC studies has been the lack of consistent cell surface marker(s) for their prospective enrichment. To directly address this issue, we evaluated two advanced tumors (colorectal cancer and glioblastoma) using high throughput flow cytometry and have identified novel CSC pathways for future functional studies.

FRIDAY, JUNE 14, 11:45 AM - 12:15 PM

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

Dr. Lee Rubin, Harvard University
Nikon Instruments
ROOM 258 A-C

BioStation CT is an advanced, automated cell culture observation and documentation instrument. Ideally suited for live cell imaging, BioStation CT maintains a stable environment during microscopic image acquisition. This strategy and revolutionary new approach will lead to improvements in cell characterization methodologies and a better understanding of the reprogramming and differentiation process.

FRIDAY, JUNE 14, 12:30 PM - 1:00 PM

NOVEL, ANIMAL-FREE FIBRONECTIN AND COLLAGEN-I ECM MIMETIC SURFACES, ENABLING THE DEFINED EXPANSION OF HMSCS AND OTHER PRIMARY AND ADULT STEM CELL TYPES

Deepa Saxena Ph.D.
Corning Incorporated
ROOM 255

As the utility of primary and adult stem cells progresses to clinical settings, the need for animal-free and scalable cell culture becomes a requirement. To enable clinical researchers achieve this, Corning has developed a line of ECM Mimetic Cultureware to support collagen-I or fibronectin dependent cell types. Here, we demonstrate expansion and functionality of hMSCs, keratinocytes and endothelial colony forming cells on ECM Mimetic surfaces. Scalable and compatible with multiple media, ECM Mimetic Cultureware is a ready-to-use option where animal-free and defined conditions are desirable.

FRIDAY, JUNE 14, 12:30 PM - 1:00 PM

INTEGRATED SOLUTIONS FOR CELL MANUFACTURING AND SCREENING

Stefan Miltenyi, CEO and founder of Miltenyi Biotec
Miltenyi Biotec GmbH
ROOM 254 A

Pluripotent stem cells hold great promise for cellular therapies and use in drug and toxicity screenings. Manufacturing of well-defined cell populations for therapeutic and screening purposes requires scalable workflows that allow closed-system or high-throughput processing. We have developed new platforms and instruments for single as well as multiple-parameter cell sorting and analysis. They help streamlining GMP-compliant cell manufacturing and integrate seamlessly into common liquid handling systems.

FRIDAY, JUNE 14, 12:30 PM - 1:00 PM

TRANSCRIPTIONAL AND EPIGENETIC DYNAMICS DURING SPECIFICATION OF HUMAN PLURIPOTENT STEM CELLS

Alex Meissner, PhD. Associate Professor of Stem Cell and Regenerative Biology, Harvard University, Broad Institute, Cambridge, MA
Life Technologies
ROOM 257 A-B

The genomic distribution of DNA methylation and diverse chromatin modifications within any cell reflects and partly determines its identity. Taking advantage of the orchestrated regulatory dynamics during the directed differentiation of human pluripotent cells we investigated the early events that contribute to the specification of all three germ layers. By carefully dissecting these initial transcriptional and epigenetic dynamics we may be able to better predict differentiation propensities and derive therapeutically relevant tissue types more efficient and safely.

FRIDAY, JUNE 14, 12:30 PM - 1:00 PM

FLOW CYTOMETRY ANALYSIS OF CELLULAR AND FUNCTIONAL PHENOTYPES OF DISEASE IN PATIENT SPECIFIC HUMAN IPSC DERIVED CELLS

Paulina Ordonez, MD, Assistant Professor of Pediatrics, Department of Pediatric Gastroenterology, Hepatology and Nutrition, University of California San Diego, La Jolla, CA
BD Biosciences
ROOM 253 A-C

Induced pluripotent stem cell (IPSC) technology is a powerful tool to model human disease in relevant cell populations. However, accurate detection of disease phenotypes will require analysis of sufficient patient lines to account for variation related to genetic background. In these regards, traditional methods of phenotypic characterization can be costly, time-consuming and not quantitative enough. Therefore, we have adapted several flow cytometry assays to rapidly screen for pathologic cellular and functional phenotypes in IPSC-derived cells.

FRIDAY, JUNE 14, 12:30 PM - 1:00 PM

TWO UNIQUE TECHNOLOGIES FOR NEURAL STEM AND INDUCED PLURIPOTENT CELL LINES: THE SYNTHEMAX® SELF-COATING SYNTHETIC SUBSTRATE AND FLOWELL™ A PERFUSION-BASED TECHNOLOGY FOR CONTINUOUS CULTURING.

Mark Rothenberg Ph.D.
Corning Incorporated
ROOM 258 A-C

This tutorial will introduce two unique technologies for stem cell research. The FloWell, allows for continuous perfusion of media and reagents from a Source Well to the Cell Well over a 3-day period in a 6-well format, removing the need for manual media exchanges. The second focuses on the Synthemax self-coating substrate. Both technologies are ideal for culturing stem cell lines including neural stem and induced pluripotent cell lines. The discussion will introduce both technologies and discuss relevant data.

INNOVATION SHOWCASES (CONT.)

SATURDAY, JUNE 15, 11:30 AM - 12:00 PM

HARNESSING THE POWER OF SINGLE CELL GENOMICS TO ACCELERATE STEM CELL DISCOVERY, PART I

Fluidigm Corporation

ROOM 255

Join us for lunch and hear scientific presentations from thought leaders in stem cell who are applying single cell approach to their search. Presentations will highlight recent advances in stem cell research, and highlight new technologies and application that promise a deeper view into underlying biological mechanisms.

SATURDAY, JUNE 15, 11:30 AM - 12:00 PM

STRETCHING EVERY CELL COUNTS: OPTIMIZING THE CULTURE OF PPSC USING NOVEL INSULIN FREE MEDIA

Rick. I. Cohen, Ph.D., Rutgers

PeproTech

ROOM 254 A

This tutorial will introduce PeproTech's PeproGrow-hESC, a new Pluripotent Stem Cell Media with companion products and the proper techniques to optimize the growth iPSC or hESC. Our presentation will include, but not limited to; the use of this novel media during iPSC generation; Cryopreservation and Thawing, Growth on Matrigel™ and/or VitroGrow-hESC (recombinant full length human Vitronectin), and ability to passage cells at wildly high dilution factors.

SATURDAY, JUNE 15, 11:30 AM - 12:00 PM

ENGINEERING OF STEM CELLS: TALEN™-MEDIATED APPROACH

Catharina Ellerström

Cellectis bioresearch

ROOM 257 A-B

Genetic modification of hPS cells has been technically challenging. These difficulties made hPS cells less amenable to genetic manipulation. Today, robust culture protocols for culture of hPS cells are available and the efficiency of targeted engineering is greatly enhanced by the use of TALEN™, the next generation gene editing tools. We will present how TALEN™ can be employed on hPSC in order to further facilitate disease modeling and drug screening.

SATURDAY, JUNE 15, 11:30 AM - 12:00 PM

NOVEL CLONALLY-PURIFIED PURESTEM EMBRYONIC PROGENITORS AND CUSTOMIZABLE HYSTEM® HYDROGEL SUBSTRATES FOR CELL CULTURE AND DELIVERY

Tom Zarembinski, PhD, and Jeffrey Janus, CEO

ES Cell International, a subsidiary of BioTime, Inc.

ROOM 253 A-C

This tutorial focuses on how novel PureStem human embryonic progenitors overcome significant challenges in hESC or adult stem-cell-based regenerative medicine and open new opportunities for research. We present their derivation, their unique characteristics, their link with the powerful LifeMap Discovery database and illustrate their chondrogenic behavior in HyStem hydrogels. Finally, we present the HyStem hyaluronan-based hydrogel platform as a customizable platform for cellular microenvironments and for implantation for preclinical animal experiments and future clinical applications.

SATURDAY, JUNE 15, 12:15 PM - 12:45 PM

HARNESSING THE POWER OF SINGLE CELL GENOMICS TO ACCELERATE STEM CELL DISCOVERY, PART II

Fluidigm Corporation

ROOM 255

Join us for lunch and hear scientific presentations from thought leaders in stem cell who are applying single cell approach to their search. Presentations will highlight recent advances in stem cell research, and highlight new technologies and application that promise a deeper view into underlying biological mechanisms.

SATURDAY, JUNE 15, 12:15 PM - 12:45 PM

LIFEMAP DISCOVERY, A POWERFUL DATABASE LINKING EMBRYONIC DEVELOPMENT WITH STEM CELL RESEARCH

Dr. Ariel Rinon

LifeMap Sciences, Inc. a subsidiary of BioTime, Inc.

ROOM 253 A-C

LifeMap Discovery is a powerful database which links embryonic development with stem cell research. We will describe its structure and present specific tissue development towards their matured fates, at anatomical and cellular levels, with their molecular signatures and signaling cascades. We will demonstrate the application of developmental information to the stem cell world, how to identify potential progenitor fates based on gene markers, and how to optimize desired cell fates through differentiation.

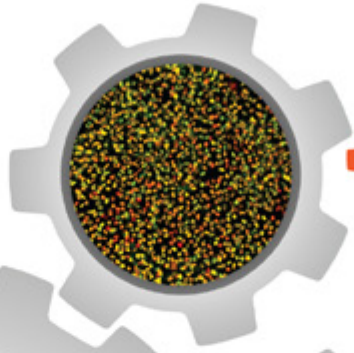
AUTHOR INDEX

NOTES

Take the "if" out of "diff"™

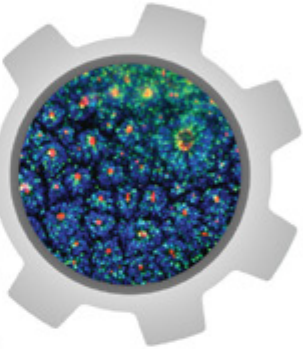
Reduce Variability by Differentiating with STEMdiff™

Even the most detailed and rigorously-followed protocols can still lead to inconsistent differentiation. Use STEMdiff™ for reproducible differentiation across multiple ES cell and iPS cell lines, and support your research with the world's most complete system of defined reagents for human pluripotent stem cells.



New

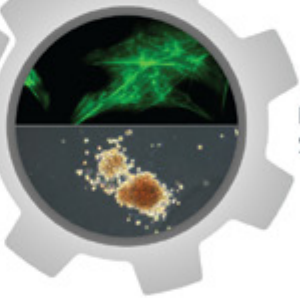
Endodermal Lineages:
STEMdiff™ Definitive Endoderm Kit



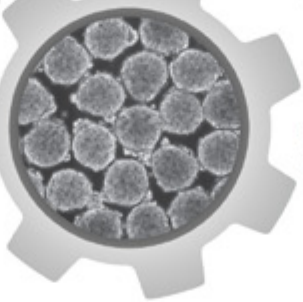
Neural Lineages:
STEMdiff™ Neural Induction Medium

STEMdiff™

Defined, serum-free products for reliable hPSC differentiation



Mesodermal Lineages:
STEMdiff™ APEL™ Medium



Embryoid Body Formation:
AggreWell™

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