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#ISSCR2020

ISSCR 2020 VIRTUAL

THE GLOBAL STEM CELL EVENT

23–27 JUNE 2020

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ISSCR INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

Dear Colleagues,

Welcome to ISSCR's first virtual meeting! Thank you for joining us for this vibrant digital experience. While the format has changed from what we originally envisioned, what has not altered is your Society's commitment to the global stem cell community. We continue to focus on bringing exceptional educational programming and networking opportunities to the stem cell field across the world. We believe we have achieved this with ISSCR 2020 Virtual!

While much has changed this year, our commitment to constantly innovate has not. This year we created a Stem Cell Research & COVID-19 session that is particularly timely and embraces how rapidly research is moving in our field to understand and combat this virus. This session is part of an ongoing, multi-faceted strategy of the society to share resources for addressing COVID-19.

You also will notice the incorporation of four themes woven throughout the meeting as they relate to individual cell types, tissues, and disease: Tissue Stem Cells and Regeneration, Cellular Identity, Modeling Development and Disease, and Clinical Applications, and you will see them noted next to sessions on the meeting platform and in this program.

Popular professional development and career-building programs are incorporated into ISSCR 2020 Virtual including the Women in Science panel discussion. This session will gather leaders in STEM to share insights and provide guidance on solving challenges many women face throughout their training and careers and all voices are invited to contribute to this conversation. A new Business of Discovery workshop is an excellent addition to the program, reflecting the growth and maturation of our field as well as the possibilities of new treatments on the horizon.

Unique to 2020, abstract submitters will be able to present their posters through a five-minute audio presentation integrated in our abstract platform. We encourage you to take full advantage of exploring those posters and interacting with your colleagues around their research.

We know that ISSCR 2020 Virtual never would have been possible to achieve without the commitment, support, and flexibility of our world-renowned speakers, our co-sponsor, Harvard Stem Cell Institute (HSCI), the dedicated ISSCR staff, and the many exhibitors, sponsors, and advertisers that patiently worked with the ISSCR to support the meeting in this digital format. We appreciate the confidence you have in the society.

We hope you will seek out the myriad opportunities this year to share research, discover new scientific approaches, expand your network of colleagues, and initiate your next collaboration. For a full 30 days, you will have the opportunity to enrich your understanding of the field through our digital event platform. Each year, this annual meeting inspires us with new ideas and reveals the significant advances taking place across the breadth of stem cell science. This year, during this extraordinary time fighting a new pandemic, will be no different.

Sincerely,

Deepak Srivastava
ISSCR President

Kevin Eggan
Program Chair

HSCI | HARVARD STEM CELL INSTITUTE

Welcome to the 2020 Annual Meeting of the International Society for Stem Cell Research.

We are at a pivotal moment in biomedicine, when stem cell technologies are moving rapidly from bench to clinic, changing the very paradigm of drug discovery and therapeutic development. Among your colleagues here at ISSCR 2020, you will see hundreds of people whose basic research has rocketed into startups, hospitals, and pharmaceutical companies. Twenty years ago, many of their ideas might have been considered outlandish; now they are the norm, and expectations have started to outpace capacity.

Disruptive change in stem cell medicine is not on the horizon; it is upon us. But as we all know, disruption is a double-edged sword. Our goal is to benefit patients and their communities, but unless everyone is brought along with us on the journey — scientists, clinicians, health care providers, payers, regulators, journalists, and patients — we could see many worthy endeavors fail.

In talking about disruption, we would be remiss not to acknowledge the fact that this is the first virtual ISSCR Annual Meeting, brought about as a result of the COVID-19 pandemic. During this time, it is more critical than ever that we use the power and insights of our science, and collaborate across borders and boundaries to seek solutions. ISSCR has been a leader in organizing collaborative teams to solve pressing issues during this public health crisis, and we should all take advantage of this Annual Meeting as an opportunity to forge connections and work together to make a real difference. As directors of HSCI, we have the great privilege of working at the center of a network of researchers, clinicians, investors, policy experts, bioethicists, and business leaders. What we have seen clearly over the past 15 years is that convening all these experts in one place has immense value, not just as intellectual fuel for great science but for human progress and societal good. Our Boston-area teaching hospitals, universities, pharma and biotech sectors, and investment communities all converge to create a unique landscape for discovery and translation. In this setting of intense innovation and demand for clinical delivery, the need for novel therapies has put unprecedented pressure on manufacturers. Responding to this need, Harvard, MIT, high-profile industry partners, and hospitals recently joined forces to launch a new non-profit facility for cell and gene therapy manufacturing and innovation to relieve the pressure. We look forward to the development of more of these types of shared resources across our global community, as they are a critical means to accelerate the field by leveraging valuable intellectual and financial capital.

You are part of something great today. Right now, at ISSCR 2020, you have a voice in changing research and medicine in ways that benefit everyone. By asking questions, participating in sessions outside your area of expertise, and talking to people you might not encounter in any other setting, you will make this conference relevant and memorable, and contribute to moving our field forward. Thank you for being here.

Douglas Melton, Ph.D.
Xander University Professor of Stem Cell and Regenerative Biology at Harvard University, Howard Hughes Medical Institute Investigator, Co-Director of HSCI

David Scadden, M.D.
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All participants are expected to treat others with respect and consideration during the virtual event. Attendees are expected to uphold standards of scientific integrity and professional ethics.

These policies comprise the Code of Conduct for ISSCR Meetings and apply to all attendees, speakers, exhibitors, staff, contractors, volunteers, and guests at the meeting and related events.

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ISSCR PUBLIC SERVICE AWARD

The ISSCR Public Service Award is given in recognition of outstanding contributions of public service to the fields of stem cell research and regenerative medicine.

The 2020 recipient is Susan L. Solomon, JD, chief executive officer and founder of The New York Stem Cell Foundation (NYSCF). Founded in 2005, NYSCF is one of the largest nonprofit organizations dedicated to stem cell research projects and is well known for breakthroughs in stem cell research. Under Solomon's leadership, NYSCF has raised and invested more than $350 million for stem cell research at the NYSCF Research Institute, through its extramural funding programs, and for educating the next generation of scientists. Ms. Solomon has made significant contributions to the field of stem cell research, has been an advocate for diversity and inclusion of young scientists and women in the field and a champion of scientific excellence.

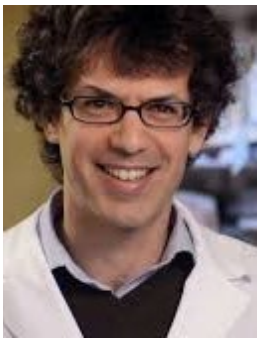
The award will be presented in Plenary I on Wednesday 24 June, 4:00 PM EDT.

ISSCR ZHONGMEI CHEN YONG AWARDS FOR SCIENTIFIC EXCELLENCE

The ISSCR Zhongmei Chen Yong Awards for Scientific Excellence recognize scientific excellence and economic need for attendees who submit abstracts for the ISSCR Annual Meetings. Thanks to the generosity of Chen Yong, prominent Chinese businessman and chairman of the Zhongmei Group, Trainee members from around the world are able to attend the ISSCR Annual Meeting to present their science at the largest global stem cell event.

The award will be presented in Plenary I on Wednesday 24 June, 4:00 PM EDT.

ISSCR DR. SUSAN LIM AWARD FOR OUTSTANDING YOUNG INVESTIGATOR



The ISSCR Dr. Susan Lim Award for Outstanding Young Investigator recognizes exceptional achievements by an ISSCR member and investigator in the early part of their independent career in stem cell research.

The 2020 recipient is Allon Klein, PhD, Associate Professor, Systems Biology, Harvard Medical School, Boston. As a multidisciplinary scientist, Dr. Klein has made ground-breaking contributions to the fields of stem cell and developmental biology. Those achievements range from the formulation of novel approaches to understand and further explain adult stem cell self-renewal, to the invention and application of an innovative cell-isolation and analysis system (inDrop) to transcriptionally profile individual cells in very high number. These approaches have allowed Dr. Klein to identify conserved patterns of stochastic adult stem cell fate across a range of epithelial tissues (e.g. skin and intestine) and cellular differentiation hierarchies during early embryonic development and in cycling adult tissues.

Dr. Klein will present his research in Plenary II, Machine Learning and Computation, on Thursday, 25 June, 9:00 AM EDT.

ISSCR MOMENTUM AWARD

The ISSCR Momentum Award recognizes the exceptional achievements of an investigator whose innovative research has established a major area of stem cell-related research with a strong trajectory for future success.

The 2020 recipient is Mitinori Saitou, MD, PhD, Kyoto University, Japan. Dr. Mitinori Saitou has focused his career on understanding mammalian germ cell development in vitro. His laboratory was the first to succeed in reconstituting the mouse germ-cell specification pathway in culture and to generate functional male and female gametes from pluripotent stem cells. Dr. Saitou has since expanded on these groundbreaking achievements by improving germ-cell technology, dissecting the molecular mechanisms, and providing fundamental insights on human germ cell development and reproduction.

Dr. Saitou will present his research in Plenary IV, Dissecting Organogenesis on Friday, 26 June, 4:00 PM EDT.

ISSCR TOBIAS AWARD LECTURE

The ISSCR Tobias Award Lecture is supported by the Tobias Foundation, and recognizes original and promising basic hematology research and direct translational or clinical research related to cell therapy in hematological disorders.

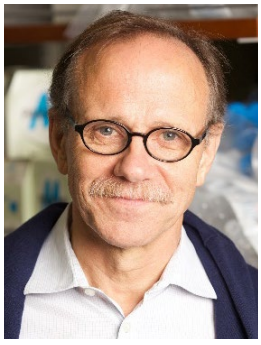
The 2020 recipient is Margaret A. (Peggy) Goodell, PhD, Chair of the Department of Molecular and Cellular Biology and Director of the Stem Cell and Regenerative Medicine Center at Baylor College of Medicine, Houston, Texas, U.S.

Dr. Goodell is an internationally recognized leader in the field of hematopoietic stem cell research and made early seminal advances in analyzing hematopoietic stem cell quiescence. She was the first to identify the importance of DMMT3 as a key epigenetic regulator of normal hematopoietic stem cell function and has since contributed major insights into our understanding of the key role of epigenetic perturbations in hematologic malignancies.

Dr. Goodell will deliver the ISSCR Tobias Award Lecture in Plenary V, Stem Cells and Aging on Friday, 26 June, 11:00 PM EDT.

JOIN US ON SATURDAY, 27 JUNE FOR THE PRESENTATION OF THE 2020 ISSCR ACHIEVEMENT AWARD

ISSCR ACHIEVEMENT AWARD



The ISSCR Achievement Award recognizes the transformative body of work of an investigator that has had a major impact on the field of stem cell research or regenerative medicine.

The 2020 recipient is Fred H. Gage, PhD, president of the Salk Institute for Biological Studies and the Vi and John Adler Chair for Research on Age-Related Neurodegenerative Disease in Salk's Laboratory of Genetics, La Jolla, California.

Dr. Gage's influential work in the fields of stem cell biology, neuroscience, and human evolution have resulted in groundbreaking discoveries that have broad implications for human health and encompassing advances that may lead to treatments for devastating diseases including Alzheimer's Disease, Parkinson's Disease, and mental health disorders.

Some of Dr. Gage's most notable scientific accomplishments include work using stem cell technologies that revealed that neurons generated from skin cells of individuals with schizophrenia are dysfunctional in early developmental stages. He applied this approach to pave new ways to detect and possibly treat the mental health disease sooner. Dr. Gage and his colleagues also discovered that neurons are generated in the brain throughout life, and that exercise can increase the brain's ability to generate more new neurons. Further, Dr. Gage and collaborators discovered that the genomic structures of individual neurons differ from each other more than was expected, which provides a model to understand evolution and disease.

Dr. Gage will present his research in Plenary VI, Reprogramming and Regeneration on Saturday, 27 June, 8:00 AM EDT.

CONGRATULATIONS TO THE 2020 ISSCR ZHONGMEI CHEN YONG AWARD WINNERS

2020 ISSCR ZHONGMEI CHEN YONG AWARDS FOR SCIENTIFIC EXCELLENCE

Supported by Chen Yong and the Zhongmei Group, the ISSCR Zhongmei Chen Yong Awards recognize scientific excellence and economic need for trainees who submit abstracts and present at the ISSCR Annual Meeting.

Deniz Aksel
Juan Alvarez
Nur Shabrina Amirruddin
Unain Ansari
Chen Atzmon
Anna Baulies
Jonathan Bayerl
Belin Selcen Beydag-Tasöz
Juliana Borsoi
Sarah Bowling
Vincenzo Calvanese
Seoyoung Choi
Joana Claudio Ribeiro
Katie Cockburn
Devanjan Dey
Lika Drakhlis
Ramy Elsaid
Camila Fernandes
Samuele Ferrari
Corey Garyn
Jianyun Ge
Zaniar Ghazizadeh
Ge Guo
Sean Harrison
Takeshi Hatani
Deirdre Hoban
Sharif Iqbal
Ryutaro Ishii
Yusuke Ito
Debabrata Jana
Adrian Janiszewski
Geraldine Jowett
Hanuman Kale
Chun-Kai Kang
Sathya Kannan
Suraj Kannan
Evgenii Kegeles
Oisín King
Marine Krzisch
Choon-Soo Lee
Shiri Levy
Elyad Lezmi
Peng Li
Wanlu Li
Bo Li
Jisun Lim
Gabriel Linares
Xiaodong Liu
Hsiao-Yun Liu
Raffaella Lucciola
Stephanie Luff
Miyeko Mana
Pietro Giuseppe Mazzara
Paul McKeever
César Meléndez
Julia Menges
Michela Milani
Tomohiro Minakawa
Matteo Maria Naldini
Natasha Hui Jin Ng

Peter Nicholls
Reina Ooka
Shinsuke Otagiri
Kathyani Parasram
Sachin Patel
Federico Pecori
Gonzalo Perez-Siles
Michael Pokrass
Qi Qiu
Norikazu Saiki
Maria Salazar-Roa
Giorgia Scapin
Enakshi Sinniah
Erin Slatery
Kyle Stokes
Dawei Sun
Sabriya Syed
Nana Takenaka-Ninagawa
Thai Tran
Larissa Traxler
Tomoya Uchimura
Silvia Velasco
Adrian Veres
Li-Tzu Wang
Samuel Wattrus
Yonglong Wei
Connor Wiegand
Ivo Woogeng
Siqin Wu
Yan Yao
Shu-Chi Yeh
Atilgan Yilmaz
Maria Patapia Zafeiriou
Roni Zaken Gallily
Wenshu Zeng
Ke Zhang
Lingling Zhang
Xiaoyi Zhang
Liu Zhiwen
Lili Zhu
Han Zhu
Yuqing Zhu

LAWRENCE GOLDSTEIN POLICY FELLOWS

ISSCR's Lawrence Goldstein Policy Fellowship, named for long-time ISSCR member and policy stalwart Lawrence Goldstein, is designed to train scientists to become stem cell policy and regulatory advocates. Recipients receive complimentary meeting registration along with hands-on experience in policy and advocacy work. ISSCR's policy fellows are:

Kirstin Matthews
Mohamed Abou El-Enein
Zubin Master

2020 ISSCR MERIT ABSTRACT AWARDS

The ISSCR recognizes outstanding abstracts with the ISSCR Merit Abstract Awards. These awards are given to ISSCR trainee members who have submitted distinguished abstracts as judged by the ISSCR 2020 abstract reviewers.

Deniz Aksel

Juan Alvarez

Nur Shabrina Amirruddin

Chen Atzmon

Sarah Bowling

Vincenzo Calvanese

Joana Claudio Ribeiro

Katie Cockburn

Samuele Ferrari

Corey Garyn

Zaniar Ghazizadeh

Ge Guo

Takeshi Hatani

Deirdre Hoban

Debabrata Jana

Adrian Janiszewski

Geraldine Jowett

Sathya Kannan

Suraj Kannan

Evgenii Kegeles

Marine Krzisch

Shiri Levy

Peng Li

Xiaodong Liu

Raffaella Lucciola

Stephanie Luff

Miyeko Mana

Paul McKeever

Tomohiro Minakawa

Matteo Maria Naldini

Peter Nicholls

Sachin Patel

Qi Qiu

Giorgia Scapin

Erin Slatery

Thai Tran

Larissa Traxler

Tomoya Uchimura

Silvia Velasco

Adrian Veres

Li-Tzu Wang

Samuel Wattrus

Yonglong Wei

Yan Yao

Shu-Chi Yeh

Atilgan Yilmaz

Wenshu Zeng

Ke Zhang

Lingling Zhang

Lili Zhu

STEVEN FINKBEINER, *GLADSTONE INSTITUTES, UNIVERSITY OF CALIFORNIA, SAN FRANCISCO, USA*

Dr. Steven Finkbeiner's research laboratory has focused on understanding the cause-and-effect relationships and finding treatments for Amyotrophic lateral sclerosis, Frontotemporal dementia, and Alzheimer's, Parkinson's and Huntington's disease. The lab pursues these goals through the use of genomics, induced pluripotent stem cells, artificial intelligence and robotic microscopy, which they invented. He has successfully partnered with industry for development of key translation discoveries into drug discovery programs. Dr. Finkbeiner trained as a neurologist and neuroscientist at Yale University, Harvard University and the University of California San Francisco. He is the Director of the Center for Systems and Therapeutics and the Taube/Koret Center for Neurodegenerative Disease Research at Gladstone Institutes and Professor of Neurology and Physiology at the University of California San Francisco.

ERIC OLSON, *UNIVERSITY OF TEXAS SOUTHWESTERN, USA*

Dr. Eric Olson is Robert A. Welch Distinguished Professor and Chair of Molecular Biology at UT Southwestern Medical Center where he is currently Professor and Chair. He and his trainees discovered many of the key genes and mechanisms that control heart and muscle development and disease. Most recently, he has pioneered a new strategy for correction of Duchenne muscular dystrophy using CRISPR gene editing. Dr. Olson is a member of the U.S. National Academy of Sciences, the National Academy of Medicine, and the American Academy of Arts and Sciences. He has co-founded multiple biotechnology companies to design new therapies for heart muscle disease.

AVIV REGEV, *BROAD INSTITUTE, MIT, HHMI USA*

Computational and systems biologist Dr. Aviv Regev is a professor of biology at MIT, Broad Institute core member and chair of the faculty, Howard Hughes Medical Institute Investigator, and founding co-chair of the organizing committee of the Human Cell Atlas (HCA). She studies how complex molecular circuits function in cells and between cells in tissues, and how to infer this using algorithms from the vast number of biological possibilities. Her lab has pioneered foundational experimental and computational methods in single-cell genomics, working toward greater understanding of the function of cells and tissues in health and disease, including autoimmune disease, inflammation, and cancer. She is a Fellow of the International Society of Computational Biology and was recently elected to the National Academy of Sciences.

ERNEST MCCULLOCH LECTURE

Wednesday 24 June, Plenary I

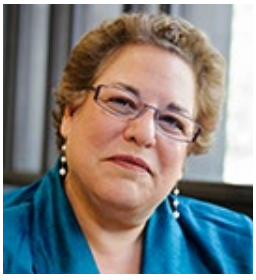
FIONA WATT, *KING'S COLLEGE LONDON, UK*

Dr. Fiona Watt obtained her first degree from Cambridge University and her DPhil from the University of Oxford. She was a postdoc at MIT, where she first began studying differentiation and tissue organization in mammalian epidermis. She led research groups at the Kennedy Institute for Rheumatology and the CRUK London Research Institute. She helped to establish the CRUK Cambridge Research Institute and the Wellcome Trust Centre for Stem Cell Research. In 2012 she moved to King's College London to found the Centre for Stem Cells and Regenerative Medicine. She is internationally recognized for her work on stem cells and their interactions with the niche in healthy and diseased skin. She is currently on secondment as Executive Chair of the UK Medical Research Council.

ANNE MCLAREN LECTURE

Saturday 27 June, Plenary VI

R. ALTA CHARO, *UNIVERSITY OF WISCONSIN, USA*



R. Alta Charo, JD is the Warren P. Knowles Professor of Law at the University of Wisconsin, and the 2019-2020 Berggruen Fellow at the Stanford Center for Advanced Studies in Behavioral Sciences. She is a member of the National Academy of Medicine, where she co-chaired (both times with Richard Hynes of MIT) the committee on guidelines for embryonic stem cell research and later the committee on genome editing. Charo was a member of President Clinton's National Bioethics Advisory Committee and a senior policy advisor at FDA during the Obama Administration, and earlier in her career she worked at the congressional Office of Technology Assessment and the US Agency for International Development. Her work focuses on the intersection of law, policy and bioethics.

PATIENT ADVOCATE

Saturday 27 June, Plenary VII

MATTHEW MIGHT, *UNIVERSITY OF ALABAMA AT BRIMINGHAM, USA*

Dr. Matthew Might has been the Director of the Hugh Kaul Precision Medicine Institute at the University of Alabama at Birmingham (UAB) since 2017. At UAB, Dr. Might is the Hugh Kaul Kaul Endowed Chair of Personalized Medicine, a Professor of Internal Medicine and a Professor of Computer Science. From 2016 to 2018, he was a Strategist in the Executive Office of the President in The White House. And, in 2015, he joined the faculty of the Department of Biomedical Informatics at the Harvard Medical School. Might is co-founder and Chief Scientific Officer of NGLY1.org, and he was a co-founder and Scientific Advisor to Pairnomix. Q State Biosciences acquired Pairnomix in October 2018 and he remains a Scientific Advisor.

JOHN MCNEISH LECTURE

Saturday 27 June, Plenary VII

BRIAN WAINGER, *HARVARD MEDICAL SCHOOL AND MASSACHUSETTS*

Dr. Brian Wainger is Assistant Professor of Anesthesiology and Neurology at Harvard Medical School and a physician scientist at Massachusetts General Hospital engaged in clinical practice, translational science, and clinical trials spanning diseases of the motor and sensory nervous systems. He received his undergraduate degree from Princeton and MD/PhD degrees from Columbia. He completed Neurology Residency and Pain Medicine Fellowship at Massachusetts General Hospital, and a Masters in Clinical Investigation at Harvard Medical School. His lab focuses on modeling motor and sensory neuron diseases using stem cell technology. He is Faculty at Harvard Stem Cell Institute and Associate Member of the Broad Institute. He received a K08 Career Development award, NIH New Innovator award, and is a New York Stem Cell Foundation – Robertson Investigator.

KEYNOTE ADDRESS

Saturday 27 June, Plenary VII

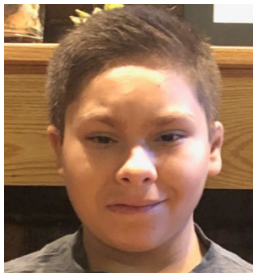
SEKAR KATHIRESAN, *VERVE THERAPEUTICS, USA*

Dr. Sakar Kathiresan is co-founder and CEO of Verve Therapeutics. He is a preventive cardiologist who has made groundbreaking discoveries of cardioprotective genetic mutations. Before joining Verve, he served as director of the MGH CGM, was the Ofer and Shelly Nemirovsky MGH Research Scholar, served as director of the Cardiovascular Disease Initiative (Broad Institute), and was professor of medicine at HMS. Dr. Kathiresan's research focuses on understanding the inherited basis for blood lipids and myocardial infarction; using these insights to improve preventive cardiac care. He was honored with a Distinguished Scientist Award from the AHA and the 2018 Curt Stern Award from the ASHG. Dr. Kathiresan graduated summa cum laude with a B.A. in history from the University of Pennsylvania and received his MD from HMS.

PATIENT ADVOCATE

DANIEL CONDE

ON DEMAND



In March 2004, at two weeks old, Daniel Conde was diagnosed with Fanconi Anemia, a genetic disorder that mainly affects the bone marrow. Daniel was three days shy of his 8th birthday when he had his stem cell transplant at Boston Children's Hospital, his donor was his sister Ava. Daniel is now a 10th grade IT student at Shawsheen Technical High School. He loves to play video games, swim, play golf, share his experience with medical students and spend time with his family.

ISSCR members gain access to global influencers in the stem cell field, career resources that open doors, and powerful scientific collaborations.

ISSCR

Membership

To join now or more information, please visit **www.isscr.org/membership**

What better way to nurture your research and career than networking? ISSCR 2020 Virtual provides many opportunities for scientists in all stages of their careers to exchange insightful and relevant advice that helps advance their research and lab work. ISSCR 2020 Virtual offers various avenues to help scientists foster and strengthen their professional networks.

EXHIBIT HALLS

Network with industry professionals from over 40 exhibiting companies. Explore the possibilities on page 80. Take advantage of casual networking with exhibitors at their virtual booths.

Exhibit Hall Hours:

Wednesday, 24 June	08:30 – 17:30 EDT
Thursday, 25 June	11:00 – 19:00 EDT
Friday, 26 June	13:00 – 21:00 EDT
Saturday, 27 June	07:30 – 13:00 EDT

POSTER SESSIONS

Click the Poster Hall in the virtual meeting Lobby to access over 1,000 posters and view the schedule of poster presenters. Chat with poster presenters during their poster presentation time.

Poster Session I: Thursday, 25 June: 14:00 – 16:00 EDT

Poster Session II: Thursday, 25 June: 22:00 – 23:59 EDT

Poster Session III: Friday, 26 June: 05:00 – 07:00 EDT

Poster Session IV: Friday, 26 June: 14:00 – 16:00 EDT

Poster Session V: Saturday, 27 June: 05:00 – 07:00 EDT

Poster Session VI: Saturday, 27 June: 12:00 – 14:00 EDT

ISSCR FORUMS

ISSCR 2020 Virtual attendees will have the opportunity to engage in conversation through virtual discussion forums. Each forum will include several chat rooms where attendees can meet socially or to discuss scientific topics. Attendees can access the ISSCR Forums through the virtual meeting lobby or in the meeting navigation bar.

THE SCIENTIFIC FORUM

Sponsored By:

STEMCELL™ TECHNOLOGIES

Five chat rooms will house conversations on a variety of topics. Attendees are encouraged to join these chats to continue conversations started in the scientific sessions. Bring your questions and comments about the research you have seen presented, discuss your own research, consider new collaborations, reach out to new people and find old friends. This is your space to connect with your stem cell science peers. Come join the conversation.

THE JUNIOR INVESTIGATOR FORUM

Sponsored By:

T-CiRA

Junior Investigators are invited to join a hosted chat on a variety of topics during the meeting, or drop in any time to join an open chat to connect socially, to collaborate scientifically, or to just relax and browse the conversations between sessions.

THE CONVERSATION FORUM

Sponsored By:

novo nordisk®

German Stem Cell Network
The German Stem Cell Network (GSCN) invites German scientists to join them to get information on what is new in the GSCN and discuss your needs and wishes.

Computational Stem Cell Biology
Computational biology is an emerging specialty within the Stem Cell Sciences. Computational stem cell biology invents and applies mathematical approaches to classifying stem cells, predicting cell behaviour, and designing reprogramming strategies or even new cell types. This forum is an opportunity to meet others working in the field, discuss opportunities and challenges for computational stem cell sciences, and highlight resources and standards that we want to work to as a discipline.

Industry Scientists Networking
The ISSCR Industry Committee invites interested attendees to join them to exchange and discuss various industry-related topics, including new developments, collaborations and potential career paths.

Policy, Ethics, and Regulatory Issues
Are policy, ethics, or regulatory issues impacting your research? Join us to discuss these issues with ISSCR leaders and find out about the ISSCR's advocacy program.

Meet the Editors of *Stem Cell Reports*
Do you have a paper nearly ready for submission? Are you looking to find more information about the ISSCR's official journal, *Stem Cell Reports*? Meet the editors to discuss your work, our recently published issues, and any topics of interest around open-access, scientific publishing.

JOB MATCH

The Job Match opportunity connects academic and industry employers with scientists looking for their next position. Annual meeting attendees can opt-in to Job Match at any time, at no additional fee. Browse the online Job Match Directory for job match profiles that meet your interests. Employers can view details of potential candidates and view CVs and presentation and poster information. Job seekers can view open position details. Then contact your favorites via email before ISSCR 2020 Virtual and/or connect virtually during the live meeting at a poster presentation or via direct messaging.

EARLY-CAREER GROUP LEADER PANEL

WEDNESDAY, 24 JUNE 18:00-19:30 EDT

Re-Defining the Success of Your Lab in Changing Times: Productivity, Motivation and Enrichment

Even prior to lab shutdowns, you might have often been awake at night feeling anxious about the success of your trainees and your projects. With bench research and much of science at a standstill, how can PIs regain perspective and purpose to re-define a thriving lab? This event is organized for early career PIs to learn tools and tips from more senior ISSCR scientists about how to get the most out of your lab. We will address topics such as setting up a strong lab culture from the beginning, enriching the lab experience to make your team the most productive, and recruiting the best people to improve motivation and promote strong research. Additionally, this event is meant to foster conversation about how to support, motivate, and train lab members under these exceptional circumstances. Come join us to learn, share, and discuss different recipes for a successful lab.

This session is designed for ISSCR members who are early-career research group leaders (principal investigators or junior faculty for eight or fewer years).

MODERATOR

Joshua Currie
Wake Forest University, USA

Co-chair, ISSCR Junior Investigators Committee

PANELISTS

Valentina Greco
Yale University School of Medicine, USA

Konrad Hochedlinger
Massachusetts General Hospital and Harvard University, USA

Joanna Wysocka
Stanford University, USA

WOMEN IN SCIENCE PANEL

FRIDAY, 26 JUNE 09:00-10:30 EDT

COLLECTIVE EXPERIENCE. COLLECTIVE SUCCESS.

Women in STEM fields face unique challenges and are often acutely aware of the role their gender plays as they progress both personally and professionally through their careers. The second annual Women in Science Panel Discussion will be an opportunity for our ISSCR community to come together to talk about these challenges and try to identify solutions to effect positive change.

An esteemed panel of successful female researchers will share their experiences, insights, and advice for overcoming gender bias and gender-related adversity. Attendees will then have the opportunity to engage in a moderated discussion.

Recognizing that it takes more than the efforts of women to achieve gender equity in the workplace, we would like to have a conversation with the entire ISSCR community. We welcome all voices to join us for this important discussion.

MODERATOR

Christine Mummery
Professor of Developmental Biology, Leiden University Medical Center, The Netherlands

PANELISTS

Sangeeta Bhatia
Director, Laboratory for Multiscale Regenerative Technologies, Massachusetts Institute of Technology, USA

Rana Dajani
Associate Professor of Molecular Cell Biology, Hashemite University, Jordan

Masayo Takahashi
President, Vision Care Inc., Japan

JUNIOR INVESTIGATOR CAREER PANEL

SATURDAY, 27 JUNE 10:00-11:30 EDT

FINDING YOUR FIT: DEFINING GOALS AND TAKING ACTION TO ACHIEVE LONG-TERM SUCCESS

Despite how it can sometimes appear, the path to a satisfying career is rarely straightforward. Are you currently asking yourself "How do I decide on a career path?" If so, join us for an in-depth panel discussion about how individual definitions for success and failure can help you identify your goals, adjust to setbacks, determine when it's time to make a change, and what steps to take now to achieve your long-term goals. This casual event hosted by the Junior Investigator Committee is intended to foster frank conversation about how to assess career decisions early on.

This session is designed for ISSCR trainee members, but any registrant is welcome to attend.

MODERATOR
Evan Graham
CELLINK, Sweden

Member, ISSCR Junior Investigators Committee

PANELISTS
Heather Duffy
Creative Innovation Consulting, USA

Steven Kattman
Sana Biotechnology, USA

Danijela Menicanin
University of Adelaide, Australia

Itedale Namro Redwan
CELLINK, Sweden

SCIENCE ADVOCACY AND COMMUNICATIONS SEMINAR

WEDNESDAY, 24 JUNE 09:00 – 10:30 EDT

Scientists are often asked to explain their work to non-scientific audiences, making effective communication skills essential, particularly when translating complex concepts into lay-friendly language. Researchers need to employ a variety of tactics to build support for evidence-based science, describe progress in the field, and highlight the impact of scientific discovery worldwide. Speakers in this seminar will discuss messages that resonate with policymakers, journalists, and the public, and share insights on how to convey the value of science with less technical audiences.

CHAIR
Sean Morrison
University of Texas Southwestern, USA

SPEAKERS
George Daley
Harvard University, USA

Fiona Watt
King's College London, UK

Sharon Begley
STAT, USA

PANEL MODERATOR
Sean Morrison
University of Texas Southwestern, USA

PANELISTS
Zubin Master
Mayo Clinic, USA

Kirstin Matthews
Rice University, USA

Mohamed Abou El-Enein
Charité Universitätsmedizin Berlin, Germany

FOCUS SESSIONS

WEDNESDAY, 24 JUNE 12:00 – 15:00
Focus sessions are parallel, in-depth educational opportunities in science, society and education organized by members and open to all annual meeting attendees.

The Science and Ethics of Human Brain Organoid Research

Presented by the ISSCR Ethics Committee

Rapid developments in human brain organoid research are likely to produce tractable new bioengineered tools for understanding functional interconnectivity of the human brain and dysfunction involved in many neurodegenerative diseases. Despite the excitement surrounding this subfield of stem cell research and its considerable scientific promise, advances could also raise novel ethical concerns. As researchers generate evermore realistic organoids that resemble human brains in vitro, it is critically important to understand what ethical boundaries may exist and where researchers and regulators should draw the line for research. In this focus session, panelists will discuss scientific, ethical, and policy issues surrounding the use of human pluripotent stem cells to generate self-organizing brain organoids and assembloids for research.

AGENDA:

WELCOME AND INTRODUCTION OF SPEAKERS
Insoo Hyun
Case Western Reserve University School of Medicine & Harvard Medical School, USA

12:05 WHAT CAN WE LEARN FROM BRAIN ORGANOIDS?
Paola Arlotta
Harvard University, USA

Giorgia Quadrato
University of Southern California, USA

12:25 HOW FAR CAN BRAIN ORGANOID RESEARCH GO?
Sergiu Pasca
Stanford University, USA

12:40 WHAT ELSE COULD WE DO WITH BRAIN ORGANOIDS?
George Church
Wyss Institute, Harvard University & the Massachusetts Institute of Technology, USA

12:55 WHAT SHOULD BRAIN ORGANOID RESEARCHERS BE MOST CONCERNED ABOUT IN THE NEAR TERM?
Jeantine Lunshof
Wyss Institute, Harvard University, USA

13:15 BREAK

13:25 WHAT IS THE PATIENT PERSPECTIVE ON BRAIN ORGANOIDS?
Karin Jongsma
UMC Utrecht, The Netherlands

13:45 STAKEHOLDER VIEWS — HOW DO SCIENTISTS AND VARIOUS PUBLICS VIEW BRAIN ORGANOID RESEARCH AND WHO SHOULD BE MAKING DECISIONS ON ITS REGULATION?
Misao Fujita
Center for iPS Cell Research and Application, Kyoto University, Japan

14:05 PANEL DISCUSSION & AUDIENCE QUESTIONS
Moderator: Insoo Hyun
Case Western Reserve University School of Medicine & Harvard Medical School, USA

14:55 CLOSING SUMMARY
Insoo Hyun
Case Western Reserve University School of Medicine & Harvard Medical School, USA

Tools for Basic and Applied Stem Cell Technology

Sponsored By: STEMCELL Technologies Inc., Thermo Fisher Scientific

Stem Cell COREdinates (www.COREdinates.org) is a consortium of human pluripotent stem cell-focused cores that share expertise with protocols, reagents, and technological advancements to establish “best practices”. The first part of our Focus Session will have selected presentations from Stem Cell COREdinate members. These presentations will cover a number of different areas of expertise including reprogramming, gene editing, disease modeling, and biobanking. The second part of the session will focus on advances in gene editing and cell therapies.

AGENDA:

12:00 – 12:05 WELCOME AND OVERVIEW
Deborah French
Children’s Hospital of Philadelphia, USA

12:05 – 12:20 LAB-BREWED TOOLS TO ASSESS ENGINEERED HEART ORGANOIDS
Jared Churko
University of Arizona, USA

12:20 – 12:35 USING AUTOMATION TO DEVELOP HUMAN STEM CELL MODELS OF DISEASE AT LARGE SCALE
Daniel Pauli
New York Stem Cell Foundation, USA

12:35 – 12:50 ADDRESSING ISSUES WITH PSC SUSPENSION CULTURE: DEVELOPMENT OF A NEW MEDIUM THAT YIELDS HIGHLY EFFICIENT SPHEROID NUCLEATION AND ROBUST EXPANSION
Michael L. Akenhead
Thermo Fisher Scientific, USA

12:50 – 13:05 THE ESSENTIAL 3 R'S OF PLURIPOTENT STEM CELL RESEARCH: RIGOR, REPRODUCIBILITY, AND REDUNDANCY
Tenneille Ludwig
WiCell Research Institute, USA

13:05 – 13:20 RECENT INSIGHTS AND TRENDS IN PLURIPOTENT STEM CELL RESEARCH
Andrew Gaffney
STEMCELL Technologies, Inc., USA

13:20 – 13:35 CRISPR GENE EDITING AT RUCDR: WORKFLOW, QC AND A CASE STUDY OF EDITING THE POLYGLUTAMINE LOCUS OF THE HD GENE
Jianhua Chu
RUCDR Infinite Biologics, USA

13:35 – 13:50 3D BIOPRINTING OF VASCULARIZED SKIN GRAFTS AND TISSUE MODELS USING HUMAN CELLS
Brigitte L. Arduini
Rensselaer Polytechnic Institute, USA

13:50 – 13:55 OVERVIEW DISEASE/THERAPY TOPICS
Barbara Corneo
Columbia University, USA

13:55 – 14:15 PROMOTING FETAL HEMOGLOBIN TO TREAT SICKLE CELL DISEASE: THE IMPORTANCE OF CAS ENZYME SELECTION IN OPTIMIZING PHENOTYPIC OUTCOME
Jack Heath
Editas Medicine, USA

14:15 – 14:35 USING INDUCED PLURIPOTENT STEM CELLS TO IMPROVE TRANSFUSION THERAPY IN PATIENTS WITH SICKLE CELL DISEASE
Hyun Hyung (Claire) An
Children's Hospital of Philadelphia, USA

14:35 – 14:55 MODELING HUMAN NATURAL KILLER CELL DEFICIENCY WITH INDUCED PLURIPOTENT STEM CELLS
Emily Mace
Columbia University Medical Center, USA

14:55 – 15:00 CONCLUDING REMARKS
Deborah French
Children's Hospital of Philadelphia, USA

12:00 – 15:00

Focus Session: Practical Considerations and Solutions in the Preclinical Development of Investigational Stem Cell Products

Presented by Covance

Human stem cell products have the potential to treat and prevent many diseases and there are growing research and development efforts underway to make next-generation stem cell therapies a reality. However, there are numerous scientific and regulatory considerations and challenges to address in the development of these investigational products, beginning in the preclinical phase prior to testing in humans. This Focus Session will present practical insights into key scientific and regulatory aspects of preclinical development for an investigational stem-cell product to justify it as safe and efficacious– critical elements of a regulatory submission to initiate human clinical studies.

AGENDA:

12:00 – 12:05 WELCOME AND OVERVIEW
Brian McIntosh
Covance, USA

12:05 – 12:40 PRECLINICAL PHARMACOLOGY
Kapil Bharti
National Eye Institute, NIH, USA

12:40 – 13:15 PRECLINICAL SAFETY ASSESSMENT OF AUTOLOGOUS PRODUCTS
Jeanne Loring
Scripps Research Institute, USA

13:15 – 13:50 REGULATORY CONSIDERATIONS AND COMPLIANCE
Paul Baldrick
Covance, USA

13:50 – 14:25 PRECLINICAL CMC
Jane Lebkowski
Regenerative Patch Technologies, USA

14:25 – 15:00 BIOMARKER SELECTION
Akanksha Gupta
Covance, USA

Focus Session: Translating Your Discovery: From the laboratory to industry

Presented by ISSCR Industry Committee

Moving a discovery from the academic laboratory towards commercialization, or the so-called "translational process," requires many steps and multiple areas of expertise that collectively advance the project toward a clinical or commercial endpoint. These include building your core team, protecting your intellectual property, financing the process, working with pharma, among other areas. This program brings together experts from industry, technology transfer, venture capital and academics who have gone through the process to help those that are currently translating discoveries or just want to learn more about the process.

AGENDA:

12:00 – 12:05 WELCOME AND OVERVIEW
Setsuko Hashimoto
CellSeed Inc, Japan

12:05 – 12:25 PROTECTING YOUR IDEA(S) FOR COMMERCIALIZATION
Victoria Sutton
Wisconsin Alumni Research Foundation, USA

12:25 – 12:45 LAUNCHING FROM THE LAB: THOUGHTS FROM ACADEMIA
Malin Parmar
Lund University, Sweden

12:45 – 13:05 FUNDING THE PROCESS: A VENTURE CAPITAL PERSPECTIVE
Nilay Thakar
Arch Venture Partners, USA

13:05 – 13:25 HOW TO BUILD YOUR TRANSLATIONAL TEAM
Masayo Takahashi
Vision Care Inc., Japan

13:25 – 13:45 CHEMISTRY, MANUFACTURING, AND CONTROLS (CMC)
Benjamin Fryer
Pluistyx Inc, USA

13:45 – 14:05 FROM LABORATORY TO BIG PHARMA
Irina Klimanskaya
Astellas, USA

14:05 – 14:25 CLINICAL TRIAL DESIGN AND OPERATIONS
Wayne Chu
Fate Therapeutics, USA

13:50 – 15:00 PANEL DISCUSSION AND ADDITIONAL Q&A
All speakers

20:00 – 23:00

Focus Session: Immune Cell Engineering from iPSCs

Presented by BlueRock Therapeutics

CAR-T cells have defined a new class of living drugs. Synthetic biology is used to "rewire" T cells to effectively target some cancers. In this Focus Session, BlueRock Therapeutics will host key opinion leaders in immune cell engineering. Here, we will expand beyond T cells and discuss more complex engineering strategies that seek to incorporate sophisticated synthetic circuitry to allow future medicines to "sense and respond" to their environment, the patient. Off-target side effects might be minimized through such targeted medicines. This combination of cell and gene therapy will continue to transform and build the cell therapy landscape.

AGENDA:

20:00 – 20:10 OPENING COMMENTS
Mark Tomishima
BlueRock Therapeutics, USA

20:10 – 20:50 THERAPEUTIC TREGS, FROM ADULT TO IPSC
Bruce Blazar
University of Minnesota, USA

20:50 – 21:30 THERAPEUTIC MACROPHAGES, FROM ADULT TO IPSC
Courtney Crane
Seattle Children's Hospital, USA

21:30 – 21:40 BREAK

21:40 – 22:20 SYNTHETIC CIRCUITS FOR SAFETY AND FUNCTION
Shaun Cordoba
Autolus, UK

22:20 – 23:00 ENGINEERING NEXT-GENERATION CELL AND GENE THERAPIES WITH SYNTHETIC BIOLOGY
Tim Lu
Senti Bio, USA

TUESDAY, 23 JUNE

09:00 – 17:05 **WORKSHOP ON CLINICAL TRANSLATION**

09:00 – 11:35 **Workshop on Clinical Translation Session I: Modeling Stem Cells for Discovery**

09:00 – 09:10 **Lee Rubin**
Harvard University, USA
Opening Workshop on Clinical Translation Remarks

09:10 – 09:15 **Ricardo Dolmetsch**
Novartis, USA
Introduction to Session I: Modeling Stem Cells for Discovery

09:15 – 09:45 **Clifford Woolf**
Boston Children's Hospital, USA

09:45 – 10:15 **Ronald Li**
Karolinksa Institutet, Sweden
NovoHeart, Canada

10:15 – 10:45 **Giorgia Quadrato**
University of Southern California, USA

10:45 – 11:15 **Ajamete Kaykas**
insitro, USA

11:15 – 11:35 **Modeling Stem Cells for Discovery Panel Discussion**

12:15 – 14:10 **Workshop on Clinical Translation Session II: Endogenous Stem Cells as Target**

12:15 – 12:20 **Chee-Yeun Chung**
Yumanity, USA
Introduction to Session II: Endogenous Stem Cells as Target

12:20 – 12:50 **Domenico Accili**
Columbia University, USA
Hua Lin
Forkhead Biotherapeutics,USA

12:50 – 13:20 **Alison McGuigan**
University of Toronto, Canada

13:20 – 13:50 **Albert Edge**
Massachusetts Eye and Ear; Decibel Therapeutics, USA

13:50– 14:10 **Endogenous Stem Cells as Targets Panel Discussion**

15:00 – 17:05 **Workshop on Clinical Translation Session III: Stem Cells as Drugs in Clinical Trials**

15:00 – 15:05 **Jerome Ritz**
Dana Farber Cancer Institute, USA
Introduction to Session III: Stem Cells as Drugs in Clinical Trials

TUESDAY, 23 JUNE *(continued)*

15:05 – 15:35 **Agnete Kirkeby**
University of Copenhagen, Denmark
Lund University, Sweden

15:35 – 16:05 **David Williams**
Boston Children's Hospital, USA

16:05 – 16:35 **Sonja Schrepfer**
University of California, San Francisco, USA

16:35 – 16:55 **Stem Cells as Drugs Panel Discussion**

17:10 – 17:20 **Stacie Weninger**
F-Prime Capital, USA
Closing Workshop on Clinical Translation Remarks

10:30 – 17:30 **BUSINESS OF DISCOVERY WORKSHOP**
Sponsored By: Advanced Regenerative Manufacturing Institute (ARMI) and BioFab USA

10:30 – 12:00 **Business of Discovery Workshop Session I: The Pitch. The Plan.**

10:30 – 10:40 **Welcome and opening remarks**
Brock Reeve
Harvard Stem Cell Institute, USA

10:40 – 11:05 **Andrea Armstrong**
Celsius Therapeutics, USA

11:05 – 11:30 **Christina Isacson**
Magenta Therapeutics, USA

11:30 – 12:00 **James (Jay) Bradner**
Novartis Institutes for BioMedical Research, USA

13:00 – 14:45 **Business of Discovery Workshop Session II: Funding**

13:00 – 13:05 **Welcome and opening remarks**
Stephen Freedman
Gladstone Institutes, USA

13:05 – 13:25 **Douglas Crawford**
Mission Bay Capital, USA

13:25 – 13:45 **Ann DeWitt**
The Engine, Massachusetts Institute of Technology, USA

13:45 – 14:05 **Michal Preminger**
Johnson & Johnson, USA

14:05 — 14:25 **Matt McMahon**
National Institutes of Health, USA

TUESDAY, 23 JUNE *(continued)*

14:25 – 14:45	**Topic 2: Discussion Panel**
15:30 – 17:30	**Business of Discovery Workshop Topic III: Stories From the Front Line**
15:30 – 15:35	**Welcome and opening remarks** **Katherine High** *Rockefeller University, USA*
15:35 – 16:00	**Tina Liu** *Ally Therapeutics, USA*
16:00 – 16:25	**Sarindr (Ik) Bhumiratana** *EpiBone, USA*
16:25 – 16:50	**Sangeeta Bhatia** *Massachusetts Institute of Technology, USA*
16:50 – 17:25	**Topic 3: Discussion Panel**
17:25 – 17:30	**Final Remarks** **Paul Tesar** *Case Western Reserve University, USA*

WEDNESDAY, 24 JUNE

8:00 – 17:30 **EXHIBIT HALL**

09:00 – 10:30 **Science Advocacy and Communication Seminar** (See page 34 for details)

12:00 – 15:00 **FOCUS SESSIONS I** (See page 35 for details)

12:00 – 15:00 **Focus Session: Practical Considerations and Solutions in the Preclinical Development of Investigational Stem Cell Products**
Sponsored by: Covance

12:00 – 15:00 **Focus Session: Tools for Basic and Applied Stem Cell Technology**
Presented by: COREdinates
Sponsored by: STEMCELL Technologies Inc. and Thermo Fisher Scientific

12:00 – 15:00 **Focus Session: The Science and Ethics of Human Brain Organoid Research**
Presented by: ISSCR Ethics Committee

12:00 – 15:00 **Focus Session: Translating Your Discovery: From the Laboratory to Industry**
Presented by: ISSCR Industry Committee

16:00 – 18:00 **Plenary I: Presidential Symposium**
Sponsored by: Fate Therapeutics

Session Chair: Deepak Srivastava
Gladstone Institutes, USA

16:00 – 16:03 **Welcome**
Mayor Martin J. Walsh
Boston MA, USA

16:03 – 16:08 **Deepak Srivastava**
Gladstone Institutes, USA
ISSCR President's Address

16:08 – 16:12 **Welcome Remarks HSCI**
David Scadden
HSCI, USA

16:12 – 16:14 **ISSCR Public Service Award Presentation to Susan L. Solomon, New York Stem Cell Foundation**

16:18 – 16:20 **ISSCR Zhongmei Chen Yong Awards for Scientific Excellence Recognition**

16:20 – 16:40 **Aviv Regev**
Broad Institute, MIT, HHMI, USA
CELL ATLASES AS ROADMAPS TO UNDERSTAND DEVELOPMENT

16:45 – 17:05 **Steven Finkbeiner**
University of California, San Francisco, USA
SEARCHING FOR SIGNATURES OF NEURODEGENERATIVE AND PSYCHIATRIC DISEASES, THERAPEUTIC STRATEGIES AND PREDICTORS OF CLINICAL OUTCOMES WITH PATIENT IPSCS

WEDNESDAY, 24 JUNE *(continued)*

17:10 – 17:30 **Eric Olson**
University of Texas Southwestern, USA
TOWARD THE GENOMIC CORRECTION OF MUSCLE AND HEART DISEASE

17:35 – 17:55 **Fiona Watt**
King's College London, UK
ERNEST MCCULLOCH MEMORIAL LECTURE: ADVENTURES IN SKIN BIOLOGY

18:00 – 19:30 **Early Career Group Leader Panel**

20:00 – 23:00 **Focus Sessions II** (See page 35 for details)

20:00 – 23:00 **Focus Session: Immune Cell Engineering From iPSCs**
Sponsored by: BlueRock Therapeutics

THURSDAY, 25 JUNE

09:00 – 11:00 **Plenary II: Machine Learning and Computational Approaches**
Sponsored by: T-CiRA Joint Program

Session Chair: Christine Wells
University of Melbourne, Australia

09:00 – 09:05 **Machine Learning and Computation Session Introduction**

Christine Wells
University of Melbourne, Australia

09:05 – 09:10 **Presentation of ISSCR Dr. Susan Lim Award for Outstanding Young Investigator**

Christina Tan
Dr. Susan Lim Endowment for Education and Research Ltd.

09:10 – 09:30 **Allon Klein**
Harvard Medical School, USA
ISSCR DR. SUSAN LIM AWARD FOR OUTSTANDING YOUNG INVESTIGATOR LECTURE: LEARNING DYNAMICS BY LINEAGE TRACING ON TRANSCRIPTIONAL LANDSCAPES

09:35 – 09:50 **Emma Lundberg**
KTH Royal Institute of Technology, Sweden
SPATIOTEMPORAL DISSECTION OF THE HUMAN PROTEOME

09:55 – 10:10 **Ajamete Kaykas**
insitro, USA
NEXT GENERATION HUMAN IPSC-DISEASE MODELS

10:15 – 10:30 **Trey Ideker**
University of California, San Diego School of Medicine, USA
UNDERSTANDING THE IMPACT OF PSYCHIATRIC GENOMES ON THE HIERARCHY OF BIOLOGICAL PATHWAYS AND SYSTEMS

10:35 – 10:50 **Hiroaki Kitano**
Okinawa Institute of Science and Technology, Japan
TOWARD THE AUTOMATION OF SCIENTIFIC DISCOVERY BY AI AND ROBOTICS

11:00 – 19:00 **EXHIBIT HALL**

THURSDAY, 25 JUNE *(continued)*

11:00 – 12:45 **Concurrent - Cellular Identity: Cardiac and Muscle**

Session Chair: Li Qian
McAllister Heart Institute, University of North Carolina, Chapel Hill, USA

11:00 – 11:05 **Overview – Cellular Identity: Cardiac and Muscle**

Li Qian
McAllister Heart Institute, University of North Carolina, Chapel Hill, USA

11:05 – 11:20 **Richard Harvey**
Victor Chang Cardiac Research Institute, Australia
ANALYSIS OF MURINE CARDIAC FIBROBLAST LINEAGE HETEROGENEITY AND DYNAMICS IN HEALTH AND DISEASE USING SINGLE CELL METHODS

11:25 – 11:33 **Rosanne Raftery**
Boston Children's Hospital, USA
SINGLE CELL TRANSCRIPTOMIC ANALYSIS OF HESC-DERIVED ARTICULAR AND GROWTH PLATE CARTILAGE REVEALS MOLECULAR CANDIDATES FOR LINEAGE COMMITMENT AND STABILITY

11:35 – 11:43 **Hao Wu**
University of Pennsylvania, USA
DECODING HUMAN CARDIAC MATURATION AND AGING WITH SINGLE-CELL MULTI-OMICS SEQUENCING

11:45 – 11:53 **Chiara Mozzetta**
National Research Council (CNR), Italy
PRDM16-MEDIATED H3K9 METHYLATION CONTROLS FIBRO-ADIPOGENIC PROGENITORS PLASTICITY AND MOUSE SKELETAL MUSCLE REPAIR

11:55 – 12:03 **Lili Zhu**
Gladstone Institutes, USA
THE TRANSCRIPTION FACTOR GATA4 REGULATES MRNA SPLICING THROUGH DIRECT INTERACTION WITH MRNA

12:05 – 12:13 **Conchi Estaras**
Temple University, USA
A NOVEL RETINOIC ACID:YAP1 SIGNALING AXIS CONTROLS ATRIAL LINEAGE ACQUISITION IN HUMAN CARDIAC PROGENITORS

12:15 – 12:30 **Helen Blau**
Stanford University School of Medicine, USA
REJUVENATING STEM CELL FUNCTION TO INCREASE MUSCLE STRENGTH

THURSDAY, 25 JUNE *(continued)*

11:00 – 12:45 **Concurrent - Clinical Applications: Early Development and Pluripotency -- Interspecies Chimeras for Stem Cell Research**

Session Chair: Martin F. Pera
The Jackson Laboratories, USA

11:00 – 11:05 **Overview- Clinical Applications: Early Development and Pluripotency -- Interspecies Chimeras for Stem Cell Research**

Martin F. Pera
The Jackson Laboratories, USA

11:05 – 11:20 **Martin F. Pera**
The Jackson Laboratories, USA
UNIQUE PROPERTIES OF A SUBSET OF HUMAN PLURIPOTENT STEM CELLS WITH HIGH CAPACITY FOR SELF-RENEWAL

11:25 – 11:33 **Laura Reinholdt**
The Jackson Laboratory, USA
GENETIC VARIATION INFLUENCES MOUSE EMBRYONIC STEM CELL STATE THROUGH A HIERARCHY OF MOLECULAR PHENOTYPES

11:35 – 11:43 **Jennifer Wang**
Boston University, USA
A NOVEL MODEL FOR STUDYING INFECTIONS AT THE MATERNAL-FETAL INTERFACE USING HUMAN IPSC-DERIVED TROPHOBLAST LINEAGES

11:45 – 11:53 **Qi Qiu**
University of Pennsylvania, USA
MASSIVELY PARALLEL, TIME-RESOLVED SINGLE-CELL RNA SEQUENCING WITH SCNT-SEQ

11:55 – 12:03 **Ivana Barbaric**
University of Sheffield, UK
CELL COMPETITION IN HUMAN PLURIPOTENT STEM CELL CULTURES

12:05 – 12:20 **Josephine Johnston**
Hastings Center, USA
WHY HUMAN-ANIMAL CHIMERA RESEARCH

12:25 – 12:40 **Jun Wu**
University of Texas, Southwestern Medical Center, USA
CELL COMPETITION CONSTITUTES A BARRIER FOR INTERSPECIES CHIMERISM

THURSDAY, 25 JUNE *(continued)*

11:00 – 12:45 **Concurrent - Modeling Development and Disease: Neural**
Sponsored by MaxWell Biosystems

Session Chair: Marianne E. Bronner
California Institute of Technology, USA

11:00 – 11:05 **Overview – Modeling Development and Disease: Neural**

Marianne E. Bronner
California Institute of Technology, USA

11:05 – 11:20 **Li-Huei Tsai**
Massachusetts Institute for Technology, USA
RECONSTRUCTION OF THE HUMAN BLOOD-BRAIN BARRIER USING INDUCED PLURIPOTENT STEM CELLS TO MODEL CEREBRAL AMYLOID ANGIOPATHY

11:25 – 11:33 **Joseph Klim**
Harvard University, USA
STEM CELL-DERIVED NEURONS PROVIDE THE MISSING LINK BETWEEN ALS PATHOLOGY AND MOTOR NEUROPATHY

11:35 – 11:43 **Maria Kousi**
Massachusetts Institute of Technology, USA
NETWORK-BASED PREDICTION AND VALIDATION THROUGH SYSTEMATIC PROFILING OF CAUSAL SCHIZOPHRENIA GENES IN HUMAN CORTICAL NEURONS

11:45 – 11:53 **Silvia Velasco**
Broad Institute, Harvard University, USA
BRAIN ORGANOIDS REPRODUCIBLY GENERATE THE CELLULAR DIVERSITY OF THE HUMAN CEREBRAL CORTEX

11:55 – 12:03 **Marine Krzisch**
Whitehead Institute for Biomedical Research, USA
INTERSPECIES CHIMERIC FRAGILE X SYNDROME MODEL REVEALS FMR1-DEPENDENT NEURONAL PHENOTYPES

12:05 – 12:13 **Larissa Traxler**
University of Innsbruck, Austria
ALZHEIMER PATIENT-DERIVED INDUCED NEURONS REVEAL A WARBURG-LIKE METABOLIC SWITCH

12:15 – 12:30 **Haruhisa Inoue**
CiRA, Kyoto University, Japan
BASIC AND TRANSLATIONAL DISEASE MODELING WITH PATIENT IPSC-DERIVED NEURAL CELLS

THURSDAY, 25 JUNE *(continued)*

11:00 – 12:45 **Concurrent - Modeling Development and Disease: Organoids of Endoderm and Kidney**

Session Chair: Takanori Takebe
Cincinnati Children's Hospital, USA and Medical Center and Yokohama City University, Japan

11:00 – 11:05 **Overview - Modeling Development and Disease: Organoids of Endoderm and Kidney**

Takanori Takebe
Cincinnati Children's Hospital, USA and Medical Center and Yokohama City University, Japan

11:05 – 11:20 **Lijian Hui**
Shanghai Institute of Biochemistry and Cell Biology, China
CELL PLASTICITY AND HUMAN LIVER DISEASE MODELS

11:25 – 11:33 **Hsiao-Yun Liu**
Columbia University Medical Center, USA
HUMAN PLURIPOTENT STEM CELL DERIVED LUNG DISTAL TIP PROGENITORS ARE STEPPING TOWARD DISEASE MODELING

11:35 – 11:43 **Jennifer Rosenbluth**
Dana-Farber Cancer Institute, USA
MAMMARY CELL LINEAGES ASSOCIATED WITH BRCA1/2 HETEROZYGOSITY IN PATIENT-DERIVED ORGANOID CULTURES

11:45 – 11:53 **Samira Musah**
Duke University, USA
ENGINEERED HUMAN KIDNEY TISSUES TO UNDERSTAND THE MECHANISMS OF DEVELOPMENT AND DISEASE PROGRESSION

11:55 – 12:03 **Sean Harrison**
University of Oslo, Norway
TISSUE SCALE CULTURE OF HUMAN PLURIPOTENT STEM CELL DERIVED LIVER ORGANOIDS AND HIGH SENSITIVITY ANALYSIS TECHNIQUES

12:05 – 12:13 **Paul Gadue**
Children's Hospital of Philadelphia, USA
A PATIENT IPSC LINE REVEALS THE PENETRANCE OF PANCREATIC AGENESIS CAUSED BY GATA6 MUTATIONS IS MODIFIED BY A NON-CODING SNP

12:15 – 12:30 **Joo-Hyeon Lee**
University of Cambridge, UK
RECONSTITUTION OF ALVEOLAR REGENERATION BY INFLAMMATORY NICHES

THURSDAY, 25 JUNE *(continued)*

11:00 – 12:45 **Concurrent - Tissue Stem Cells and Regeneration: Hematopoietic and Endothelial Cells**
Sponsored by Kuhner Shaker

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

11:00 – 11:05 **Overview- Tissue Stem Cells and Regeneration: Hematopoietic and Endothelial Cells**

Leonard I. Zon
Boston Children's Hospital, USA

11:05 – 11:20 **Claudia Waskow**
Leibniz Institute on Aging- FLI, Germany
CROSS COMMUNICATION OF HEMATOPOIETIC CELLS OF ADULT AND EMBRYONIC ORIGIN

11:25 – 11:33 **Kostandin Pajcini**
University of Illinois at Chicago, USA
JAGGED1 IS REQUIRED FOR THE TRANSITION OF HEMATOPOIETIC STEM CELLS FROM THE FETAL LIVER TO THE ADULT BONE MARROW NICHE.

11:35 – 11:43 **Jiajing Qiu**
Icahn School of Medicine at Mount Sinai, USA
IDENTIFICATION OF POTENT HUMAN HEMATOPOIETIC STEM CELLS USING MITOCHONDRIAL PROFILE TO IMPROVE BONE MARROW TRANSPLANTATION

11:45 – 11:53 **Yan Yao**
Columbia University Medical Center, USA
REGULATION OF HEMATOPOIETIC STEM CELL FUNCTION BY MITOCHONDRIAL DYNAMICS

11:55 – 12:03 **Julianne Smith**
Case Western Reserve University, USA
15-PGDH INHIBITION POTENTIATES MURINE HEMATOPOIETIC REGENERATION VIA SPLENIC NICHE ACTIVATION

12:05 – 12:13 **Sachin Patel**
Boston Children's Hospital, USA
EMBRYONIC MULTIPOTENT PROGENITORS SPECIFIED DURING THE DEFINITIVE WAVE OF HEMATOPOIESIS REPRESENT A SIGNIFICANT SOURCE OF ADULT BLOOD

12:15 – 12:30 **Matt Porteus**
Stanford Medical School, USA
GENOME EDITING TO CREATE SAFE AND EFFECTIVE STEM CELL BASED MEDICINES

THURSDAY, 25 JUNE *(continued)*

INNOVATION SHOWCASES (See page 91 for details)

13:00 – 13:30 *Presented by: Cell MicroSystems*
EVALUATION OF CLONAL ORGANOID HETEROGENEITY AT SINGLE GASTROID RESOLUTION

Scott Magness
University of North Carolina, Chapel Hill, USA

Jarrett Bliton
University of North Carolina, Chapel Hill, USA

Jessica Hartman
Cell Microsystems, Inc., USA

13:00 – 13:30 *Presented by: NanoSurface Biomedical*
BUILDING A BETTER STEM CELL: IMPROVING THE MATURITY OF HUMAN STEM CELLS IN VITRO THROUGH BIOENGINEERING

Nicholas Geisse
NanoSurface Bio, USA

13:00 – 13:30 *Presented by: Solentim*
NOVEL WORKFLOW FOR HIGH EFFICIENCY SINGLE CELL ISOLATION OF IPSCS WITH CONCURRENT DOCUMENTATION OF CLONALITY

Ian Taylor
Solentim, UK

13:00 – 14:00 *Presented by: Biological Industries*
ACADEMIA AND INDUSTRY GO HAND-IN-HAND: FROM ICM AND TROPHOBLASTS , THROUGH BBB-ON-A-CHIP, TO ALS AND DIABETES TREATMENTS

Gad Vatine
Ben-Gurion University of the Negev, Israel

Yossi Buganim
The Hebrew University-Hadassah Medical School, Israel

Kfir Molakandov
Kadimastem Ltd, Israel

Michal Izrael
Kadimastem Ltd, Israel

THURSDAY, 25 JUNE *(continued)*

13:00 – 14:00 *Presented by: Bio-Techne*
OPTIMIZING IPSC REPROGRAMMING, ENGINEERING, AND CULTURING FOR ANIMAL-FREE GMP MANUFACTURING

Kevin Flynn
Bio-Techne, USA

Travis Cordie
Bio-Techne, USA

Beau Webber
University of Minnesota, USA

13:00 – 14:00 *Presented by: Miltenyi*
FROM BENCH TO BEDSIDE – PLURIPOTENT STEM CELL DIFFERENTIATION

Agnete Kirkeby
University of Copenhagen, Denmark

Sebastian Knoebel
Miltenyi Biotec B.V. & Co. KG, Bergisch Gladback, Germany

13:00 – 14:00 *Presented by: STEMCELL Technologies*
APPLICATIONS OF ORGANOID AND ORGANOTYPIC CULTURES IN INFECTIOUS DISEASES, NEPHROTOXICITY, AND HIGHLY-RELEVANT CELL-BASED ASSAY DEVELOPMENT

Philipp Kramer
STEMCELL Technologies, Canada

Juan Hou
STEMCELL Technologies, Canada

Martin Stahl
STEMCELL Technologies, Canada

13:00 – 14:00 *Presented by: Thermo Fisher Scientific*
LEVERAGING A NOVEL PSC SUSPENSION MEDIA SYSTEM TO SCALE UP FOR GENERATION OF DIFFERENTIATED CELLS

Rebecca Sereda
Harvard University-Stem Cell and Regenerative Bio Department, USA

David Kuninger
Thermo Fisher Scientific, USA

14:00 – 16:00 **Poster Session I**

THURSDAY, 25 JUNE *(continued)*

16:00 – 18:00 **Plenary III: Embryogenesis and Development**
Sponsored by: Vertex Cell and Gene Therapies

Session Chair: Ruth Lehmann
HHMI/Skirball Institute, NYU School of Medicine, USA

16:00 – 16:05 **Embryogenesis and Development Session Introduction**

Ruth Lehmann
HHMI/Skirball Institute, NYU School of Medicine, USA

16:05 – 16:20 **Kathy Niakan**
The Francis Crick Institute, UK
GENETIC APPROACHES TO STUDY EARLY LINEAGE SPECIFICATION IN HUMAN EMBRYOS

16:25 – 16:40 **Kenneth Zaret**
University of Pennsylvania, USA
PROSPECTS FOR CHANGING CELL FATE AT WILL

16:45 – 17:00 **Benoit Bruneau**
Gladstone Institutes, USA
CHROMATIN SAFEGUARD FOR CARDIAC MESODERM DIFFERENTIATION

17:05 – 17:13 **Vincent Pasque**
KU Leuven, Belgium
REVEALING THE CRITICAL REGULATORS OF CELL IDENTITY IN HUMAN PRE-IMPLANTATION EMBRYOS AND HUMAN NAÏVE AND PRIMED PLURIPOTENT STEM CELLS USING SINGLE-CELL MULTI-OMICS

17:15 – 17:23 **Giorgia Scapin**
Nationwide Children's Hospital / The Ohio State University College of Medicine, USA
PULSATION ACTIVATES PIEZO1 TO FORM LONG-TERM HUMAN HEMATOPOIETIC STEM CELLS

17:25 – 17:45 **Elaine Fuchs**
HHMI, The Rockefeller University, USA
STEM CELLS: MAKING AND MAINTAINING TISSUES EVEN IN TIMES OF STRESS

THURSDAY, 25 JUNE *(continued)*

INNOVATION SHOWCASES (See page 91 for details)

19:00 – 19:30

Presented by: STEMCELL Technologies,Canada
DIFFERENTIATING IMMUNE CELLS FROM HUMAN PLURIPOTENT STEM CELLS IN FEEDER- AND SERUM-FREE CULTURES

Nooshin Tabatabaei-Zavareh
STEMCELL Technologies, Canada

19:30 – 20:00

Presented by: STEMCELL Technologies, Canada
OPTIMIZED, SERUM-FREE, IN VITRO CULTURE CONDITIONS FOR CRISPR-MEDIATED GENOME EDITING OF CD34+ CELLS

Amanda Fentiman
STEMCELL Technologies, Canada

THURSDAY, 25 JUNE *(continued)*

20:00 – 21:45 **Concurrent - Clinical Applications: Hematopoietic and Endothelial Cells**
Sponsored by: Editas Medicine

Session Chair: Michel Sadelain
Memorial Sloan-Kettering Cancer Center, USA

20:00 – 20:05 **Overview - Clinical Applications: Hematopoietic and Endothelial Cells**

Michel Sadelain
Memorial Sloan-Kettering Cancer Center, USA

20:05 – 20:20 **Alessandro Aiuti**
San Raffaele Hospital, Italy
IN VIVO CLONAL TRACKING AND LINEAGE MODELING IN HEMATOPOIETIC STEM CELL GENE THERAPY

20:25 – 20:33 **Anna Villa**
Ospedale San Raffaele, Italy
COUPLING EXPANSION AND TRANSDUCTION OF HUMAN HEMATOPOIETIC STEM AND PROGENITOR CELLS TO TREAT AUTOSOMAL RECESSIVE OSTEOPETROSIS

20:35 – 20:43 **Miki Ando**
Juntendo University School of Medicine, Japan
DEVELOPMENT OF IPSC-DERIVED EXTENDED CTL THERAPY FOR CERVICAL CANCER

20:45 – 20:53 **Tobias Deuse**
University of California San Francisco, USA
DE NOVO MUTATIONS IN MITOCHONDRIAL DNA OF IPSCS PRODUCE IMMUNOGENIC NEOEPITOPES IN MICE AND HUMANS

20:55 – 21:03 **Esra Karaca**
Stanford University, USA
ENHANCED SURVIVAL OF HUMAN IPSC-DERIVED ENDOTHELIAL CELLS ON NANOPATTERNED SCAFFOLDS FOR TREATMENT OF PERIPHERAL ARTERIAL DISEASE

21:05 – 21:13 **Alan Trounson**
Monash University, Australia
TARGETING HUMAN OVARIAN CANCER WITH IMMUNE CELLS DERIVED FROM THE PATIENT OR FROM HOMOZYGOUS HLA HAPLOTYPE IPS CELLS

21:15 – 21:30 **Judith Shizuru**
Stanford University School of Medicine, USA
BLOOD STEM CELL TRANSPLANTATION WITHOUT GENOTOXICITY: TRANSLATION FROM MICE TO HUMAN TRIALS OF ANTI-CD117 ANTIBODY CONDITIONING

THURSDAY, 25 JUNE *(continued)*

20:00 – 21:45 **Concurrent - Clinical Applications: Neural**

Session Chair: Kevin C. Eggan
Harvard University and Broad Institute of MIT and Harvard, USA

20:00 – 20:05 **Overview- Clinical Applications: Neural**

Kevin C. Eggan
Harvard University, USA

20:05 – 20:20 **Malin Parmar**
Lund University, Sweden
DEVELOPING A STEM CELL BASED THERAPY FOR PARKINON'S DISEASE

20:25 – 20:33 **Christophe Heinrich**
INSERM, France
SEIZURE RELIEF IN EPILEPTIC MICE THROUGH GABAERGIC NEURONS DERIVED FROM LINEAGE REPROGRAMMING OF GLIAL CELLS

20:35 – 20:43 **Wanlu Li**
Shanghai Jiao Tong University, China
OLIGODENDROCYTE PRECURSOR CELL TRANSPLANTATION PROMOTES OLIGODENDROGENESIS AND SYNAPTOGENSIS IN MICE AFTER CEREBRAL ISCHEMIA

20:45 – 20:53 **Leslie Caron**
The University of Sydney, Australia
HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED GABAERGIC INTERNEURON TRANSPLANTS ATTENUATE NEUROPATHIC PAIN

20:55 – 21:03 **Julia Oswald**
Schepens Eye Research Institute, USA
GROWTH FACTOR ENHANCED MOUSE IPSC-DERIVED RETINAL ORGANOIDS AS CELL SOURCE FOR RETINAL CELL REPLACEMENT

21:05 – 21:13 **Andrea Barabino**
Maisonneuve-Rosemont Hospital's Research Centre, Universite de Montreal (UDEM), Canada
FIRST TRANSPLANTATION TRIAL OF A POLARIZED IPSC-DERIVED RETINAL SHEET IN A NON-HUMAN PRIMATE MODEL FOR THE TREATMENT OF MACULAR DEGENERATIONS

21:15 – 21:30 **Megan Munsie**
University of Melbourne, Australia
STEM CELL MIRACLE OR RUSSIAN ROULETTE?: EXPLORING CHOICE IN THE CONTEXT OF MS

THURSDAY, 25 JUNE *(continued)*

20:00 – 21:45 **Concurrent - Modeling Development and Disease: Cardiac and Muscle**

Session Chair: Christine L. Mummery
Leiden University Medical Center, The Netherlands

20:00 – 20:05 **Overview- Modeling Development and Disease: Cardiac and Muscle**
Christine L. Mummery
Leiden University Medical Center, The Netherlands

20:05 – 20:20 **Tim Hoey**
Tenaya Therapeutics, USA
DEVELOPING AN OPTIMIZED GENE THERAPY FOR DIRECT CARDIAC REPROGRAMMING

20:25 – 20:33 **Xinxiu Xu**
University of Pittsburgh, School of Medicine, USA
IPSC MODEL OF HYPOPLASTIC LEFT HEART SYNDROME REVEALS METABOLIC AND CELL STRESS PATHWAYS LINKED TO CLINICAL OUTCOME

20:35 – 20:43 **Zaniar Ghazizadeh**
Yale University, School of Medicine, USA
A METASTABLE ATRIAL STATE UNDERLIES THE PRIMARY GENETIC SUBSTRATE FOR MYL4 MUTATION-ASSOCIATED ATRIAL FIBRILLATION

20:45 – 20:53 **Tomoya Uchimura**
Kyoto University, Japan
GENERATION OF SKELETAL MUSCLE FIBERS FROM HUMAN PLURIPOTENT STEM CELLS RECAPITULATING A FATIGUE SYMPTOM OF DUCHENNE MUSCULAR DYSTROPHY IN VITRO

20:55 – 21:03 **Lika Drakhlis**
Hannover Medical School, Germany
RECAPITULATING EARLY EMBRYONIC HEART DEVELOPMENT WITH HUMAN PLURIPOTENT STEM CELLS

21:05 – 21:13 **Oisín King**
Imperial College London, UK
INFLUENCE OF PERFUSABLE MICROVASCULATURE ON EXCITATION-CONTRACTION COUPLING IN IPSC-DERIVED MYOCARDIUM

21:15 – 21:30 **Stephanie Protze**
McEwen Stem Cell Institute, University Health Network, Canada
MODELING DEVELOPMENT AND FUNCTION OF THE CARDIAC CONDUCTION SYSTEM FOR BIOLOGICAL PACEMAKER APPLICATIONS

THURSDAY, 25 JUNE *(continued)*

20:00 – 21:45 **Concurrent - Tissue Stem Cells and Regeneration: Early Development and Pluripotency**
Sponsored by: CREM: Center for Regenerative Medicine, Boston University and Boston Medical Center

Session Chair: Konrad Hochedlinger
Massachusetts General Hospital, USA

20:00 – 20:05 **Overview - Tissue Stem Cells and Regeneration: Early Development and Pluripotency**

Konrad Hochedlinger
Massachusetts General Hospital, USA

20:05 – 20:20 **Fuchou Tang**
Peking University, China
DECODING GENE REGULATION NETWORK IN HUMAN GERMLINE CELLS BY SINGLE-CELL FUNCTIONAL GENOMICS APPROACHES

20:25 – 20:33 **Derk Ten Berge**
Erasmus Medical Center, University Medical Center, Netherlands
IN VITRO CAPTURE AND CHARACTERIZATION OF EMBRYONIC ROSETTE-STAGE PLURIPOTENCY, A NOVEL NAIVE-PRIMED PLURIPOTENCY INTERMEDIATE WITH A UNIQUE EPIGENOME

20:35 – 20:43 **Xi Chen**
University of Southern California, USA
DERIVATION OF EMBRYONIC STEM CELLS FROM MULTIPLE AVIAN SPECIES

20:45 – 20:53 **Peng Li**
Nankai University, China
COPS5 SAFEGUARDS GENOMIC STABILITY OF EMBRYONIC STEM CELLS THROUGH REGULATING CELLULAR METABOLISM AND DNA REPAIR

20:55 – 21:03 **Chen Dong**
Washington University in St. Louis, USA
DERIVATION OF TROPHOBLAST STEM CELLS FROM NAÏVE HUMAN PLURIPOTENT STEM CELLS

21:05 – 21:13 **Michael Pokrass**
Johns Hopkins University School of Medicine, USA
ERK SIGNALING BIFURCATION AT MITOTIC EXIT PATTERNS THE MOUSE PREIMPLANTATION EMBRYO

21:15 – 21:30 **Janet Rossant**
Hospital for Sick Children, Canada
DEFINING TOTIPOTENCY IN MOUSE STEM CELLS USING CRITERIA OF INCREASING STRINGENCY

THURSDAY, 25 JUNE *(continued)*

20:00 – 21:45 **Concurrent - Tissue Stem Cells and Regeneration: Epithelial Stem Cells**

Session Chair: Valentina Greco
Yale Stem Cell Center, Yale Medical School, USA

20:00 – 20:05 **Overview - Tissue Stem Cells and Regeneration: Epithelial Stem Cells**

Valentina Greco
Yale Stem Cell Center, Yale Medical School, USA

20:05 – 20:20 **Danelle Devenport**
Princeton University, USA
FEEDBACK BETWEEN MORPHOGENESIS AND CELL FATE DURING EPITHELIAL PATTERNING

20:25 – 20:33 **Yonglong Wei**
University of Texas Southwestern Medical Center, USA
LINEAGE TRACING OF ZONAL POPULATIONS REVEALS THAT MID-LOBULAR HEPATOCYTES PREFERENTIALLY REPOPULATE THE LIVER

20:35 – 20:43 **Farnaz Shamsi**
Joslin Diabetes Center, USA
VASCULAR SMOOTH MUSCLE-DERIVED PROGENITORS CONTRIBUTE TO BROWN ADIPOCYTE DEVELOPMENT

20:45 – 20:53 **Sharif Iqbal**
University of Helsinki, Finland
AGE AND INJURY-INDUCED STROMAL ASPORIN MODULATES REGENERATION OF THE SMALL INTESTINAL EPITHELIUM

20:55 – 21:03 **Valerie Gouon-Evans**
Boston University, USA
INJECTIONS OF NUCLEOSIDE MODIFIED MRNA ENCODING VEGF-A INDUCE CHOLANGIOCYTE-DRIVEN LIVER REGENERATION IN MOUSE MODELS OF ACUTE AND CHRONIC LIVER INJURIES

21:05 – 21:13 **Ya-Chieh Hsu**
Harvard University, USA
STRESS HORMONE CORTICOSTERONE GOVERNS HAIR FOLLICLE STEM CELL QUIESCENCE BY SUPPRESSING A DERMAL NICHE ACTIVATOR GAS6

21:15 – 21:30 **Eugenia Piddini**
University of Bristol, UK
CELL COMPETITION IN EPITHELIAL REPAIR

22:00 – 23:59 **Poster Session II**

FRIDAY, 26 JUNE

05:00 – 07:00 **Poster Session III**

09:00 – 10:30 **Women in Science Discussion Panel**

11:00 – 12:45 **Concurrent - Cellular Identity: Hematopoietic and Endothelial Cells**
Sponsored by Agilent Technologies

Session Chair: Hongkui Deng
Peking University, Beijing, China

11:00 – 11:05 **Overview- Cellular Identity: Hematopoietic and Endothelial Cells**
Hongkui Deng
Peking University, Beijing, China

11:05 – 11:20 **Shahin Rafii**
Cornell Medical College & Angiocrine Bioscience, USA
ADAPTABLE AND HEMODYNAMIC H MAN VASCULOGENIC ENDOTHELIAL CELLS FOR ORGANOGENESIS AND TUMORIGENESIS

11:25 – 11:33 **Justin Brumbaugh**
University of Colorado Boulder, USA
SUPPRESSING METHYLATION AT H3K9 AND H3K36 REVEALS DISTINCT ROLES FOR HISTONE MODIFICATIONS DURING DIFFERENTIATION AND HOMEOSTASIS

11:35 – 11:43 **Rong Lu**
University of Southern California, USA
MOLECULAR SIGNATURES DISTINGUISH FUNCTIONAL HETEROGENEITY OF MOUSE HEMATOPOIETIC STEM CELLS

11:45 – 11:53 **Carlos-Filipe Pereira**
Lund University, Sweden
GENERATING HUMAN TYPE 1 DENDRITIC CELLS BY DIRECT CELL REPROGRAMMING

11:55 – 12:03 **Marlies Rossmann**
Harvard Stem Cell Institute, USA
TRANSCRIPTIONAL REGULATION OF MITOCHONDRIAL METABOLISM BY TIF1GAMMA DRIVES ERYTHROID PROGENITOR DIFFERENTIATION

12:05 – 12:13 **Luke Harland**
University of Oxford, UK
EOMESODERMIN GOVERNS THE HEMOGENIC COMPETENCE OF MURINE YOLK SAC MESODERMAL PROGENITORS

12:15 – 12:30 **Shangqin Guo**
Yale University, USA
AN UNUSUAL CELLULAR STATE OCCUPIED BY THE FAST CYCLING HEMATOPOIETIC PROGENITORS

FRIDAY, 26 JUNE *(continued)*

11:00 – 12:45 **Concurrent - Clinical Applications: Cardiac and Muscle**

Session Chair: Charles E. Murry
University of Washington, USA

11:00 – 11:05 **Overview- Clinical Applications: Cardiac and Muscle**
Charles E. Murry
University of Washington, USA

11:05 – 11:20 **Milica Radisic**
University of Toronto, Canada
ADVANCES IN HEART-ON-A-CHIP ENGINEERING

11:25 – 11:33 **Seraina Domenig**
ETH Zürich, Switzerland
GENERATION OF IPSC-DERIVED MYOGENIC PRECURSORS TO TREAT DUCHENNE MUSCULAR DYSTROPHY IN A MOUSE MODEL

11:35 – 11:43 **Irene De Lazaro**
Harvard University, USA
TRANSIENT REPROGRAMMING OF RAT AND MOUSE CARDIOMYOCYTES IN VITRO AND IN VIVO

11:45 – 11:53 **Xujie Liu**
Boston Children's Hospital, USA
INHIBITION OF ROS-MEDIATED CAMKII ACTIVATION RESTORES ABNORMAL CALCIUM HANDLING AND IMPAIRED CONTRACTION IN BTHH SYNDROME

11:55 – 12:03 **Frank Secreto**
Mayo Clinic, USA
SUCCESSFUL ENGRAFTMENT OF HUMAN IPSC-DERIVED CARDIAC LINEAGE CELLS IN A MODEL OF RIGHT VENTRICLE HEART DISEASE USING IMMUNOSUPPRESSED MACAQUES

12:05 – 12:13 **Sebastien Uzel**
Harvard University, USA
ENGINEERED HUMAN CARDIAC TISSUES WITH PERFUSABLE EMBEDDED VASCULATURE

12:15 – 12:30 **Rick Horwitz**
Allen Institute for Cell Science, USA
CREATING A LANDSCAPE OF STEM CELL SIGNATURES

FRIDAY, 26 JUNE *(continued)*

11:00 – 12:45 **Concurrent - Modeling Development and Disease: Gene Editing in Early Embryo and Pluripotent Stem Cells**

Session Chair: Rudolf Jaenisch
Whitehead Institute for Biomedical Research, USA

11:00 – 11:05 **Overview- Modeling Development and Disease: Gene Editing in Early Embryo and Pluripotent Stem Cells**

Rudolf Jaenisch
Whitehead Institute for Biomedical Research, USA

11:05 – 11:20 **Giuseppe Testa**
University of Milan, Italy
DEVELOPMENTAL DISEASE MODELS AT SINGLE-CELL RESOLUTION: SCIENTIFIC AND ETHICAL BENCHMARKING

11:25 – 11:33 **Roni Zaken Gallily**
The Hebrew University of Jerusalem, Israel
ANALYSIS OF HAPLOINSUFFICIENCY DISORDERS IN HUMAN EMBRYONIC STEM CELLS

11:35 – 11:43 **Kristina Godek**
Geisel School of Medicine at Dartmouth, USA
MECHANISMS OF ANEUPLOIDY IN HUMAN EMBRYONIC STEM CELLS

11:45 – 11:53 **Jonathan Bayerl**
Weizmann Institute of Science, Israel
DEFINING ALTERNATIVE HUMAN NAÏVE PLURIPOTENCY CONDITIONS DEVOID OF MEK/ERK INHIBITORS

11:55 – 12:03 **Deniz Aksel**
Harvard University, USA
MEASURING AND CONTROLLING THE SPATIOTEMPORAL DYNAMICS OF FGF SIGNALING IN A HUMAN GASTRULATION MODEL

12:05 – 12:13 **Kirstin Matthews**
Rice University, USA
EMBRYOIDS, SYNTHETIC EMBRYOS AND CELL-CULTURE MODELS OF EARLY HUMAN DEVELOPMENT: THE NEED FOR IMPROVED NAMING CONVENTIONS

12:15 – 12:30 **Shoukhrat Mitalipov**
Oregon Health & Science University, USA
DNA REPAIR RESPONSE IN HUMAN EMBRYOS

FRIDAY, 26 JUNE *(continued)*

11:00 – 12:45 **Concurrent - Tissue Stem Cells and Regeneration: Cancer and Stem Cells**

Session Chair: Hans Clevers
Hubrecht Institute, Netherlands

11:00 – 11:05 **Overview - Tissue Stem Cells and Regeneration: Cancer and Stem Cells**

Hans Clevers
Hubrecht Institute, Netherlands

11:05 – 11:20 **Robert Blelloch**
University of California, San Francisco, USA
MICRORNA REGULATION OF PLURIPOTENCY AND DEVELOPMENT

11:25 – 11:33 **Katie Cockburn**
Yale University, USA
CO-OCCURING PROLIFERATION AND DIFFERENTIATION BEHAVIORS DEFINE EPIDERMAL REGENERATION

11:35 – 11:43 **Qi Sun**
New York University, School of Medicine, USA
ONCOGENIC MELANOCYTE STEM CELLS, DRIVEN BY REGENERATIVE NICHE SIGNALS, GIVE RISE TO HETEROGENEOUS MELANOMA RESEMBLING HUMAN MELANOMA

11:45 – 11:53 **Alicia McConnell**
Boston Children's Hospital, USA
AN ENCOMPASSING PRECURSOR LESION ATTRACTOR STATE PRECEDES NEURAL CREST REACTIVATION IN MELANOMA

11:55 – 12:03 **Phillip Karpowicz**
University of Windsor, Canada
THE CIRCADIAN CLOCK GENE, BMAL1, SUPPRESSES TUMORIGENESIS BY REGULATING INTESTINAL STEM CELL SIGNALING

12:05 – 12:13 **Nick Barker**
Institute of Medical Biology, Singapore
AQP5 ENRICHES FOR STEM CELLS AND CANCER ORIGINS IN THE DISTAL STOMACH

12:15 – 12:30 **Carla Kim**
Boston Children's Hospital, USA
LUNG EPITHELIAL CELL ORGANOIDS MODEL CELL-CELL INTERACTIONS IN LUNG DISEASE AND LUNG CANCER

FRIDAY, 26 JUNE *(continued)*

11:00 – 12:45 **Concurrent - Tissue Stem Cells and Regeneration: Neural**

Session Chair: Fiona Doetsch
University of Basel, Biozentrum, Switzerland

11:00 – 11:05 **Overview - Tissue Stem Cells and Regeneration: Neural**

Fiona Doetsch
University of Basel, Biozentrum, Switzerland

11:05 – 11:20 **Alejandro Schinder**
Leloir Institute, Argentina
MECHANISMS OF NEUROGENESIS-INDUCED REMODELING IN THE ADULT AND AGING HIPPOCAMPUS

11:25 – 11:33 **Reilly Kidwell**
University of California San Diego, USA
FUNCTIONAL CHARACTERIZATION OF A NOVEL GENE IN THE ANTIGEN PRESENTATION PATHWAY ALLOWS SPECIFIC IMMUNOTHERAPEUTIC TARGETING OF GLIOBLASTOMA STEM CELLS

11:35 – 11:43 **Hongjun Liu**
ShanghaiTech University, China
REGENERATION OF FUNCTIONAL RETINAL GANGLION CELLS BY NEURONAL IDENTITY REPROGRAMMING

11:45 – 11:53 **Deirdre Hoban**
Lund University, Sweden
HUMANIZED MODELS FOR ASSESSING IN VIVO NEURAL CONVERSION

11:55 – 12:03 **Michelle Vincendeau**
Helmholtz Zentrum München, Germany
ELEVATED HERV-K(HML-2) EXPRESSION NEGATIVELY IMPACTS HUMAN CORTICAL DEVELOPMENT

12:05 – 12:13 **Panteleimon Rompolas**
University of Pennsylvania, USA
TISSUE WIDE COORDINATION OF CORNEAL HOMEOSTASIS REVEALED AT THE SINGLE STEM CELL LEVEL BY 2-PHOTON LIVE IMAGING

12:15 – 12:30 **Kapil Bharti**
National Eye Institute, NIH, USA
INITIATING A PHASE I /IIA CLINICAL TRIAL FOR AN AUTOLOGOUS IPS CELL THERAPY FOR MACULAR DEGENERATION: FROM BENCH-TO-BEDSIDE

13:00 – 21:00 **EXHIBIT HALL**

FRIDAY, 26 JUNE *(continued)*

INNOVATION SHOWCASES (See page 91 for details)

13:00 – 13:30 *Presented by: Ajinomoto*
TOWARDS THERAPIES COMBINING REPROGRAMMING AND GENETIC ENGINEERING

Hajime Onuki
Ajinomoto, Japan

Marius Wernig
Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, USA

13:00 – 13:30 *Presented by: Olympus*
FROM PLURIPOTENCY TO ORGANOIDS: LIVE INCUBATION MONITORING IDENTIFIES CLUES FOR REPRODUCIBILITY

Takanori Takebe
Tokyo Medical and Dental University, Japan

13:00 – 14:00 *Presented by: 10x Genomics*
IDENTIFICATION OF DRUGS BLOCKING SARS-COV-2 INFECTION USING HUMAN PLURIPOTENT STEM CELL-DERIVED COLONIC ORGANOIDS

Shuibing Chen
Weill Cornell Medical College, USA

13:00 – 14:00 *Presented by: MaxWell Biosystems*
NEXT GENERATION HIGH-CONTENT ELECTROPHYSIOLOGY SYSTEMS FOR EFFICIENT FUNCTIONAL CHARACTERIZATION OF HUMAN IPSC-DERIVED NEURONS

Urs Frey
MaxWell Biosystems, USA

Jan Mueller
MaxWell Biosystems, USA

Silvia Ronchi
Bio Engineering Lab, ETH Zurich, Switzerland

Xinyue Yuan
Bio Engineering Lab, ETH Zurich, Switzerland

13:00 – 14:00 *Presented by: Molecular Devices, LLC*
GAIN DEEPER INSIGHTS INTO CELLULAR 3D STRUCTURES WITH WATER IMMERSION OBJECTIVES FOR HIGH-CONTENT IMAGING

Tim Baranowski
Molecular Devices, LLC, USA

FRIDAY, 26 JUNE *(continued)*

13:00 – 14:00 *Presented by: STEMCELL Technologies, Canada*
ADVANCED BRAIN ORGANOID CO-CULTURE SYSTEMS

Erin Knock
STEMCELL Technologies, Canada

13:00 – 14:00 *Presented by: Thermo Fisher Scientific*
ALEXANDER DISEASE AND GIANT AXONAL NEUROPATHY: CELL BIOLOGICAL INSIGHTS FROM IPSC-DERIVED ASTROCYTE AND NEURON DISEASE MODELS

Natasha T. Snider
Dept of Cell Biology and Physiology, UNC-Chapel Hill, USA

14:00 – 16:00 **Poster Session IV**

16:00 – 18:00 **Plenary IV: Dissecting Organogenesis**
Sponsored by: Vertex Cell and Gene Therapies

Session Chair: Melissa Little
Murdoch Childrens Research Institute, Australia

16:00 – 16:05 **Dissecting Organogenesis Session Introduction**

Session Chair: Melissa Little
Murdoch Childrens Research Institute, Australia

16:05 – 16:20 **Madeline Lancaster**
Medical Research Council Laboratory of Molecular Biology, UK
NEW DIRECTIONS FOR HUMAN BRAIN ORGANOIDS

16:25 – 16:40 **Miki Ebisuya**
EMBL Barcelona, Spain
HUMAN TIME VS. MOUSE TIME WITH RECAPITULATED SYSTEMS

16:45 – 17:00 **Hans Snoeck**
Columbia University Medical Center, USA
LUNG ORGANOIDS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

17:05 – 17:13 **April Craft**
Boston Children's Hospital, USA
KNEE IN A DISH: SPECIFICATION OF ESC-DERIVED JOINT PROGENITORS WITH DIFFERENTIAL JOINT TISSUE POTENTIAL

17:15 – 17:23 **Todd McDevitt**
University of California San Francisco, USA
AXIAL ELONGATION OF CAUDALIZED HUMAN PLURIPOTENT STEM CELL ORGANOIDS MIMICS NEURAL TUBE DEVELOPMENT

17:25 – 17:30 **ISSCR Momentum Award Presentation**

17:30 – 17:50 **Mitinori Saitou**
Institute for the Advanced Study of Human Biology, Kyoto University, Japan
ISSCR MOMENTUM AWARD LECTURE: MECHANISM AND IN VITRO RECONSTITUTION OF MAMMALIAN GERM-CELL DEVELOPMENT

FRIDAY, 26 JUNE *(continued)*

20:00 – 21:45 **Concurrent - Cellular Identity: Early Development and Pluripotency**

Session Chair: Hans R. Schöler
Max Planck Institute for Molecular Biomedicine, Germany

20:00 – 20:05 **Overview - Cellular Identity: Early Development and Pluripotency**
Hans R. Schöler
Max Planck Institute for Molecular Biomedicine, Germany

20:05 – 20:20 **Richard Young**
Whitehead Institute for Biomedical Research, USA
NUCLEAR CONDENSATES IN GENE CONTROL AND CELL IDENTITY

20:25 – 20:33 **Atilgan Yilmaz**
The Hebrew University of Jerusalem, Israel
ESSENTIAL GENES FOR DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS INTO ALL THREE GERM LAYERS

20:35 – 20:43 **Peter Nicholls**
Whitehead Institute, USA
TWO 129-STRAIN LOCI ARE SUFFICIENT TO CONFER SUSCEPTIBILITY TO SPONTANEOUS TERATOMAS IN MICE

20:45 – 20:53 **Zsuzsanna Izsvak**
Max Delbrück Center, Germany
ANTAGONISM BETWEEN YOUNG AND OLD TRANSPOSABLE ELEMENTS MEDIATES INTRA-ORGANISM SELECTION IN THE EARLY HUMAN EMBRYO

20:55 – 21:03 **Erin Slatery**
University of Cambridge, UK
PRIMATE EMBRYO PROFILING FROM ZYGOTE TO GASTRULATION DEMARCATES PLURIPOTENT STATES IN VITRO

21:05 – 21:13 **Sicong Wang**
Medical College of Wisconsin, USA
APEX2-BASED SPATIAL PROTEOMIC ANALYSIS OF A HUMAN PLURIPOTENT STEM CELL-DERIVED EPIBLAST-LIKE MODEL

21:15 – 21:30 **Joanna Wysocka**
Stanford University, USA
A HILL ON WADDINGTON'S EPIGENETIC LANDSCAPE: HOW CAN CELLS EXPAND THEIR DIFFERENTIATION POTENTIAL DURING DEVELOPMENT?

FRIDAY, 26 JUNE *(continued)*

20:00 – 21:45 **Concurrent - Cellular Identity: Neural**
Arnold R. Kriegstein
University of California, San Francisco, USA

20:00 – 20:05 **Overview- Cellular Identity: Neural**

Arnold R. Kriegstein
University of California, San Francisco, USA

20:05 – 20:20 **Justin Ichida**
University of Southern California, USA
MITIGATING ANTAGONISM BETWEEN TRANSCRIPTION AND PROLIFERATION ALLOWS NEAR-DETERMINISTIC CELLULAR REPROGRAMMING

20:25 – 20:33 **Marta Zuzic**
Technische Universität Dresden, Germany
FORWARD PROGRAMMING OF PHOTORECEPTORS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

20:35 – 20:43 **Kristen Fread**
University of Virginia, USA
CELL TRAJECTORY MAPPING OF MOUSE IN VITRO NEUROGENESIS BY SINGLE-CELL MASS CYTOMETRY IDENTIFIES DISTINCT NEURAL SUBTYPES AND PROVIDES A COMPARATIVE ANALYSIS OF IN VITRO AND IN VIVO NEURAL DEVELOPMENT

20:45 – 20:53 **Lize Meert**
Erasmus Medical Center, Netherlands
THE ROLE OF AUTISM-RELATED CHROMATIN REMODELER CHD8 AND ITS INTERACTORS IN HUMAN NEURAL STEM CELL IDENTITY

20:55 – 21:03 **Janghwan Kim**
Korea Research Institute of Bioscience and Biotechnology (KRIBB), Korea
SINGLE-CELL ANALYSIS ON DIRECT REPROGRAMMING TO NEURAL PROGENITORS BY PLURIPOTENCY FACTORS

21:05 – 21:13 **Kate Galloway**
Massachusetts Institute of Technology (MIT), USA
HARNESSING P53 TO STABILIZE ACCELERATED, DUAL-PHASE REPROGRAMMING

21:15 – 21:30 **Don W. Cleveland**
University of California, San Diego, USA
IDENTITY THEFT: CONVERSION OF ASTROCYTES INTO NEURONS REVERSES PARKINSON'S-LIKE DISEASE

FRIDAY, 26 JUNE *(continued)*

20:00 – 21:45 **Concurrent - Clinical Applications: Pancreas**

Session Chair: Douglas A Melton
Harvard University, USA

20:00 – 20:05 **Overview - Clinical Applications: Pancreas**

Douglas A Melton
Harvard University, USA

20:05 – 20:20 **Kevin D'Amour**
ViaCyte, USA
DEVELOPMENT OF STEM-CELL DERIVED, ISLET REPLACEMENT FOR TYPE 1 DIABETES

20:25 – 20:33 **Jeffrey Millman**
Washington University, School of Medicine, USA
DEVELOPMENT OF FUNCTIONAL HUMAN ISLETS FOR CELL REPLACEMENT THERAPY TO TREAT DIABETES

20:35 – 20:43 **Samantha Collins**
Harvard University, USA
BETA AND ALPHA CELL ENRICHMENT IMPROVES STEM CELL GRAFT FUNCTION IN MICE

20:45 – 20:53 **Xi Wang**
Cornell University, USA
A NANOFIBER-SKIN, HYDROGEL-CORE ENCAPSULATION DEVICE FOR SAFE DELIVERY OF INSULIN-PRODUCING CELLS

20:55 – 21:03 **Han Zhu**
University of California San Diego, USA
MUILTIOMIC SINGLE CELL ANALYSIS TO IDENTIFY MECHANISMS OF PANCREATIC BETA CELL MATURATION DURING HUMAN PLURIPOTENT STEM CELL DIFFERENTIATION

21:05 – 21:13 **Connor Wiegand**
University of Pittsburgh, USA
ENGINEERING VASCULARIZED ISLET ORGANOIDS FROM HUMAN INDUCED PLURIPOTENT STEM CELL

21:15 – 21:30 **Hongkui Deng**
Peking University, China
GENERATING FUNCTIONAL PANCREATIC ENDOCRINE CELLS FROM EXTENDED PLURIPOTENT STEM CELL

FRIDAY, 26 JUNE *(continued)*

20:00 – 21:45 **Concurrent - Modeling Development and Disease: Hematopoietic and Endothelial Cells**

Kristin Baldwin
Scripps Research Institute, USA

20:00 – 20:05 **Overview - Modeling Development and Disease: Hematopoietic and Endothelial Cells**

Kristin Baldwin
Scripps Research Institute, USA

20:05 – 20:20 **Steve McCarroll**
Harvard Medical School and Broad Institute of MIT and Harvard, USA
MONOGENIC AND POLYGENIC INHERITANCE BECOME INSTRUMENTS FOR CLONAL SELECTION IN HEMATOPOIETIC STEM CELLS

20:25 – 20:33 **Ashlee Conway**
Harvard Medical School, USA
ENGINEERING ADULT RED BLOOD CELLS FROM HUMAN IPSCS THAT SICKLE IN VITRO: A NOVEL THERAPEUTIC PLATFORM FOR SICKLE CELL ANEMIA

20:35 – 20:43 **Stephanie Luff**
Washington University in St Louis, USA
GENERATION OF RETINOIC ACID-DEPENDENT DEFINITIVE HEMATOPOIETIC PROGENITORS FROM HUMAN PLURIPOTENT STEM CELLS

20:45 – 20:53 **Geraldine Jowett**
King's College London, UK
HUMAN TYPE-1 INNATE LYMPHOID CELLS SECRETE TGFB1, DRIVING EPITHELIAL AND MATRIX REMODELLING IN HIPSC-DERIVED INTESTINAL ORGANOIDS

20:55 – 21:03 **Aaron Burberry**
Harvard University, USA
THE ALS/FTD ASSOCIATED GENE PRODUCT C9ORF72 FUNCTIONS IN MURINE HEMATOPOIETIC STEM AND PROGENITOR CELLS

21:05 – 21:13 **Andrea Ditadi**
Ospedale San Raffaele, Italy
MOUSE EMBRYONIC PRE-HSCS LYMPHOCYTES CONTRIBUTE TO AUTOIMMUNITY

21:15 – 21:30 **Ravindra Majeti**
Stanford University, USA
MODELING HUMAN PRE-LEUKEMIC HEMATOPOIETIC STEM CELLS USING CRISPR ENGINEERING

FRIDAY, 26 JUNE *(continued)*

20:00 – 21:45 **Concurrent - Tissue Stem Cells and Regeneration: Cardiac and Muscle**

Session Chair: Stephanie Protze
McEwen Stem Cell Institute, University Health Network, Canada

20:00 – 20:05 **Overview- Tissue Stem Cells and Regeneration: Cardiac and Muscle**

Stephanie Protze
McEwen Stem Cell Institute, University Health Network, Canada

20:05 – 20:20 **Kristy Red-Horse**
Stanford University, USA
USING DEVELOPMENTAL BIOLOGY TO INFORM TISSUE REGENERATION

20:25 – 20:33 **Lauren Koepke**
Stanford University, USA
MOUSE AND HUMAN ARTICULAR CARTILAGE REGENERATION BY ACTIVATION OF SKELETAL STEM CELLS

20:35 – 20:43 **Carlos Aguilar**
University of Michigan, USA
SINGLE CELL DECONSTRUCTION OF MUSCLE STEM CELL SENSITIVITY TO THE NEURO-MUSCULAR SYNAPSE DURING AGING

20:45 – 20:53 **Sahar Tavakoli**
Harvard University, USA
ZEBRAFISH CHEMICAL COMPOUND SCREEN UNCOVERS INDUCERS OF SKELETAL MUSCLE ENGRAFTMENT ACROSS SPECIES

20:55 – 21:03 **Wenshu Zeng**
Hong Kong University of Science and Technology, Hong Kong
TRANSLATIONAL CONTROL OF MOUSE MUSCLE STEM CELLS QUIESCENCE EXIT BY CPEB1

21:05 – 21:13 **Suraj Kannan**
Johns Hopkins School of Medicine, USA
TRANSCRIPTOMIC ENTROPY ENABLES CROSS-STUDY AND CROSS-SPECIES QUANTIFICATION OF CARDIOMYOCYTE MATURATION AT SINGLE CELL LEVEL

21:15 – 21:30 **Masaki Ieda**
University of Tsukuba, Japan
DIRECT REPROGRAMMING INTO CARDIOVASCULAR LINEAGES

FRIDAY, 26 JUNE *(continued)*

23:00 – 01:15 **Plenary V: Stem Cells and Aging**

Session Chair: Urban Lendahl
Karolinska Institute, Sweden

23:00 – 23:05 **Stem Cells and Aging Session Introduction**

Urban Lendahl
Karolinska Institute, Sweden

23:05 – 23:20 **Beth Stevens**
Boston Children's Hospital, USA
MAPPING MICROGLIA STATE AND FUNCTION IN ALZHEIMER'S DISEASE

23:25 – 23:40 **Guanghui Liu**
Chinese Academy of Sciences, China
PROGRAMMING AND REPROGRAMING OF AGING

23:45 – 23:59 **Emi Nishimura**
Tokyo Medical and Dental University, Japan
STEM CELL COMPETITION FOR SKIN HOMEOSTASIS AND AGING

SATURDAY, 27 JUNE

00:05 – 00:20 **MUSCLE STEM CELL SELF RENEWAL IS REGULATED BY ACETYLATION OF PAX7**
Rudnicki, Michael
Ottawa Hospital Research Institute, Canada

00:25 – 00:33 **Raffaella Lucciola**
The Salk Institute for Biological Studies, USA
PROFILING OF CHROMATIN ACCESSIBILITY IN HUMAN INDUCED NEURONS IN ALZHEIMER'S DISEASE

00:35 – 00:43 **Miyeko Mana**
Massachusetts Institute of Technology (MIT), USA
A DIET-INDUCED PPAR/FAO AXIS ENHANCES STEMNESS AND ESTABLISHES A METABOLIC LIABILITY IN ADULT INTESTINAL STEM CELLS

00:45 – 00:50 **ISSCR Tobias Award Presentation**

00:50 – 01:10 **Margaret A. Goodell**
Baylor College of Medicine, USA
ISSCR TOBIAS AWARD LECTURE: IMMORTAL HEMATOPOIETIC STEM CELLS IN AGING AND CANCER

05:00 – 07:00 **Poster Session V**

07:30 – 13:00 **EXHIBIT HALL**

08:00 – 10:05 **Plenary VI: Reprogramming and Regeneration**
Sponsored by: FUJIFILM

Session Chair: Amander Clark
Department of Molecular Cell and Developmental Biology, University of California, Los Angeles, USA

08:00 – 08:05 **Reprogramming and Regeneration Session Introduction**

Amander Clark
Department of Molecular Cell and Developmental Biology, University of California, Los Angeles, USA

08:05 – 08:20 **Botond Roska**
Institute of Molecular and Clinical Ophthalmology Basel, Switzerland
CELL TYPES OF THE HUMAN RETINA AND ITS ORGANOIDS AT SINGLE-CELL RESOLUTION

08:25 – 08:40 **Li Qian**
University of North Carolina, Chapel Hill, USA
CARDIAC REPROGRAMMING: LEVERAGING BASIC SCIENCE FOR TRANSLATIONAL APPLICATION

SATURDAY, 27 JUNE *(continued)*

08:45 – 08:53 **Thai Tran**
Merck & Co Inc., USA
METABOLIC CONTROL OF WNT SIGNALING AND STEMNESS IN COLORECTAL CANCER

08:55 – 09:10 **Shinya Yamanaka**
Gladstone Institutes and Center for IPS Cell Research & Application, Kyoto University, Japan
RECENT PROGRESS IN iPS CELL RESEARCH AND APPLICATION

09:15 – 09:30 **R. Alta Charo**
University of Wisconsin, USA
ANNE MCLAREN MEMORIAL LECTURE: WHAT'S NEXT FOR HUMAN GENOME EDITING POLICY?

09:35 – 09:40 **ISSCR Achievement Award Presentation**

09:40 – 10:00 **Fred H. Gage**
The Salk Institute for Biological Studies, USA
ISSCR ACHIEVEMENT AWARD LECTURE: DNA DAMAGE AND REPAIR IN THE NEURONAL LINEAGE

10:00 – 11:30 **JI Career Panel**

12:00 – 14:00 **Poster Session VI**

14:00 – 15:10 **Special Session: ISSCR Response to COVID-19**
Sponsored by: BlueRock Therapeutics

Session Chair: Deepak Srivastava
Gladstone Institutes, USA

14:03 – 14:06 **Address to ISSCR: COVID-19**

Anthony S. Fauci
National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), USA

14:06 – 14:23 **Jennifer Doudna**
University of California, Berkeley and HHMI, USA

14:23 – 14:30 **Jayaraj Rajagopal**
MGH/Harvard Medical School, USA

14:30 – 14:37 **Benjamin tenOever**
Icahn School of Medicine at Mount Sinai, USA

14:37 – 15:10 **COVID-19 Panel Discussion**

SATURDAY, 27 JUNE *(continued)*

16:00 – 18:30 **Plenary VII: Clinical Innovation and Gene Editing**
Sponsored by: BlueRock Therapeutics

Session Chair: Jane S. Lebkowski
Regenerative Patch Technologies, USA

16:00 – 16:05 **Clinical Innovation and Gene Editing Session Introduction**

Jane S. Lebkowski
Regenerative Patch Technologies, USA

16:05 – 16:10 **Christine L. Mummery**
Leiden University Medical Center, Netherlands
PRESIDENT-ELECT REMARKS

16:10 – 16:25 **PATIENT ADVOCATE ADDRESS**

Matthew Might
University of Alabama, Birmingham, USA

16:25 – 16:40 **Brian Wainger**
Harvard Medical School and Massachusetts General Hospital, USA
JOHN MCNEISH MEMORIAL LECTURE: HUMAN STEM CELL MODELING TO CLINICAL TRIAL: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED PHASE II TRIAL OF EZOGABINE ON CORTICAL AND SPINAL MOTOR NEURON EXCITABILITY IN AMYOTROPHIC LATERAL SCLEROSIS

16:45 – 16:53 **Benjamin Reubinoff**
Hadassah Hebrew University Medical Center, Israel
PHASE I/IIA CLINICAL TRIAL OF HUMAN EMBRYONIC STEM CELL (HESC)-DERIVED RETINAL PIGMENTED EPITHELIUM (RPE) TRANSPLANTATION IN ADVANCED DRY FORM AGE-RELATED MACULAR DEGENERATION (AMD): INTERIM RESULTS

16:55 – 17:03 **Bernhard Gentner**
IRCCS San Raffaele Scientific Institute, Italy
SUPRAPHYSIOLOGIC ENZYME RECONSTITUTION BY TRANSPLANTATION OF GENETICALLY-ENGINEERED AUTOLOGOUS HEMATOPOIETIC STEM AND PROGENITOR CELLS SHOWS PRELIMINARY BENEFITS IN CHILDREN WITH HURLER DISEASE

17:05 – 17:20 **Michel Sadelain**
Memorial Sloan-Kettering Cancer Center, USA
CAR T CELLS: THE EMERGENCE OF SYNTHETIC IMMUNITY

17:25 – 17:40 **Katherine High**
Rockefeller University, USA
GENE THERAPY FOR GENETIC DISEASE: LESSONS LEARNED FROM CLINICAL DEVELOPMENT PROGRAMS

SATURDAY, 27 JUNE *(continued)*

17:45 – 18:15 **Sekar Kathiresan**
Verve Therapeutics, USA
KEYNOTE ADDRESS: GENOME EDITING MEDICINES TO MIMIC MUTATIONS PROTECTIVE AGAINST HEART ATTACK

18:25 – 18:30 **Closing Remarks**

Christine L. Mummery
Leiden University Medical Center, The Netherlands

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EXHIBIT HALL 1 - BIOTECH INSTRUMENTATION A-K	EXHIBIT HALL 3- CELL & TISSUE CULTURE REAGENTS A-K
10x Genomics	Able
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THE NEW YORK STEM CELL FOUNDATION RESEARCH INSTITUTE

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The NYSCF Research Institute accelerates cures for the major diseases of our time through stem cell research and technology. NYSCF invented a fully automated platform that reprograms hundreds of iPSC lines monthly, available to laboratories worldwide. As an independent, privately funded non-profit, NYSCF takes a team science approach, building and funding a collaborative stem cell community to advance the understanding and treatment of disease.

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THURSDAY, 25 JUNE

13:00 – 13:30

CELL MICROSYSTEMS

EVALUATION OF CLONAL ORGANOID HETEROGENEITY AT SINGLE GASTROID RESOLUTION

Scott Magness *and* **Jarrett Bliton**, *University of North Carolina – Chapel Hill, and* **Jessica Hartman**, *Cell Microsystems, Inc., USA*

Organoids are small multi-cellular stem-cell-driven tissues that self-organize, self-renew, and possess differentiated lineages consistent with those found in vivo. For these reasons, organoids represent excellent in vitro models of functional heterogeneity found in primary tissues. However, many challenges preclude statistically meaningful analysis of functional heterogeneity in single organoids. Thus, platforms that enable analysis of functional properties of individual organoids are needed. Here we use Cytosort? Arrays (CRA) and the CellRaft AIR System from Cell Microsystems to characterize the functional heterogeneity found in clonally-derived gastroids. Gastroid forming efficiency, growth dynamics, and morphology were used as measures of phenotypic heterogeneity. Growth rates of single organoids ranged from 2.4 to 34.3 um/day in the observed population, indicating heterogeneous proliferative capacity in clonally-derived gastroids. K-means clustering of gastroids from two models of gastric preneoplasia show recapitulation of in vivo markers of gastric metaplasia (Muc6+, Tff2+; Gif+) and heterogeneous expression of markers associated with gastric cancer progression (Trp53, Foxq1, Cdh17), indicating gastroid progeny can recapitulate in vivo expression patterns and are derived from a multipotent cell-of-origin. These data highlight the variation inherent in organoid populations and suggest functional heterogeneity at the single-cell level and the divergence of biological processes that ultimately give rise to heterogenous organoids.

13:00 – 13:30

NANOSURFACE BIOMEDICAL

BUILDING A BETTER STEM CELL: IMPROVING THE MATURITY OF HUMAN STEM CELLS IN VITRO THROUGH BIOENGINEERING

Nicholas Geisse, *NanoSurface Bio, USA*

The adoption of stem cells as models of in vitro human function is often hampered by their lack of phenotypic maturity. Novel bioengineering strategies hold great promise in addressing the maturity gap, especially for cell types that are highly reliant on extracellular factors for their growth, development, and function. For example, cardiomyocytes (CMs) and skeletal muscle cells (SkMs) rely on essential cues from the extracellular matrix to control a variety of structural and functional phenotypes, and reproducing these cues in vitro is a goal of many bioengineering strategies. We will present recent advances in improving iPSC-CM and iPSC-SkM maturity that use a combinatorial approach of coupling extracellular matrix engineering with exogenous maturation cues. We will demonstrate how these strategies improve not only baseline structural and functional phenotypes, but also the ability of these cells to reproduce known in vivo toxicities that would otherwise be undetectable in traditional models. We will also show how maturity can influence the stratification of disease phenotypes in vitro, greatly improving their relevance and translation to ameliorating human disease. The strategies presented are utilized in a cell, assay, and instrument-agnostic fashion and can enable high-throughput microscopy and electrophysiology studies on bioengineered stem cell models.

13:00 – 13:30

SOLENTIM

NOVEL WORKFLOW FOR HIGH EFFICIENCY SINGLE CELL ISOLATION OF IPSCS WITH CONCURRENT DOCUMENTATION OF CLONALITY

Ian Taylor, *Solentim, UK*

Isolation of single clonal iPSCs is becoming highly desirable for research groups studying disease models or companies developing iPSC-based therapeutics and who will need to demonstrate clonality to the regulator. FACS does not work well for stem

cells, and current approaches of diluting cells into petri dishes for subsequent colony picking is tedious and highly inefficient. It is also desirable to get away from the use of Matrigel which contains mouse components. In this talk we will describe how using the VIPS instrument (acronym for Verified in situ Plate Seeding), we can isolate and document single iPSCs in well plates using a soluble peptide matrix. Data will be presented to show that a large proposition of these clones go on to form colonies and maintain their pluripotency over multiple passages.

13:00 – 14:00

BIOLOGICAL INDUSTRIES

ACADEMIA AND INDUSTRY GO HAND-IN-HAND: FROM ICM AND TROPHOBLASTS, THROUGH BBB-ON-A-CHIP, TO ALS AND DIABETES TREATMENTS

Gad Vatine, *Ben-Gurion University of the Negev, Israel,* **Yossi Buganim**, *The Hebrew University-Hadassah Medical School, Israel,* **Kfir Molakandov**, *and* **Michal Izrael**, *Kadimastem Ltd, Israel*

Pluripotent Stem cells are used as tools for basic research and to treat life threatening conditions. Cutting edge technologies and specialty media allow this to happen. Here we present 3 distinct cases of hPSC usage: (1) In order to pre-clinically test the potential of BBB-targeted gene therapy for MCT8-deficient patients, an AAV9-MCT8 was injected intravenously into a mouse model of MCT8-deficiency. The treatment resulted in re-expression of MCT8 in the in blood vessels and neural cells, rescued a deficiency in cortical parvalbumin+ inhibitory interneurons, improved TH-brain content and gene expression as well as behavioral performance; (2) Kadimastem technology enables to produce functioning cells differentiated from clinical grade pluripotent stem cells (embryonic and induced pluripotent stem cells). The company had two main cell therapy projects for the treatment of Amyotrophic Lateral Sclerosis and Diabetes; (3) During early embryogenesis, totipotent cells undergo asymmetric cell divisions, resulting in two compartments in the early embryo: the inner cell mass (ICM) that gives rise to pluripotency cells and an outer layer of trophectoderm (TE). Here, we sought to understand whether cells acquiring pluripotency or trophectoderm state, by reprogramming factors, share similar processes such as those of the early embryo.

13:00 – 14:00

BIO-TECHNE

OPTIMIZING IPSC REPROGRAMMING, ENGINEERING, AND CULTURING FOR ANIMAL-FREE GMP MANUFACTURING

Kevin Flynn, **Travis Cordie**, *Bio-Techne and* **Beau Webber**, *University of Minnesota, USA*

Tissues and cells derived from induced pluripotent stem cells (iPSCs) are on the precipice of use as autologous and allogeneic therapeutic strategies for degenerative disease, tissue regeneration, and correction of hematologic genetic diseases, such as beta-thalassemia and sickle-cell anemia. Encouraging preclinical and early clinical trial data across multiple indications has hastened the need for improvements in GMP manufacturing of iPSCs, from optimizing the efficiency of iPSC reprogramming and gene editing to minimizing the safety profile of the therapeutic by incorporating animal-free raw materials into a closed system culture process. During this session we will introduce raw material and technology advancements from Bio-Techne that address current challenges facing GMP iPSC manufacturing. In three focused presentations we will discuss: 1) novel animal-free GMP reagents for iPSC reprogramming, expansion, and differentiation, 2) efficient integration of iPSC reprogramming into GMP manufacturing, and 3) advances in iPSC gene editing methodologies and how they manage downstream product safety and process scale-up.

13:00 – 14:00

MILTENYI

FROM BENCH TO BEDSIDE – PLURIPOTENT STEM CELL DIFFERENTIATION

Agnete Kirkeby, *University of Copenhagen, Denmark and* **Sebastian Knoebel**, *Miltenyi Biotec B.V. & Co. KG, Bergisch Gladback, Germany*

Pluripotent stem cell (PSC) research is rapidly moving towards therapeutic applications. This clinical outlook requires the implementation of workflows which ensure a seamless transition from bench to bedside.

Sebastian Knoebel will present recent developments with regards to PSC maintenance, characterization, and the latest innovations in PSC differentiation.

Specifically, he will highlight how PSC workflows such as expansion and differentiation can be implemented using the new

CliniMACS Prodigy® Adherent Cell Culture System together with high-quality cell culture reagents and reliable quality control solutions.

Agnete Kirkeby will give a special focus on strategies pursued in a H2020 consortium to bring hESC-derived cell products to clinical trial for Parkinson's disease (PD). The treatment of PD has for more than 50 years relied on dopaminergic therapies that are effective early in the disease, but which are limited by severe side effects. Restoring the lost dopaminergic innervation of the striatum would represent a major advance in treating PD. Methods for producing authentic midbrain dopaminergic neurons from pluripotent cells have advanced to a stage which makes it possible to efficiently produce dopaminergic progenitors at a high purity and quality.

13:00 – 14:00

STEMCELL TECHNOLOGIES

APPLICATIONS OF ORGANOID AND ORGANOTYPIC CULTURES IN INFECTIOUS DISEASES, NEPHROTOXICITY, AND HIGHLY-RELEVANT CELL-BASED ASSAY DEVELOPMENT

Juan Hou, *Senior Scientist,* **Philipp Kramer**, *Senior Scientist,* **Martin Stahl**, *Scientist, STEMCELL Technologies, Canada*

Organoid and organotypic cultures have expanded the limit to which *in vitro* cell cultures can be used to answer scientific questions. In addition to continuously defining new ways to culture organoids from a plethora of different tissue types, as was the focus in the last decade, researchers are now actively exploring how organoids can be used as tools to expedite life sciences discoveries and the development of potential treatments. This session will provide an overview of how different types of organoids are utilized to better understand human diseases, such as COVID-19, and how organoids can be used as a more physiologically relevant method for assay development in place of immortalized cell lines. We will highlight available and upcoming fully optimized and complete organoid cell culture media and protocols from STEMCELL Technologies that enable researchers to focus efforts on addressing their scientific questions quickly, rather than on optimizing and trouble-shooting reagents to ensure consistency and quality.

13:00 – 14:00

THERMO FISHER

LEVERAGING A NOVEL PSC SUSPENSION MEDIA SYSTEM TO SCALE UP FOR GENERATION OF DIFFERENTIATED CELLS

Rebecca Sereda, *Harvard University- Stem Cell and Regenerative Bio Department, USA and* **David Kuninger**, *Thermo Fisher Scientific, USA*

3D cellular models like organoids and spheroids offer an opportunity to better understand complex biology in a more physiologically relevant context than traditional 2D models. Here we will discuss general principles and practices of 3D suspension culture of pluripotent stem cells and are pleased to introduce our new 3D suspension culture medium – Gibco? StemScale? PSC Suspension Medium, a culture system that enables efficient self-aggregation of singularized PSCs into spheroids with a robust and straightforward protocol without the need for filtration steps or microcarriers. Spheroids grown in this medium maintain robust cell expansion (typically ? 8 fold) over multiple passages with high viability and pluripotency (? 90% of cells). Efficient expansion has been demonstrated in a variety of culture formats (well plates to bioreactors). We will provide an overview of our design approach, tips and tricks learned thus far and discuss scale up strategies, including approached to cell characterization and banking. Additional data demonstrating differentiation directly from expanded PCS spheroids, maintained in suspension, will be shared for neural and cardiac lineages.

19:00 – 19:30

STEMCELL TECHNOLOGIES

DIFFERENTIATING IMMUNE CELLS FROM HUMAN PLURIPOTENT STEM CELLS IN FEEDER- AND SERUM-FREE CULTURES

Nooshin Tabatabaei-Zavareh, *Senior Scientist, STEMCELL Technologies, Canada*

Human pluripotent stem cells (hPSCs) may provide an unlimited source of immune cells for disease modeling, gene editing, and cell therapy development applications. Differentiation of hPSCs into im-

mune cells is challenging and often involves the use of serum and feeder cells, which can be difficult to standardize. We have developed multi-step feeder- and serum-free culture systems for differentiating hPSCs into T cells, NK cells, or monocytes. To generate T and NK cells, hPSCs were first induced to differentiate to mesoderm and hematopoietic lineages by formation of 3D aggregates. $CD34^+$ hematopoietic progenitors were then isolated and differentiated through intermediate $CD7^+CD5^+$ lymphoid progenitors into either $CD4^+CD8^+$ double-positive (DP) T cells or $CD56^+$ NK cells using lineage-specific culture conditions. DP T cells were matured to $CD8^+$ T cells. Monocytes were generated in a 2D culture system in which hPSCs were differentiated in sequential steps to mesoderm, hematopoietic progenitors, and then monocytes by performing medium changes in monolayer cultures and harvesting $CD14^+$ monocytes from the supernatant. hPSC-derived T cells, NK cells, and monocytes are similar to their counterparts in peripheral blood. These results show that hPSCs can be differentiated under feeder- and serum-free conditions into immune cells for basic and translational research.

19:30 – 20:00

STEMCELL TECHNOLOGIES

OPTIMIZED, SERUM-FREE, IN VITRO CULTURE CONDITIONS FOR CRISPR-MEDIATED GENOME EDITING OF CD34+ CELLS

Amanda Fentiman, *Product Manager, Hematology, STEMCELL Technologies, Canada*

The ability to genetically manipulate hematopoietic stem and progenitor cells (HSPCs) can facilitate gains in our understanding of the mechanisms that regulate hematopoiesis and contribute to the development of novel cellular therapies. Optimal culture conditions are key to maintaining the viability, proliferation and differentiation potential of HSPCs and achieve high editing efficiencies in gene editing experiments. In this presentation we will discuss STEMCELL's products for in vitro expansion of primary human hematopoietic cells, including primitive stem and progenitor cell populations. We will also outline our optimized protocol for gene editing of cord blood-derived CD34+ cells and discuss our upcoming cGMP animal component-free StemSpan™ medium manufactured to relevant 21 CFR Part 820 guidelines.

FRIDAY, 26 JUNE

13:00 – 13:30

AJINOMOTO

TOWARDS THERAPIES COMBINING REPROGRAMMING AND GENETIC ENGINEERING

Marius Wernig, *Institute for STEMCELL Biology and Regenerative Medicine, USA and* **Hajime Onuki**, *Ajnomoto, Japan*

Reprogramming holds the promise to generate autologous cells for cell transplantation-based tissue repair. In addition to simply derived and regenerate lost cells, the advent of facile genetic engineering allows now to not only repair genetic defects causing disease ex vivo for cell transplantation but also the engineering of disease interfering systems to bestow transplanted cells with therapeutic interventions.

As a first step, we have been focusing on rare monogenetic diseases that have a solid scientific basis for a beneficial cell transplantation. We have begun with the rare skin disease Epidermolysis Bullosa to implement induced pluripotent stem (iPS) and efficient Cas9-based genetic engineering. Manufacturing process optimized in the laboratory need to transition into good manufacturing practice (GMP) and all reagents need to be certified or exchanged to certifiable reagents to satisfy FDA-regulations of conducting Phase 1 clinical trials.

13:00 – 13:30

OLYMPUS CORPORATION

FROM PLURIPOTENCY TO ORGANOIDS: LIVE INCUBATION MONITORING IDENTIFIES CLUES FOR REPRODUCIBILITY

Takanori Takebe, *Tokyo Medical and Dental University, Japan*

Despite its translational value, organoid based approaches are generally inefficient and irreproducible. Understanding the quality control attributes for differentiation is vital to facilitate downstream bio-

medical applications. To approach this challenge, we employed the cutting-edge monitoring system "OLYMPUS Provi CM20*" for tracing organoid differentiation process to reverse engineer human organoid protocols. By identifying image-guided manipulatable factors at pluripotent stage, we are developing a human liver organoid differentiation protocol that minimizes inter-clone, -batch and -donor dependent variabilities. Applying this protocol into over 20 different iPSC library allows us for the investigation and interrogation of genotype-phenotype correlational studies against Non-alcoholic Fatty Liver Disease (NAFLD) in vitro, thus informing an essential genetic trait that counter-balances insulin responses and steatosis. Presented strategy will aid building highly reproducible platform to study personalized mechanism of health and disease with the use of PSC.

*The OLYMPUS Provi CM20 incubation monitoring system is incubator compatible imaging system that automates the scanning plates and the quantification of cell numbers and confluency. The data are wirelessly transferred to a tablet/PC accessed even remotely. The CM20 system significantly reduces the labor associated with routine monitoring and quantification, that will help improve quality management of tissue culture process.

13:00 – 14:00

10X GENOMICS

IDENTIFICATION OF DRUGS BLOCKING SARS-COV-2 INFECTION USING HUMAN PLURIPOTENT STEM CELL-DERIVED COLONIC ORGANOIDS

Shuibing Chen, *Weill Cornell Medical College, USA*

Here, we report using human pluripotent stem cell-derived colonic organoids (hPSC-COs) to explore the permissiveness of colonic cell types to SARS-CoV-2 infection. Single cell RNA-seq and immunostaining showed that the putative viral entry receptor ACE2 is expressed in multiple hESC-derived colonic cell types, but highly enriched in enterocytes. Multiple cell types in the COs can be infected by a SARS-CoV-2 pseudo-entry virus, which was further validated in vivo using a humanized mouse model. We used hPSC-derived COs in a high throughput platform to screen 1280 FDA-approved drugs against viral infection. Mycophenolic acid and quinacrine dihydrochloride were found to block the infection of SARS-CoV-2 pseudoentry virus in COs both in vitro and in vivo, and confirmed to block infection of SARSCoV-2 virus. This study established both in vitro and in vivo organoid models to investigate infection of SARS-CoV-2 disease-relevant human colonic cell types and identified drugs that blocks SARS-CoV-2 infection, suitable for rapid clinical testing.

13:00 – 14:00

MAXWELL BIOSYSTEMS

NEXT GENERATION HIGH-CONTENT ELECTROPHYSIOLOGY SYSTEMS FOR EFFICIENT FUNCTIONAL CHARACTERIZATION OF HUMAN IPSC-DERIVED NEURONS

Urs Frey *and* **Jan Mueller**, *MaxWell Biosystems, USA,* **Silvia Ronchi** *and* **Xinyue Yuan**, *Bio Engineering Lab, ETH Zurich*

Human induced pluripotent stem cell (h-iPSC)-derived neurons enable pre-clinical drug discovery and toxicology using human cells in vitro. To optimize the use of h-iPSC-derived neurons in these fields, reproducible functional assessment and high sensitivity detection of compound effects are needed.

In this innovation showcase, we will highlight next generation high-content electrophysiology technologies, novel functional readouts, and their applications to h-iPSC-derived neurons. We will introduce MaxTwo, the first high-throughput electrophysiology platform using high-density microelectrode array (HD-MEA) technology. MaxTwo can run cell-based functional assays that provide readouts on neuronal dynamics at subcellular, cellular, and network levels. We will also present the latest updates on the software, MaxLab Live, with a focus on built-in easy-to-use analysis tools. Additionally, latest comparison results on healthy and disease models of different h-iPSC-derived neurons, namely, dopaminergic, glutamatergic, and motor neurons, will be described. As an outlook, a new dual-mode HD-MEA system will be shown. Overall, the presentations will provide an overview on how HD-MEA technology can efficiently advance iPSC-derived neuron research and accelerate drug development for neurodegenerative diseases.

13:00 – 14:00

MOLECULAR DEVICES, LLC

GAIN DEEPER INSIGHTS INTO CELLULAR 3D STRUCTURES WITH WATER IMMERSION OBJECTIVES FOR HIGH-CONTENT IMAGING

Tim Baranowski, *Molecular Devices, LLC, USA*

High-content screening systems have evolved over the past decade to optimize assay sensitivity, flexibility, resolution, and throughput. The high-performance water immersion objectives on the ImageXpress Micro Confocal system facilitates these growing demands by enhancing the resolution, sensitivity and throughput for a variety of complex cell-based assays for biologic research and drug discovery. Water immersion objectives have higher numerical apertures allowing 4 times better light collection and providing higher resolution in X, Y and Z. This results in sharper cellular detail, better detection and improved analysis. With the increasing interest in using three-dimensional (3D) cultures for assay development and phenotypic screening for a range of cellular models, water immersion objectives are essential in capturing more data greater depths in 3D structures, such as spheroids and thick tissues.

13:00 – 14:00

STEMCELL TECHNOLOGIES

ADVANCED BRAIN ORGANOID CO-CULTURE SYSTEMS

Erin Knock, *Senior Scientist, Stem Cell Technologies, Canada*

Organoid technology is developing at a rapid pace with the field working to develop disease relevant models that are as close to human tissue as possible. In this talk I will walk through some critical factors to consider before embarking on a study using organoid models. Beginning with a brief note on the importance of pluripotent stem cell (PSC) quality, I will touch on how PSC-based models can inform and build upon work using animals. I will then review some published examples of how increasingly complex PSC-derived culture systems are being used for disease modeling. Finally, I will outline the performance of three new PSC-based culture systems: the STEMdiff™ Dorsal and Ventral Forebrain Organoids and STEMdiff™ Microglia Culture Systems. I will outline how to use these systems and show how to combine them as co-cultures relevant to disease modeling.

13:00 – 14:00

THERMO FISHER SCIENTIFIC

ALEXANDER DISEASE AND GIANT AXONAL NEUROPATHY: CELL BIOLOGICAL INSIGHTS FROM IPSC-DERIVED ASTROCYTE AND NEURON DISEASE MODELS

Natasha T. Snider, *Dept of Cell Biology and Physiology, UNC-Chapel Hill, USA*

Intermediate filaments (IFs) form a major component of the cytoskeleton important for structural and functional integrity of cells. Abnormal accumulations of glial and neuronal IFs are frequently observed in neurodegenerative diseases. These accumulations impair vital cellular functions, but the molecular processes that govern the reshaping of the IF cytoskeleton from a dynamic system in healthy cells to rigid inclusions in disease, are poorly understood. Induced pluripotent stem cells and Crispr/Cas9 technology are transforming our understanding of IF disease pathogenesis. Our research addresses shared mechanisms of the pediatric neurodegenerative diseases Alexander Disease (AxD) and Giant Axonal Neuropathy (GAN). Although they have different genetic causes and clinical presentations, AxD and GAN display similar white matter abnormalities and astrogliosis caused by the massive accumulation and aggregation of glial fibrillary acidic protein (GFAP) within cytoplasmic aggregates termed Rosenthal fibers. Illuminating the dynamics between GFAP filaments and aggregates provides a framework for testing novel treatments for AxD and GAN. We anticipate that the insights and tools developed around AxD and GAN will also advance our understanding of more common neurodegenerative diseases involving IF protein aggregation, including Alzheimer Disease, Parkinson Disease and Amyotrophic Lateral Sclerosis (ALS).

WEDNESDAY, JUNE 24, 16:00 – 18:00

PLENARY I: PRESIDENTIAL SYMPOSIUM

16:20 – 16:40
CELL ATLASES AS ROADMAPS TO UNDERSTAND DEVELOPMENT

Regev, Aviv

Broad Institute, MIT, HHMI, Cambridge, MA, USA

Three of the fundamental questions in biology are how do individual cells differentiate to form complex tissues, how do such tissues function in a coordinated and flexible fashion to maintain homeostasis, and what are the gene regulatory mechanisms that support these processes. Single cell and spatial genomics have now opened new ways to approach these questions by combining the throughput and comprehensive nature of genomics with the microscopic resolution that is required for description of complex multi-cellular phenomena. The first generation of single cell genomics techniques provided us with remarkably rich phenomenology of heterogeneous cellular behaviors. In this talk I will describe how we combine machine learning algorithms and perturbation experiments to transform observational studies to models of dynamics and causal mechanism in developing cells and tissues.

Keywords: single cell genomics, development, machine learning

16:45 – 17:05
SEARCHING FOR SIGNATURES OF NEURODEGENERATIVE AND PSYCHIATRIC DISEASES, THERAPEUTIC STRATEGIES AND PREDICTORS OF CLINICAL OUTCOMES WITH PATIENT IPSCS

Finkbeiner, Steven

Gladstone Institutes, University of California, San Francisco, CA, USA

The lack of effective therapeutic options for the major adult neurodegenerative diseases is disappointing, and the poor predictive value of preclinical models has been a major obstacle to translating promising discoveries from the laboratory to success with patients in the clinic. In this talk, we will describe strategies and progress of our group, and consortia to which we belong, that are using patient-derived and engineered iPSCs, often in combination with information from the patients from whom the cells are derived, to better bridge that gap and to understand the predictive value of these models. With deep phenotypic data from patients and by deeply characterizing the iPSC-derived models with genomics, transcriptomics, epigenomics, proteomics and robotic longitudinal single cell analysis, we can begin to elucidate links between these models and the diseases we seek to understand and treat. To help uncover signatures from these massive datasets and link them to patient features, we are increasingly applying specialized artificial intelligence computational techniques such as machine learning and deep learning. The goal is to identify signatures of disease that might be the basis for rational therapeutic intervention and patient stratification schemes that improve clinical trial design and increase the chances of finding safe and effective treatments.

Keywords: patient-derived iPSC, engineered iPSCs, neurodegenerative diseases,

17:10 – 17:30
TOWARD THE GENOMIC CORRECTION OF MUSCLE AND HEART DISEASE

Olson, Eric

University of Texas Southwestern, Dallas, TX, USA

We seek to delineate the mechanisms that govern development, disease and regeneration of the heart and other muscles and to build upon this knowledge to restore muscle function during disease and aging. There are hundreds of debilitating muscle diseases caused by single gene mutations. In an effort to correct the underlying causes of such disorders, we are deploying gene editing strategies to bypass errors in the genome, a strategy we refer to as Myoediting. Duchenne muscular dystrophy (DMD) is a fatal, progressive muscle disease caused by mutations in the Dystrophin gene, which encodes a large intracellular protein that maintains integrity of muscle cell membranes. Thousands of DMD mutations have been identified in humans. Through delivery of CRISPR/Cas9 gene editing components with adeno-associated viruses, we have corrected DMD in mice harboring a broad collection of the most common human mutations. We have also optimized Myoediting of many types of DMD mutations in human muscle cells derived from iPS cells generated from blood samples of DMD patients. With the ultimate goal of clinical translation of gene editing for DMD, current efforts are directed toward correction of DMD mutations in large mammals and to further optimizing Myoediting and confirming its safety. Opportunities and challenges in the path toward permanent correction of disease-causing mutations responsible for DMD and other monogenic disorders through the normalization of muscle gene expression by genomic editing will be discussed.

Keywords: Gene editing, Monogenic disorders, Duchenne muscular dystrophy

17:35 – 17:55

ERNEST MCCULLOCH MEMORIAL LECTURE: ADVENTURES IN SKIN BIOLOGY

Watt, Fiona M.

King's College London, UK

Mammalian skin is a highly tractable tissue for experimental analysis. Genetic manipulation and lineage tracing in mice have provided important insights into the heterogeneity and plasticity of the epidermal stem cell compartment and revealed a surprising complexity in the cell types of the dermis. Complementing mouse models, human epidermis and dermal fibroblasts can be expanded in culture for therapeutic applications and experimental analysis. Advances in single cell technologies such as RNA-sequencing now provide an important opportunity to re-evaluate the extent to which the different experimental models reflect the true cellular heterogeneity of the skin. Complemented by mechanistic studies of how cells transition between different states, we are starting to gain new understanding of this fascinating tissue.

Keywords: Epidermis, Skin, Differentiation

THURSDAY, JUNE 25, 09:00 – 11:00

PLENARY II: MACHINE LEARNING AND COMPUTATIONAL APPROACHES

09:10 – 09:30
ISSCR DR. SUSAN LIM AWARD FOR OUTSTANDING YOUNG INVESTIGATOR LECTURE: LEARNING DYNAMICS BY LINEAGE TRACING ON TRANSCRIPTIONAL LANDSCAPES

Klein, Allon

Harvard Medical School, Boston, MA, USA

Single cell genome-wide profiling offers several unbiased approaches to identify molecular differences between cells in tissues. But genomic assays kill cells, limiting our ability to construct long-term dynamic processes in stem cell differentiation. Computational single cell biology offers tools to infer genome-wide dynamics in cells from static snapshots. But computational inference can suffer from several stereotyped errors. How can we now evaluate computational methods that infer dynamics? Or whether our measurements even capture sufficient features for predicting a cell's behavior? I will briefly review our development of droplet microfluidic single cell profiling, and then discuss how genetic lineage tracing, combined with single cell RNA-Seq and machine learning approaches allows addressing these questions. I will use hematopoiesis and embryonic development as model systems, leading to a revised model for the dynamics and hierarchy of hematopoietic differentiation.

Keywords: Single cell genomics; lineage tracing; computational genomics

09:35 – 09:50
SPATIOTEMPORAL DISSECTION OF THE HUMAN PROTEOME

Lundberg, Emma

KTH Royal Institute of Technology, Stockholm, Sweden

Resolving the spatial distribution of the human proteome at a subcellular level increases our understanding of human biology and disease. In the Human Protein Atlas project, we are systematically mapping the human proteome in a multitude of human cells and organs using microscopy. We have generated a high-resolution map of the subcellular distribution of the human proteome and have shown that as much as half of all proteins localize to multiple compartments. Such proteins may have context specific functions and 'moonlight' in different parts of the cell, thus increasing the functionality of the proteome and the complexity of the cell from a systems perspective. Furthermore, I will present unpublished data on the extent of cell-to-cell variations of the human proteome. Using imaging proteogenomics we performed a spatiotemporal dissection of the human cell cycle. We could identify 17% of the human proteome to display cell-to-cell variability, of which we could attribute 26% as correlated to cell cycle progression and present the first evidence of cell cycle association for 234 proteins. Interestingly, we can attribute only 15% of proteomic cell cycle regulation to transcriptomic cycling with single-cell RNA sequencing, which points to other means of regulation such as post-translational modifications. All this work is critically dependent on computational image analysis, and I will discuss machine learning approaches for classification of spatial data as well as the citizen science effort "Project Discovery" integrated into a massively-multiplayer online game that has engaged more than 300,000 players world-wide. In summary, I will demonstrate the importance of spatial proteomics data for improved single cell biology and present how the freely available Human Protein Atlas database (www.proteinatlas.org) can be used as a resource for life science.

Keywords: Human Protein Atlas, Spatial Proteomics, Cell Cycle

09:55 – 10:10
NEXT GENERATION HUMAN IPSC-DISEASE MODELS

Kaykas, Ajamete

insitro, South San Francisco, CA, USA

Human cell-based invitro disease models are powerful tools, but they currently suffer from lack of reproducibility, scale, and connection to patient level data. The current paradigm is to generate patient or CRISPR engineered iPSC lines for a small number of patients [often 3 controls and 3 patients for high penetrant mutations (3x3 experiments)] and look for obvious cellular phenotypes that mark human disease. While a plethora of studies have been conducted and published with marked success, it is not yet clear if these models yield models that are predictive for drug discovery success and have a higher likelihood of efficacy when put in man. We are taking a multipronged approach to build next level human iPSC-based models to validate the "disease in a dish" approach and demonstrate that these models can predict human clinical outcome from cellular phenotypes, across humans with a broad genetic diversity. First, we are industrializing the process by automating iPSC engineering, culture and differentiation. We are developing a machine learning-based automation system that tracks, cultures and differentiates iPSCs at scale. We are generating a diverse iPSC cell bank with deep genomic and patient level data. We are further increasing the genetic diversity of our line with CRISPR-based engineering to well beyond the standard 3X3 experiment. Finally, we are using machine learning and computer vision to identify which

features of high dimensional morphological and transcriptomic data captured from differentiated iPSCs are most predictive clinical outcome. We plan on using these predictive models to generate next generation disease models and use them to screen for therapeutic intervention. I will present an overview of our platform and the progress we have made to date on a few disease indications.

Keywords: Disease-model Machine Learning

10:15 – 10:30

UNDERSTANDING THE IMPACT OF PSYCHIATRIC GENOMES ON THE HIERARCHY OF BIOLOGICAL PATHWAYS AND SYSTEMS

Ideker, Trey

University of California, San Diego School of Medicine, La Jolla, CA, USA

Our goal is to bridge the gap between the human genome and disease outcomes by systematic mapping of gene and protein interaction networks. These network maps are driving the construction of intelligent systems for translation of the genome, leveraging recent advances in deep machine learning. Initially developed for cancer, recently we have begun to apply these approaches to neuropsychiatric and neurological behavior research, as part of two NIH-funded research centers: The Psychiatric Cell Mapping Initiative and the National Center of Excellence for GWAS in Outbred Rats. Through collaborative work in these centers we are unravelling the pathways underlying autism spectrum disorders, epilepsy, intellectual disability, and schizophrenia.

Keywords: Psychiatric, Cell Mapping, Autism

10:35 – 10:50

TOWARD THE AUTOMATION OF SCIENTIFIC DISCOVERY BY AI AND ROBOTICS

Kitano, Hiroaki[1], Yachie, Ayako[2], Ghosh, Samik, Palaniappan, Sucheendra and Matsuoka, Yukiko

[1]Okinawa Institute of Science and Technology, Onnason, Japan, [2]The Systems Biology Institute, Tokyo, Japan

Biomedical research involves diverse and multiple experiments and data analysis methods. Flexible and scalable support of the research process by semi-automating the process will significantly improve the efficiently of research. In addition, use of machine learning and large-scale natural language processing contribute to research by augment capabilities of researchers for recognize hidden patterns behind the data as well as enabling them to access knowledge sparsely encoded in scientific literature and data-bases. In order the ensure connectivities of analysis modules and data acquisition, we have developed Garuda Connectivity Platform that enable us to design “recipe” that connects and automate process of data analysis, and Gandhara AI Framework supporting use of NLP, ML, and other AI technologies. Our final goal is to automate a process of scientific discovery by AI and robotics that includes hypothesis generation , experimental design and protocol generation, execution by robotics, and data analysis for verification and falsification.

Keywords: Artificial Intelligence, Scientific Discovery, Software Platform, Machine Learning, Robotics

THURSDAY, JUNE 25, 11:00 – 12:45

CONCURRENT - CELLULAR IDENTITY: CARDIAC AND MUSCLE

11:05 – 11:20

ANALYSIS OF MURINE CARDIAC FIBROBLAST LINEAGE HETEROGENEITY AND DYNAMICS IN HEALTH AND DISEASE USING SINGLE CELL METHODS

Harvey, Richard P., Patrick, Ralph, Farbehi, Nona, Janbandhu, Vaibhao and Tallipragada, Vikram

Victor Chang Cardiac Research Institute, Sydney, Australia

Cardiovascular (CV) disease remains the most significant cause of mortality and morbidity in the Western world, and the individual and societal costs are staggering. One of the hallmarks of virtually all forms of CV disease and progression to heart failure (HF) is myocardial fibrosis. Central players are cardiac fibroblasts - enigmatic, changeable cells that play normal roles as sentinels, paracrine signalers, mechanical and electrical transducers, matrix factories, and lineage progenitors. Cardiac fibrosis is the target of extensive drug development efforts; however, progress in this area is limited by assay design, model assumptions, non-specific markers, and poorly defined cell states and networks. A Linnaean framework of cell taxonomies based on morphology and marker expression has served biologists well in cataloguing cells types in the body; however, in tissues undergoing constant renewal (e.g. blood), or adaptation and remodeling (e.g. heart), this framework is inadequate. Single cell transcriptomics has revolutionized our ability to characterize cell states in complex tissues, overcoming the limitations of bulk cell analyses and revealing a diversity of cell states representing different time scales of change, thus lending itself to new computational methods for inferring cell dynamics and causality. Our work seeks to develop a new conceptual framework for understanding cardiac fibrosis in HF. scRNAseq analysis of cardiac interstitial cells in a murine model of myocardial infarction revealed unexpected cell complexity and dynamics in cardiac interstitial cell populations including >9 fibroblast lineage states, mostly novel. In other models, scRNAseq can also discern subtle changes in

fibroblast population proportions and states, for example, during aging or after remote injury. We will briefly also describe a novel algorithm for analysis of differential transcript use in scRNAseq data, and its application to a model of augmented cardiac repair.

Keywords: cardiac fibrosis; heart failure; single cell RNAseq

11:25 – 11:33

SINGLE CELL TRANSCRIPTOMIC ANALYSIS OF HESC-DERIVED ARTICULAR AND GROWTH PLATE CARTILAGE REVEALS MOLECULAR CANDIDATES FOR LINEAGE COMMITMENT AND STABILITY

Raftery, Rosanne M., Craft, April, Pregizer, Steven and Raj, Suyash

Department of Orthopedic Surgery, Boston Children's Hospital, Boston, MA, USA

The stark lack of therapies that target degenerative joint disease is in part due to our incomplete understanding of the precise mechanisms controlling the development of the joint-lining articular cartilage. While adult stem cells can form cartilage, they fail to maintain an articular chondrocyte (AC) phenotype, and instead possess a propensity to differentiate into growth plate chondrocytes (GPCs), which undesirably induces osteogenesis. We pioneered methods for inducing human pluripotent stem cells (hPSCs) to differentiate into either ACs or GPCs and respective cartilage tissues. Long-term culture with TGFb3 induces an AC phenotype, while BMP4 induces a hypertrophic GPC phenotype. We are using this model to define the molecular characteristics of chondrocytes as they become lineage committed and elucidate the factors responsible for articular versus growth plate cartilage specification. To identify when ACs become stable, meaning unable to undergo hypertrophy, we challenged TGFb3-treated ACs with BMP4 at sequential time-points throughout a 6-month culture period. Based on the ability/inability of ACs to upregulate GPC-specific genes and/or acquire a hypertrophic phenotype, we found that <8-week old tissues were still able to respond to BMP4, but >12-week old tissues were resistant to this challenge, maintaining an AC identity. To identify factors that may be responsible for this stable lineage choice, we performed single cell transcriptomic analyses on hESCs as they differentiate into ACs or GPCs. We found that end stage tissues consist of transcriptionally distinct chondrocytes consistent with embryonic cartilage development. Analysis of TGFb3-treated cells revealed two populations of cells, representing the intermediate (COL2A1 and ACAN) and superficial (PRG4) zones of articular cartilage. Encouragingly, known genes associated with cartilage stability were also expressed. Ongoing work is focused on validating differentially expressed genes between ACs that are permissive to hypertrophy and those that are fully committed to the AC lineage, which will likely identify important cell fate decisions points in the chondrogenic lineages. Our work thus provides crucial information regarding AC specification that will ultimately provide a robust cell source for articular cartilage repair.

Keywords: Pluripotent stem cells, Articular cartilage, Joint development

11:35 – 11:43

DECODING HUMAN CARDIAC MATURATION AND AGING WITH SINGLE-CELL MULTI-OMICS SEQUENCING

Wu, Hao[1], Hu, Peng[1], Qiu, Qi[1], Bedi, Kenneth[2] and Margulies, Kenneth[2]

[1]Department of Genetics, University of Pennsylvania, Philadelphia, PA, USA, [2]Department of Medicine, University of Pennsylvania, Philadelphia, PA, USA

The life of all animals, from the beginning of their life and throughout adulthood, depends on the normal function of the heart. As the mammalian hearts have very limited regenerative potential, the loss or pathological remodeling of specific cardiac cell types, such as ventricular cardiomyocytes, during injury and diseases often leads to heart failure and sudden death. However, limited understanding of the precise composition and functional heterogeneity of cardiac cell-types during in vitro cardiac differentiation of human pluripotent stem cells (hPSCs) and developing/mature human hearts represents a major barrier to the development of effective cell replacement therapies to treat heart diseases. Here, we use single-cell/nucleus RNA sequencing (RNA-seq) and epigenomic analysis (chromatin accessibility and DNA methylome) to comprehensively analyze major human cardiac structures (ventricle and atrium) at single-cell resolution. By generating >300,000 single cell or nuclear transcriptome/epigenomes from human hearts as well as from long-term hPSC cardiac differentiation (up to 9 months), we generate a cellular taxonomy of cardiac cell-types and their functional/maturation states during early cardiac progenitor commitment and differentiation, and across human life-span (from fetal to adult [22 to 91 year old]). These results reveal maturation state- and age-dependent changes in gene expression, epigenomes, and gene regulatory networks in cardiomyocytes (CMs) and non-CM cell-types such as cardiac fibroblasts, endothelial cells and resident macrophages. Furthermore, we linked cardiac disease associated genetic variants to cell type specific gene expression patterns to infer the functionally relevant cell types. Taken together, our comprehensive single-cell/nucleus multi-omics analysis not only provides critical molecular insights into human cardiac maturation and aging, but also paves the way to identify new therapeutic targets for congenital and adult heart diseases.

Keywords: Human cardiac maturation and aging, Single-cell transcriptomics & epigenomics, Human pluripotent stem cells

11:45 – 11:53

PRDM16-MEDIATED H3K9 METHYLATION CONTROLS FIBRO-ADIPOGENIC PROGENITORS PLASTICITY AND MOUSE SKELETAL MUSCLE REPAIR

Mozzetta, Chiara

Institute of Molecular Biology and Pathology (IBPM), National Research Council (CNR), Rome, Italy

Fibro-Adipogenic Progenitors (FAPs) are crucial regulators of skeletal muscle homeostasis as they possess the intrinsic ability to either support muscle regeneration or to contribute to fibro-adipogenic degeneration of dystrophic muscles. Hence, the elucidation of the molecular mechanisms controlling their phenotypical plasticity holds therapeutic potential. Here, we identified Prdm16 as a FAPs-enriched factor that mediates their developmental capacities by modulating heterochromatin organization at the nuclear periphery. Deletion of Prdm16 prevents FAPs adipogenic differentiation and unlocks a myogenic capacity. Mechanistically, we found that Prdm16 localizes at the nuclear lamina where it cooperates with the H3K9 methyltransferases (KMTs), G9a and GLP, to mediate H3K9me2 deposition and silencing of muscle-specific genes. Pharmacological inhibition of G9a/GLP induces FAPs to directly participate to myogenesis in vivo, thus inducing an overall amelioration of skeletal muscle regeneration. Together, our findings reveal a FAPs-specific epigenetic axis important to control their identity and highlight the possibility to exploit it therapeutically to reprogram FAPs fate in vivo to prevent fibro-adipogenic degeneration of dystrophic muscles.

Keywords: Fibro-adipogenic progenitors, nuclear lamina, H3K9 methylation

11:55 – 12:03

THE TRANSCRIPTION FACTOR GATA4 REGULATES MRNA SPLICING THROUGH DIRECT INTERACTION WITH MRNA

Zhu, Lili, Choudhary, Krishna, Thomas, Reuben, Ang, Yen Sin, Stone, Nicole, Teran, Barbara, Liu, Lei, Gifford, Casey, Bemmel, Joke and Srivastava, Deepak

GICD, Gladstone Institutes, San Francisco, CA, USA

Alternative RNA splicing plays critical roles in differentiation, development, and disease, with tissue and temporal specificity having functional consequences. Despite growing knowledge of the mechanisms involved in regulation of splicing, relatively little is known about how tissue-specific alternative splicing arises. GATA4 encodes a zinc-finger transcription factor expressed in developing mesendodermal cells that plays a critical role in heart development and the cardiac stress response. Identification of the protein-protein interactome of GATA4 in hiPSC-derived cardiomyocytes using affinity purification followed by mass spectrometry revealed an unexpected interaction between GATA4 and RNA binding proteins belonging to the spliceosome complex. Knockdown of GATA4 in human cardiomyocytes resulted in >1000 differential alternative mRNA splicing events in genes involved in cytoskeleton organization and calcium ion import, such as TTN, CACNA1C, and CACNA1D. Enhanced crosslinking and immunoprecipitation (eCLIP) assay demonstrated that GATA4 directly interacts with RNA molecules, with enrichment of specific binding motifs. 104 of the differentially spliced RNAs were bound by GATA4, and this subset was involved in muscle stretch, cardiac conduction, and cardiac muscle cell contraction. Incubation of putative pre-mRNA targets and spliceosome-containing cellular extracts, with or without GATA4 protein, validated GATA4's direct role in regulating alternative splicing events that precisely mimicked those dysregulated upon GATA4 knockdown. These findings suggest that tissue-enriched transcription factors not only guide cell-type-specific transcription, but can also direct mRNA splicing events to achieve the required transcriptome for appropriate tissue and organ function.

Keywords: GATA4, RNA Splicing, RNA binding

12:05 – 12:13

A NOVEL RETINOIC ACID:YAP1 SIGNALING AXIS CONTROLS ATRIAL LINEAGE ACQUISITION IN HUMAN CARDIAC PROGENITORS

Estaras, Conchi[1], Huang, Ling[2] and Stronati, Eleonora[3]

[1]Center for Translational Medicine/Department of Physiology, Temple University, Philadelphia, PA, USA, [2]Razavi Newman Integrative Genomics and Bioinformatics Core, Salk Institute for Biological Studies, La Jolla, CA, USA, [3]Center for Translational Medicine, Temple University, Philadelphia, PA, USA

Both Vitamin A deficiency and overexposure gives rise to developmental abnormalities of the heart. Moreover, specific genetic backgrounds, such as the 22q11.2 deletion (which causes Digeorge Syndrome) also alter the Vitamin A/Retinoic Acid (RA) homeostasis contributing to congenital heart defects (CHD). One of the earliest roles of RA signaling in heart development is to promote posterior specification of the cardiac progenitor cells (CPCs) and the acquisition of an atrial cell fate. Perturbation of the RA signaling during the deployment of the CPCs gives rise to CHD, including conotruncal and septum defects. Nevertheless, the majority of patients with CHD (~55%), do not carry CHD-related mutations and had normal nutritional exposure to Vitamin A. Therefore, whether there are unknown regulators governing the specification of CPCs in concert with RA signaling, and how they integrate into the molecular circuitries that control cardiomyocyte (CM) identity represent a major gap in knowledge. Here, we adopted a functional genomic approach at single-cell resolution

to investigate the RA-induced regulatory network that controls the specification of human pluripotent stem cell-derived CPCs toward an atrial cell fate. Unexpectedly, our data show that in hCPCs, RA signaling induces genome-wide activation of YAP:TEAD enhancers. Our ongoing studies show that this novel RA:YAP1 signaling axis is essential for the acquisition of an atrial CM identity. Our transcriptome analysis found that the RA-induced YAP:TEAD enhancers are negative regulators of transcription of nearby genes. For instance, we found that YAP1 acts as a corepressor of key ventricular lineage genes, such as HAND1. Accordingly, the cardiomyocytes derived from RA-treated YAP KO cells express higher levels of ventricular genes, compared to WT. Interestingly, our preliminary data show that the 22q11.2-iPSCs carry significantly lower levels of active YAP compared to the WT iPSCs, that highly correlates with lower expression of YAP-target genes in the syndrome cells. We are currently investigating whether disruption of the RA:YAP signaling axis contributes to CHD in the 22q11.2 syndrome. Overall, we identified a novel RA:YAP1 signaling axis in the context of regulatory networks essential for human cardiomyocyte identity and associated with CHD.

Keywords: Retinoic Acid cardiomyocyte differentiation, YAP1 TEAD transcription, atrial identity digeorge 22q11.2

12:15 – 12:30

REJUVENATING STEM CELL FUNCTION TO INCREASE MUSCLE STRENGTH

Blau, Helen M.

Stanford University School of Medicine, Stanford, CA, USA

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

Keywords: regeneration, muscle, stem cells

THURSDAY, JUNE 25, 11:00 – 12:45

CONCURRENT - CLINICAL APPLICATIONS: EARLY DEVELOPMENT AND PLURIPOTENCY -- INTERSPECIES CHIMERAS FOR STEM CELL RESEARCH

11:05 – 11:20

UNIQUE PROPERTIES OF A SUBSET OF HUMAN PLURIPOTENT STEM CELLS WITH HIGH CAPACITY FOR SELF-RENEWAL

Pera, Martin F.

The Jackson Laboratories, Bar Harbor, ME, USA

Archetypal human pluripotent stem cells (hPSC) are widely considered to be equivalent in developmental status to mouse epiblast stem cells, which correspond to pluripotent cells at a late post-implantation stage of embryogenesis. Heterogeneity within hPSC cultures complicates this interspecies comparison. A subpopulation of archetypal hPSC enriched for high self-renewal capacity (ESR) has distinct properties relative to the bulk of the population, including a cell cycle with a very low G1 fraction and a metabolomic profile that reflects a combination of oxidative phosphorylation and glycolysis. ESR cells are pluripotent and capable of differentiation into primordial germ cell-like cells. Chromatin accessibility analysis revealed a unique set of open chromatin sites in ESR cells. RNA-seq at the subpopulation and single cell levels shows that, unlike mouse epiblast stem cells, the ESR subset of hPSC displays no lineage priming, and that it can be clearly distinguished from gastrulating and extraembryonic cell populations in the primate embryo. ESR hPSC correspond to an earlier stage of post-implantation development than mouse epiblast stem cells, more similar to the formative state. The ESR subpopulation expresses high levels of the components of the Nodal pathway, and Nodal signaling is critical to the maintenance of this self-renewing state. The development of improved methods to maintain the ESR subpopulation in a pure form will enhance stability and differentiation potential of hPSC.

Keywords: human pluripotent stem cells, self-renewal, NODAL

11:25 – 11:33

GENETIC VARIATION INFLUENCES MOUSE EMBRYONIC STEM CELL STATE THROUGH A HIERARCHY OF MOLECULAR PHENOTYPES

Reinholdt, Laura[1], Skelly, Dan[1], Czechanski, Anne[1], Byers, Candice[1], Aydin, Selcan[1], Spruce, Catrina[1], Olivier, Chris[1], O'Connor, Callan[1], Martin, Whitney[1], Choi, Kwangbom[1], Gatti, Daniel[1], Raghupathy, Narayanan[1], Stanton, Alexander[1], Keele, Gregory[1], Vincent, Matthew[1], Pankratz, Matthew[2], Porter, Devin[2], Dionne, Stephanie[1], Harrill, Allison[3], Choi, Ted[1], Churchill, Gary[1], Munger, Steven[1] and Baker, Christopher[1]

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For decades, germ line competent embryonic stem cell lines could only be derived from a limited set of "permissive" inbred mouse strains. This essential phenotypic difference was eventually attributed to differential activation of the core signaling pathways that respond to exogenous leukemia inhibitory factor (LIF) in permissive vs. non-permissive strains. Implementation of "2i" culture conditions in which inhibitors of MEK/ERK and GSK3β signaling are added to standard mESC culture media circumvents this problem, allowing for successful derivation and propagation of germ line competent mESCs from non-permissive strain backgrounds. Yet, the underlying genetics driving these interstrain differences in LIF responsiveness, and whether these genetic differences also influence directed differentiation remain a mystery. And this problem is not limited to mouse embryonic stem cells. Genetics also influences molecular phenotypes and differentiation capacity in hESCs and hiPSCs where genetic background has been shown to be a dominant source of inter-line variability. Despite significant a priori knowledge of the core regulatory gene networks that maintain pluripotency in vitro, the sheer number of genetic variants present in these genes, regardless of species, precludes reverse genetic approaches. Using an unbiased, forward genetic approach we sought to reveal the genetic variation that influences the response of ESCs to the culture environment. We profiled gene expression and chromatin accessibility in 185 genetically heterogeneous mESCs. We mapped thousands of loci affecting chromatin accessibility (caQTL) and/or transcript abundance (eQTL). Among these loci, we identified Lifr transcript abundance as the causal intermediate regulating 122 distant genes enriched for roles in maintenance of pluripotency. We identified a variant in a novel enhancer ~10kb upstream of Lifr that alters chromatin accessibility, influences Lifr transcript abundance, and precipitates a cascade of molecular events affecting expression of pluripotency markers. We validated this hypothesis using reciprocal allele swaps, revealing mechanistic details of the genetic variation that drives differential activation of signaling pathways in permissive and nonpermissive mESCs.

Keywords: systems genetics, pluripotency, mESCs

11:35 – 11:43

A NOVEL MODEL FOR STUDYING INFECTIONS AT THE MATERNAL-FETAL INTERFACE USING HUMAN IPSC-DERIVED TROPHOBLAST LINEAGES

Wang, Jennifer R.[1], Huang, Wan[2], Connor, John[3], Ingalls, Robin[4], Kuohung, Wendy[4] and Mostoslavsky, Gustavo[5]

[1]Center for Regenerative Medicine (CReM), Boston University, Brookline, MA, USA, [2]Department of Graduate Medical Sciences, Boston University, Boston, MA, USA, [3]National Emerging Infectious Laboratories, Boston University, Boston, MA, USA, [4]Department of Medicine, Boston University, Boston, MA, USA, [5]Center of Regenerative Medicine, Boston University, Boston, MA, USA

The fetal placenta is a multifunctional organ derived from the extraembryonic layer. Trophoblasts are composed of three cell populations: cytotrophoblasts (CT), extravillous cytotrophoblasts (EVT), and syncytiotrophoblasts (ST). CT cells are placental stem cells that can give rise to EVT and ST cells. Here we report a novel protocol to differentiate human iPSC into trophoblasts that will serve as a platform for modeling infections at the maternal-fetal interface. We also seek to derive trophoblast stem cells from iPSC as a novel method for maintaining CT cells in long-term culture. iPSC lines were cultured on Matrigel-coated wells in mTeSR1 medium. On day 2 after plating, the medium was changed to contain BMP4 and inhibitors of activin A and FGF-signaling (BMP4/A83-01/PD173074; BAP treatment). Putative trophoblast lineages emerge after 8 days of BAP-treatment. These cells demonstrate formation of syncytium, invasive capacity, and robust secretion of placental hormone hCG. They also express characteristic markers of extraembryonic cell lineages, including TFAP2A, hCSI, CGB7, and PGF. Alternatively, in our attempt to derive a trophoblast stem cell line, iPSC were exposed to BAP-treatment for 72 hours and then switched to a media containing inhibitors of TGF-b (EGF/A83-01/SB431542), activator of Wnt (CHIR99021), and Rho-associated protein kinase (ROCK), which enabled the robust differentiation into putative CT cells. These cells can proliferate extensively while expressing high levels of TFAP2A, and give rise to differentiated terminal ST and EVT cell types. Finally, we also demonstrated our ability to infect these cells with several relevant pathogens, including a rVSV-EBOV-GFP recombinant virus, as a proof of principle demonstrating the potential use of this novel human in vitro

platform for modeling infections of the maternal-fetal interface.

Keywords: iPSC, trophoblast, placenta

11:45 – 11:53
MASSIVELY PARALLEL, TIME-RESOLVED SINGLE-CELL RNA SEQUENCING WITH SCNT-SEQ

Qiu, Qi, Hu, Peng, Govek, Kiya, Camara, Pablo and Wu, Hao

Department of Genetics, University of Pennsylvania, Philadelphia, PA, USA

Single-cell RNA sequencing offers snapshots of whole transcriptomes but obscures the temporal dynamics of RNA biogenesis and decay. Here we present single-cell new transcript tagging sequencing (scNT-Seq), a method for massively parallel analysis of newly-transcribed and pre-existing RNAs from the same cell. This droplet microfluidics-based method enables high-throughput chemical conversion on barcoded beads, efficiently marking metabolically labeled newly-transcribed RNAs with T-to-C substitutions. By simultaneously measuring new and old transcriptomes, scNT-Seq reveals neuronal subtype-specific gene regulatory networks and time-resolved RNA trajectories in response to brief (minutes) versus sustained (hours) neuronal activation. Integrating scNT-Seq with genetic perturbation reveals that DNA methylcytosine dioxygenases may inhibit stepwise transition from pluripotent embryonic stem cell state to intermediate and totipotent two-cell-embryo-like (2C-like) states by promoting global RNA biogenesis. Furthermore, pulse-chase scNT-Seq enables transcriptome-wide measurements of RNA stability in rare 2C-like cells. Time-resolved single-cell transcriptomic analysis thus opens new lines of inquiry regarding cell-type-specific RNA regulatory mechanisms.

Keywords: Single-cell RNA sequencing, Metabolic labeling, mRNA stability

11:55 – 12:03
CELL COMPETITION IN HUMAN PLURIPOTENT STEM CELL CULTURES

Barbaric, Ivana

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The occurrence of genetic changes in human pluripotent stem cells (hPSCs) upon extensive culture presents one of the potential safety concerns for their use in cell replacement therapies. Recurrent karyotypic abnormalities in hPSC cultures include gains of chromosomes 1, 12, 17 and 20. Variant cells harbouring recurrent karyotypic abnormalities display selective growth advantage over normal cells. The rapid overtake of variants has been attributed to their increased growth rates, reduced propensity for apoptosis and decreased levels of spontaneous differentiation. Nonetheless, little is known about the interactions of normal and variant cells and how they may contribute to the variant cells spreading throughout the culture. Here we show that the dominance of variant clones in mosaic cultures is enhanced through competitive interactions resulting in elimination of wild-type cells. This elimination occurs through corralling and mechanical compression by faster growing variants, causing a redistribution of F-actin and sequestration of YAP in the cytoplasm that induces apoptosis in wild-type cells. Importantly, YAP overexpression in wild-type cells is sufficient to alleviate their loser phenotype. Our results demonstrate that hPSC fate is coupled to mechanical cues imposed by neighbouring cells and reveal that hijacking this mechanism allows variants to achieve clonal dominance in cultures.

Funding source: This work was supported by the Medical Research Council MR/N009371/1 and the UK Regenerative Medicine Platform, MRC reference MR/R015724/1.

Keywords: human pluripotent stem cells, culture acquired variants, cell competition

12:05 – 12:20
WHY HUMAN-ANIMAL CHIMERA RESEARCH

Johnston, Josephine

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Chimera research is accelerating. Advances in stem cell science and gene editing techniques are enabling scientists to more extensively and precisely insert stem cells into one species into another at any stage of development, from in vitro experiments with chimeric embryos to in vivo studies that create chimeric animals. Ethics and governance questions have been raised about various kind of chimera research, with a particular focus on research in which human cells are mixed with those of non-human animals. Perhaps most controversially, news reports, policy changes, and journal articles over the past year have raised the possibility that scientists will mix human and non-human primate (NHP) cells to create human-NHP chimeric embryos and human-NHP chimeric animals. Proponents of this research argue that it could advance understanding of early human development, advance understanding of neurological and psychiatric disease, and improve generation of human-compatible organs in nonhuman animals. Opponents critique the scientific justification for the research, arguing that it involves unacceptable harms to NHPs, raises unresolved ethical and oversight questions, and threatens public trust in science. This presentation describes key ethical issues in chimera research, with a focus on the creation of human-animal chimera and, within that, on the possibility of human-NHP chimeric embryos and human-NHP chimeric animals. From animal rights and welfare concerns, to questions about "humanization," to weaknesses in oversight and governance mechanisms, chimera research touches on significant—and in many ways, profound—questions about our relationships with

other animals, the goals of scientific research, and the basis for public trust in science.

Keywords: Chimeras, ethics, policy

12:25 – 12:40
CELL COMPETITION CONSTITUTES A BARRIER FOR INTERSPECIES CHIMERISM

Wu, Jun

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Pluripotent stem cells (PSCs) provide an invaluable in vitro model to study early mammalian development and hold great potential in revolutionizing regenerative medicine. More recently, progresses in interspecies blastocyst complementation have opened new possibilities for PSC research. Through genetic manipulation, a developmental organ niche of the host species could be brought to service exclusively for PSCs from the donor species, and thereby, generating organ-enriched chimeras, which bodes hope for solving the severe shortage of organ donors. One of the keys to success for interspecies blastocyst complementation is the ability of donor PSCs to contribute to chimera formation in the host species. Rat and mouse PSCs can robustly contribute to interspecies chimera formation in mouse and rat, respectively, which enabled the generation of rat pancreas in mice, and mouse pancreas in rats via interspecies blastocyst complementation. To date, however, robust chimerism between evolutionary more distant species has not been achieved, which suggests the existence of xenogeneic barrier between evolutionary distant species during early development. Cell competition entails an evolutionary conserved fitness-sensing process during which fitter cells eliminate their neighboring less-fit but otherwise viable cells. Cell competition has been proposed as a surveillance mechanism to ensure normal development and maintain tissue homeostasis. During interspecies chimera formation, xenogeneic donor PSCs may be treated as unfit or aberrant cells and targeted for elimination. Our central hypothesis is that cell competition constitutes a major component of the xenogeneic barrier and overcome interspecies cell competition during early development improves chimerism between evolutionary distant species. To address this, we develop an interspecies PSC co-culture strategy and uncover a previously unknown mode of cell competition between species. Suppressing interspecies PSC competition significantly improved the survival of human cells in early animal embryos.

Keywords: Cell competition, interspecies blastocyst complementation, interspecies chimeras, pluripotent stem cells

THURSDAY, JUNE 25, 11:00 – 12:45

CONCURRENT - MODELING DEVELOPMENT AND DISEASE: NEURAL

11:05 – 11:20
RECONSTRUCTION OF THE HUMAN BLOOD-BRAIN BARRIER USING INDUCED PLURIPOTENT STEM CELLS TO MODEL CEREBRAL AMYLOID ANGIOPATHY

Tsai, Li-Huei[1] and Blanchard, Joel[2]

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The majority of Alzheimer's disease patients and 20–40% of non-demented elderly experience amyloid deposits along their cerebral vasculature, a condition known as cerebral amyloid angiopathy (CAA). CAA impairs the function of the blood-brain barrier (BBB) leading to ischemia, hemorrhages, and accelerated cognitive dysfunction. The APOE4 allele is the strongest known risk factor for CAA and sporadic Alzheimer's disease (AD), however the pathogenic mechanisms underlying this predisposition are unknown. Using human iPSC-derived cells we recreate the human BBB in vitro generating a highly tractable model that recapitulates key anatomical and physiological properties of the BBB. Similar to the human brain BBB, we find that amyloid accumulates on our in vitro BBB (iBBB) and both APOE4 homozygous and heterozygous iBBBs exhibit significantly more amyloid accumulation than APOE3/3 iBBBs. We then used reciprocal isogenic iPSC-derived iBBBs to dissect the mechanisms underlying APOE4 risk for CAA. Through combinatorial experiments we pinpoint the causal cells through which APOE4 predisposes CAA. This revealed that APOE4 causes cell type specific dysregulation of APOE gene expression. We identify the pathways underlying APOE dysregulation and find that inhibiting these pathways with FDA-approved drugs prevent the build up of amyloid in APOE4 iBBBs. Collectively, this work establishes a human model of the BBB, defines a mechanism through which APOE4 predisposes amyloid deposition, and uncovers new therapeutic opportunities for CAA and potentially AD.

Keywords: n/a

11:25 – 11:33

STEM CELL-DERIVED NEURONS PROVIDE THE MISSING LINK BETWEEN ALS PATHOLOGY AND MOTOR NEUROPATHY

Klim, Joseph and Eggan, Kevin

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Aggregation of the RNA-binding protein TDP-43 in vulnerable neurons is the diagnostic pathology for most patients with amyotrophic lateral sclerosis (ALS), which is characterized by the selective loss of motor neurons. Furthermore, mutations in the gene TARDBP, which encodes for TDP-43, is a cause of familial ALS. Although it has been proposed that these genetic and pathological perturbations disrupt normal RNA metabolism, the identity of the RNAs regulated by TDP-43 in human neurons remains poorly understood. Here, we used RNA sequencing to identify transcripts whose abundances in purified human stem cell-derived motor neurons (hMNs) were sensitive to reduced TDP-43 levels. We found that transcript levels of Stathmin 2 (STMN2), a regulator of microtubule stability and neurite extension normally highly expressed in motor neurons, were reproducibly and sharply decreased. This reduction was also the case in hMNs differentiated from patient-derived induced pluripotent stem cell lines with pathogenic TDP-43 mutations. STMN2 loss upon altered TDP-43 function was due to altered splicing, which is functionally important, as we demonstrate STMN2 is necessary for normal axonal outgrowth and regeneration. Although hMNs generated in vitro share key molecular and functional properties with bona fide hMNs, the in vivo validation of discoveries from stem cell-based models of ALS is a critical test of their relevance to disease mechanisms. To this end, we used ALS patient spinal cord tissues to provide in vivo evidence corroborating our disease modeling studies that TDP-43 dysregulation alters the expression of STMN2 through altered splicing. We further leveraged this molecular information of altered STMN2 splicing to develop a potential ALS biomarker assay, and we have identified compounds that can correct this splicing defect that could serve as an ALS therapeutic. In conclusion, findings from human stem cell-based models can be used to discover unique aspects of human biology underlying disease pathomechanisms and can illuminate potential therapeutic targets and disease biomarkers.

Funding source: Project ALS

Keywords: Disease Modeling, Cryptic Exon, Antisense oligonucleotide therapy

11:35 – 11:43

NETWORK-BASED PREDICTION AND VALIDATION THROUGH SYSTEMATIC PROFILING OF CAUSAL SCHIZOPHRENIA GENES IN HUMAN CORTICAL NEURONS.

Kousi, Maria[1], Smith, Kevin[2], Davila-Velderrain, Jose[1], Mohammadi, Shahin[1], Eggan, Kevin[2] and Kellis, Manolis[1]

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The recent increase in genetic discovery for psychiatric disorders has uncovered an undisputed genetic basis for schizophrenia (SCZ) and more than 100 robustly associated loci. However, the driver genes, variants, and mechanism of action of these loci remain uncharacterized, hindering the ability to translate genetic findings into novel drug targets and treatments for SCZ patients. To overcome this limitation, we integrate genetic, transcriptional and epigenomic evidence to prioritize driver genes and regulatory regions for functional studies using excitatory human cortical neurons. Towards this, we first defined the "Brain regulatory genomic space" (NRGS) as the union of the genomic loci with putative gene-regulatory roles in the brain, by integrating epigenomic, chromatin interaction, genomic and evolutionary evidence. We next intersected these regions with 108 SCZ-associated genetic loci, resulting in 1062 putative disease-associated regulatory regions, corresponding to 0.12% of the genome. By linking each of these regions to their downstream target genes using chromatin conformation and eQTL genetic evidence we identified a total of 300 novel SCZ candidate target genes. Finally, we harnessed adult human brain single-cell expression profile data from 10,000 cells and intersected those with global human interactome maps using the Single-Cell Imputation and NETwork (SCINET) algorithm, to generate cell-type specific interactome maps and score the candidate genes through their connectivity profiles. We are currently conducting experiments to test the functional impact of perturbing high-scoring candidate genes through the use of a programmable CRISPR-Cas9 system in NGN2 cortical neurons. To assess disease relevant phenotypes, we developed a functional pipeline that evaluates number of synapses through immunocytochemistry and electrophysiological properties through multi-electrode arrays. Overall, our computational integration of multi-omic and single-cell datasets enables informed and multidisciplinary prioritization of novel candidate therapeutic targets. At the same time, the use of humanized neuronal models for functional validation, aims to provide biological insights towards

elucidating the disease-relevant mechanisms and ultimately designing novel therapies.

Funding source: R01 MH109978

Keywords: Neuropsychiatric disease, Schizophrenia-associated neuronal models, Computational prioritization of candidate genes

11:45 – 11:53
BRAIN ORGANOIDS REPRODUCIBLY GENERATE THE CELLULAR DIVERSITY OF THE HUMAN CEREBRAL CORTEX

Velasco, Silvia[1,2], Kedaigle, Amanda[2], Simmons, Sean[2], Quadrato, Giorgia[3], Adiconis, Xian[2], Paulsen, Bruna[4], Regev, Aviv[5], Levin, Joshua[2] and Arlotta, Paola[4]

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Experimental models of the human brain are needed for basic understanding of its development and disease. Human brain organoids hold unprecedented promise for this purpose; however, their use as experimental systems has been limited by their poor characterization and inherent reproducibility. Here, we show that a newly optimized organoid model pre-patterned to form the dorsal forebrain can achieve highly reproducible generation of a rich diversity of cell types appropriate for the developing human cerebral cortex. Using single-cell RNA sequencing of 166,242 cells isolated from 21 individual organoids, from different experimental batches and stem cell lines, we find that 95% of the organoids generate a virtually indistinguishable compendium of cell types, through the same developmental trajectories, and with organoid-to-organoid variability comparable to that of individual endogenous brains. The data demonstrate that reproducible development of complex central nervous system cellular diversity does not require the context of the embryo, and that establishment of terminal cell identity is a highly constrained process that can emerge from diverse stem cell origins and growth environments. The work paves the way for modeling aspects of human cortical development and disease that have never been experimentally accessible before.

Keywords: 3D human brain organoids, Neurodevelopment, Disease modeling

11:55 – 12:03
INTERSPECIES CHIMERIC FRAGILE X SYNDROME MODEL REVEALS FMR1-DEPENDENT NEURONAL PHENOTYPES

Krzisch, Marine A.[1], Wu, Hao[2], Yuan, Bingbing[3], Fu, Dongdong[1], Liu, Shawn[4], Garrett-Engele, Carrie[1], Ng, Carrie[2], Ling, Ling[2], Kakumanu, Akshay[2], Chang, Aaron[2], Warren, Steven[2], Cacace, Angela[2], Wallace, Owen[2], Rietjens, Rosalie[1], Andrykovich, Kristin[1], Jain, Bhav[1] and Jaenisch, Rudolf[1]

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Fragile X syndrome (FXS) is the leading cause of inherited intellectual disability. It stems from an expansion of the CGG triplet repeats in the FMR1 gene, leading its transcriptional silencing. FMR1 protein (Fmrp) is a potential translational repressor at the synapse and its absence is thought to cause defects in neuronal development. Data on FXS patients remains scarce, and so far, findings from FXS animal models have failed to translate into successful therapies. This highlights the need to develop human cell-based models of FXS. Culture conditions fail to fully recapitulate the morphological and functional characteristics of neurons in the human brain and in vitro models show important intrinsic variation. Here, we co-transplanted neural precursor cells (NPCs) from FXS patient-derived induced pluripotent stem cell (iPSC) lines and isogenic control iPSC lines where the Fmr1 mutation was corrected in the brain of neonatal immune-deprived mice. The cells migrated away from the transplantation site and differentiated into neurons and glial cells. We assessed cell-autonomous developmental phenotypes of transplanted neurons at different timepoints post-transplantation. Transplanted FXS neurons showed accelerated morphological and functional maturation compared to isogenic control, whereas we failed to detect significant differences in the maturation of FXS and control neurons in vitro. Single cell RNA sequencing of transplanted cells and bulk RNA sequencing of cultured neurons both revealed alterations in synaptogenesis and neuronal maturation pathways. Finally, principal component analysis suggested that transplanted neurons are more similar to neurons from the human primary cortex than neurons from human brain organoids. Together, these results indicate that modeling FXS using the transplantation of NPCs derived from patients into the mouse brain may allow to better mimick the development of human neurons than traditional culture models, and reveal phenotypes not observed in culture. Additionally, our transplantation model provides a readout for testing potential therapeutic compounds in vivo.

Keywords: Fragile X syndrome, Disease modeling, Neuron

12:05 – 12:13

ALZHEIMER PATIENT-DERIVED INDUCED NEURONS REVEAL A WARBURG-LIKE METABOLIC SWITCH

Traxler, Larissa[1], Herdy, Joseph[2], Stefanoni, Davide[3], Schlachetzki, Johannes[4], Kim, Yongsung[2], Agarwal, Ravi[2], Böhnke, Lena[5], Edenhofer, Frank[6], Gage, Fred[2], D'Alessandro, Angelo[3] and Mertens, Jerome[5]

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Old age is the most significant risk factor for several disease, including diabetes, cancer, and a majority of dementias including Alzheimer's disease (AD). A dominating research focus in the AD field has been the characterization of potentially toxic protein oligomers and aggregates. Based on genetic models for the rare familial forms of the disease, a prevailing view that encompasses protein toxicity followed by cell autonomous and inflammation-related neuronal cell death has been established. However, following over a one-hundred failed clinical trials in the past twenty years based on this view, skepticism in the existent hypotheses and its model systems is growing. Due to the inaccessibility of live human brain tissue, direct conversion of patient fibroblasts into induced neurons (iNs) offers a path towards the next generation of disease models. As opposed to rejuvenated iPSC-derived neurons, direct iN conversion yields neurons with preserved signatures of epigenetic and functional aging. We previously detected AD-specific changes in age-equivalent iNs from AD patients, which were absent in isogenic but rejuvenated iPSC-derived neurons, and that revealed aberrant activation of signaling pathways and a hypo-mature state of AD iNs. Here, comparison of iNs from 10 AD patients and 10 non-demented controls revealed a metabolic energy crisis in AD iNs, characterized by decreased ATP/ADP levels and increased consumption of NAD+; all despite functional mitochondrial respiration. Paired mass-spectrometry-based metabolomics and transcriptomics revealed a global metabolic transformation of AD iNs towards increased glycolysis, serine biosynthesis, and fatty acid synthesis, which correlates with the hypo-mature neuronal state and increased competence for neuronal apoptosis. Notably, similar metabolic events are a well-known hallmark of cancer, where aberrant aerobic glycolytic activity is known as the Warburg effect. Interestingly, Warburg-related changes and metabolites have been postulated to drive cellular de-differentiation and transformation. We thus conclude that an age-dependent but AD-specific metabolic switch in neurons might ultimately lead to a neuronal identity crisis that is accompanied by increased cellular vulnerability and impaired functionality.

Keywords: Induced neurons (iNs), Alzheimer's Disease, Metabolism

12:15 – 12:30

BASIC AND TRANSLATIONAL DISEASE MODELING WITH PATIENT IPSC-DERIVED NEURAL CELLS

Inoue, Haruhisa

CiRA, Kyoto University, Kyoto, Japan

The iPSC technology was established a decade or so ago, and enormous progress in stem cell medicine has since been made. Human iPSC-derived neuronal cells, which had previously been inaccessible, now show the exciting promise of multiple applications. Various disease pathomechanisms have been revealed, and new drugs originating from iPSC screens are in the pipeline. In conjunction with that, recent advances in new technologies are providing opportunities to elevate iPSC-based platforms ever higher in the challenge of neurodegenerative disease modeling and drug discovery. I'd like to talk about our recent efforts and discuss various perspectives of iPSC-based disease modeling and drug discovery.

Keywords: translational research, patient iPSCs, neurodegenerative diseases

THURSDAY, JUNE 25, 11:00 – 12:45

CONCURRENT - MODELING DEVELOPMENT AND DISEASE: ORGANOIDS OF ENDODERM AND KIDNEY

11:05 – 11:20

CELL PLASTICITY AND HUMAN LIVER DISEASE MODELS

Hui, Lijian

Shanghai Institute of Biochemistry and Cell Biology, Shanghai, China

Cell plasticity provides a strategy to generate functional cells independent of progenitor cells. We recently developed measures to culture and expand functional hepatocytes through either transdifferentiation or de-differentiation. These functional hepatocytes are now under characterization for their potential in human liver disease treatment. In this talk, I will present our progress in applying functional hepatocytes for modeling human liver diseases in vitro, e.g. the development of

human liver cancers, and underlying mechanisms we learned from these new models.

Keywords: Cell plasticity, functional hepatocytes, liver disease models

11:25 – 11:33

HUMAN PLURIPOTENT STEM CELL DERIVED LUNG DISTAL TIP PROGENITORS ARE STEPPING TOWARD DISEASE MODELING

Liu, Hsiao-Yun, Chen, Ya-Wen and Snoeck, Hans-Willem

Department of Medicine, Columbia University Medical Center, New York, NY, USA

Engraftment of progenitor cells in the lung remains a challenge. It has been shown that isolated mouse fetal distal tip cells can engraft in bleomycin or naphthalene-injured lungs. We showed previously that we can generate 3D lung organoids from human pluripotent stem cells. We describe here the generation from hPSCs, including induced pluripotent stem cells, of a population of putative fetal lung distal tip progenitors (pDTPs) that can be expanded continuously (up to now 26 months) and efficiently repopulate most or all lineages in bleomycin-injured lungs of immunodeficient NSG mice. We also observed engraftment of submucosal glands, which contain the reserve stem cells of the airways and are believed to play major role in the pathogenesis of the pulmonary manifestations of cystic fibrosis. Furthermore, these cells can be converted in vitro to cells with characteristics of SMG myoepithelial cells which exclusively repopulate SMGs in vivo. As a fraction of cystic fibrosis (CF) patients does not express any CFTR or show folding and trafficking abnormalities that are not fully corrected by current corrector drugs, stem cell therapy for CF may become envisageable and might one day provide a definitive cure in such patients. The pDTPs we generated might be one candidate cell population to achieve a definitive cure for such patients. Proper conditioning of the host, however, remains a major challenge that has to be met.

Keywords: fetal lung distal tip progenitors, progenitor cells engraftment, cystic fibrosis

11:35 – 11:43

MAMMARY CELL LINEAGES ASSOCIATED WITH BRCA1/2 HETEROZYGOSITY IN PATIENT-DERIVED ORGANOID CULTURES

Rosenbluth, Jennifer M[1], Li, Carman[2], Gray, G. Kenneth[2], Goh, Walter[2], Schackmann, Ron[2], Garber, Judy[1], Dillon, Deborah[3] and Brugge, Joan[2]

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The human mammary gland is comprised of multiple epithelial cell lineages including mature luminal, luminal progenitor, and basal/stem cells. We previously found that all three major mammary lineages can be preserved in patient-derived organoid cultures. The relative proportion of these cell types is known to be altered in certain clinical conditions, such as heterozygosity for the breast cancer predisposition gene BRCA1. We used single-cell mass cytometry for a panel of 38 markers associated with breast development and tumorigenesis to obtain a high-dimensional view of cell lineages present in patient-derived mammary organoids. Breast organoids heterozygous for BRCA1/2 showed enrichment for a specific subtype of luminal cell as compared to wild-type control organoids. An increase in this cell population is also associated with aging, and was found in situ to be in association with terminal ductal lobular units, which have been proposed to be the site of origin of breast cancer. Using our system, we have identified growth factors and signaling pathways that promote the expansion of these cells in the BRCA1/2–mutated organoid. Thus, we have established a breast organoid system for modeling the earliest stages of breast cancer, and have expanded our tissue repository to characterize organoids derived from over 100 patients with and without inherited mutations in breast cancer predisposition genes.

Keywords: breast cancer, organoid, BRCA1

11:45 – 11:53

ENGINEERED HUMAN KIDNEY TISSUES TO UNDERSTAND THE MECHANISMS OF DEVELOPMENT AND DISEASE PROGRESSION

Musah, Samira

Department of Biomedical Engineering & Medicine/ Nephrology, Duke University, Durham, NC, USA

Molecular signals can function synergistically with mechanical forces to guide tissue development and organ function. In this work, we employed strategies to modulate human stem cell biology by using molecular and mechanical cues with the goal of addressing some of the most challenging problems in renal medicine. More than 10% of the world's population suffer from kidney disease, and targeted therapeutic strategies are still needed. Functional in vitro models of the human kidney could facilitate therapeutic discovery and enable mechanistic studies of renal disease. Efforts to develop such models are limited by the lack of human kidney cells such as podocytes, which are the specialized epithelial cells that regulate selective permeability in the glomerulus. In this study, we developed a highly efficient method for the differentiation of human induced pluripotent stem (iPS) cells into kidney glomerular podocytes. By using Organ Chip (organ-on-a-chip) microfluidic devices, we developed an in vitro model of the human kidney glomerular capillary wall that supports podocyte differentiation and recapitulate the nor-

mal tissue-tissue interface and selective permeability of the glomerulus. In an extension of this study, we developed an in vitro disease model that enabled the discovery of a new pathway for modulating podocyte cell fate transitions under healthy and diseased conditions. We are extending this work to further understand the mechanisms of human kidney disease, with the goal of developing a robust platform for the identification of novel disease biomarkers and targeted therapeutics.

Funding source: This work was supported by an Incubation Fund from The Duke Innovation and Entrepreneurship Initiative, and a Burroughs Wellcome Fund PDEP Career Transition Ad Hoc Award for S.M.

Keywords: Organs-on-chips, Kidney podocyte, disease models

11:55 – 12:03

TISSUE SCALE CULTURE OF HUMAN PLURIPOTENT STEM CELL DERIVED LIVER ORGANOIDS AND HIGH SENSITIVITY ANALYSIS TECHNIQUES

Harrison, Sean P.[1], Wesseler, Milan[2], Skottvoll, Frøydis[3], Hansen, Frederik[4], Mrsa, Ago[3], Pedersen-Bjergaard, Stig[4], Baumgarten, Saphira[1], Bogen, Inger[5], Larsen, Niels[2], Wilson, Steven[3] and Sullivan, Gareth[1]

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Human pluripotent stem cell derived liver cells are of interest due to their xenobiotic metabolism, the role of drug-induced-liver-injury during development of pharmaceuticals and as models for disease and development. While their incorporation into microfluidic devices and online systems can bring with them the benefits of a high degree of control and lower variability. Current liver-on-chip devices often use simple hepatocyte models; here we present multi hepatic cell type containing liver organoids exhibiting features of tissue-like complexity with an easily scaled volume based growth factor free production method. These organoids show long-term maintenance of hepatic phenotype such as inducible CYP450 activity for at least 80 days and can be grown at densities of >500/ml in volumes >300ml, allowing use in tissue scale cultures not feasible with current organoid production methods. In perfused tissue chips with synthetic vasculature and tuneable oxygen gradients they show longevity and viability with culture periods exceeding 4 months. The resulting fused cell masses are on a millimetre scale, providing a more in vivo like model of hepatic tissue than is possible with smaller or simpler cultures. At the other end of the scale spectrum, techniques to interrogate smaller numbers of the liver organoids can increase their applicability for use in drug development or toxicity screening. Here we show the use of electro-membrane-extraction (EME), on small sample sizes of 50 organoids in 100ul volumes, for separation of heroin metabolites from potential contaminants and confounding factors in the culture medium. These EME separated extracts are then compatible with various mass-spectrometry (MS) techniques including the highly sensitive nano-liquid chromatography (LC) which is capable of detecting metabolites in samples 1000 fold more dilute than conventional ultrahigh pressure LC-MS. NanoLC-MS was also used to assess the proteome of the organoids revealing a high degree of similarity compared to primary liver tissue particularly among the most abundantly expressed proteins. This method for generating large amounts of complex organoids, along with highly sensitive separation and analysis, allows for the formation of a diversity of hepatic model systems highly amenable to chip-based and/or online culture.

Funding source: This work was partially supported by the Research Council of Norway through its Centres of Excellence scheme, project number 262613. Financial support from UiO:Life Science is gratefully acknowledged.

Keywords: Liver Organoid, Pluripotent stem cell, microfluidic-chip

12:05 – 12:13

A PATIENT IPSC LINE REVEALS THE PENETRANCE OF PANCREATIC AGENESIS CAUSED BY GATA6 MUTATIONS IS MODIFIED BY A NON-CODING SNP

Gadue, Paul J.

Department of Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, PA, USA

GATA6 is a critical regulator of pancreas development, with heterozygous mutations in this transcription factor being the most common cause of pancreas agenesis. However, patients harboring GATA6 mutations exhibit variability in disease penetrance. We have used a pancreatic agenesis patient-induced pluripotent stem cell (iPSC) model to study this disorder, using the CRISPR-Cas9 system to correct the coding mutation and pancreatic in vitro differentiation to monitor pancreas development. We found that while the corrected iPSC line demonstrated GATA6 protein expression that was indistinguishable from other wild type (WT) stem cell lines at the definitive endoderm stage of development, GATA6 protein expression was only partially restored at the pancreatic progenitor stage. To investigate this finding, we screened the regulatory regions of the GATA6 gene and identified a SNP in a 3' regulatory region of GATA6, with the patient being homozygous for the minor allele variant. We tested the SNP in 32 further patients with pancreatic agenesis caused by

GATA6 mutations and found that the frequency of the minor allele was significantly enriched in the pancreatic agenesis cohort. Using genome editing, we introduced the minor allele SNP in the context of WT GATA6 or a heterozygous coding mutation into WT ESC and iPSC lines. We found the minor allele SNP depressed GATA6 expression specifically at the pancreas progenitor stage of development, working additively with the heterozygous coding mutation. The lines harboring both the coding mutation and the minor allele SNP had the lowest levels of GATA6 expression and lowest efficiency of pancreas development as assayed by generation of PDX1+NKX6-1+ cells amongst other markers of pancreas development. The minor allele SNP disrupted binding of the orphan nuclear receptor RORA as demonstrated by ChIP. Finally, the patient iPSC line with the corrected coding mutation was also genome edited to introduce a RORA consensus binding site at the SNP location and demonstrated enhanced GATA6 expression and pancreas differentiation. Our work reveals a genetic modifier contributing to the pancreatic agenesis phenotype in patients with GATA6 mutations and shows the benefits of using pluripotent stem cells to study the effects of non-coding genetic variants in modifying disease penetrance.

Keywords: Pancreas, Pluripotent Stem Cells, Disease Modifier

12:15 – 12:30
RECONSTITUTION OF ALVEOLAR REGENERATION BY INFLAMMATORY NICHES

Lee, Joo-Hyeon

University of Cambridge, UK

Tissue regeneration involves a multi-step process composed of diverse cellular hierarchies and states that are also implicated in tissue dysfunction and pathogenesis. Here we identified the lineage trajectory from AT2 toward AT1 cells during alveolar regeneration after injury. Single cell RNA-sequencing analysis of in vivo AT2 lineage-labeled cells and ex vivo AT2 cell-derived organoids allowed us to delineate a precise differentiation trajectory in which AT2 cells adopt a 'priming' state followed by transition into 'Damage-Associated Transition Progenitors (DATPs)' prior to conversion into terminally differentiated AT1 cells. Importantly, we demonstrated that inflammatory niches driven by IL-1? signaling pathways orchestrate the regeneration process by triggering state-specific differentiation programs of AT2-lineage cells. Our study reveals essential functions of inflammation in alveolar regeneration, providing new insights into how chronic inflammation impairs tissue restoration and leads to lung diseases.

Funding Source: Wellcome Trust/Royal Society Sir Henry Dale Fellowship (107633/Z/15/Z), European Research Council Starting Grant (679411)

Keywords: Inflammatory niche, alveolar regeneration, Damage-Associated Transient Progenitors

THURSDAY, JUNE 25, 11:00 – 12:45

CONCURRENT - TISSUE STEM CELLS AND REGENERATION: HEMATOPOIETIC AND ENDOTHELIAL CELLS

11:05 – 11:20
CROSS COMMUNICATION OF HEMATOPOIETIC CELLS OF ADULT AND EMBRYONIC ORIGIN

Waskow, Claudia

Leibniz Institute on Aging- FLI, Jena, Germany

Embryo-derived macrophages persist in many tissues throughout life but their turn-over kinetic, precise function and importance for adult hematopoiesis and tissue integrity over time is underexplored. It remained unclear how the differentiation of dendritic cells (DCs) is regulated in vivo. We show that Csf1r-mediated signals control the spleen DC pool size by a cell-extrinsic and non-hematopoietic mechanism engaging embryo-derived tissue-resident red pulp macrophages (RP-Mp), providing a novel regulatory mechanism controlling the differentiation of blood cells from definitive hematopoietic stem cells in the adult mouse. The RP-Mp – DC interaction axis remains physiologically of great importance during the regeneration of DCs after activation-induced depletion in adult mice in vivo. We further show that bone-resorbing osteoclasts that are crucial regulators of bone integrity and tooth eruption are of embryonic origin and their maintenance depends on iterative fusion of circulating monocytes with long-lived osteoclast syncytia. Consequently, monocyte transfusion results in gene transfer that can be exploited to rescue adult-onset osteoclast pathologies such as osteopetrosis mediated by cathepsin K-deficiency. Thus, we show that cross communication between hematopoietic cell types of distinct developmental origins is required for steady-state hematopoiesis throughout life, providing evidence for a novel layer of complexity for the understanding of hematopoietic differentiation processes in vivo and for potential future interference options.

Keywords: Hematopoiesis, Embryo, Adult, Mouse, macrophage, dendritic cell, osteoclast, immune cell homeostasis

11:25 – 11:33

JAGGED1 IS REQUIRED FOR THE TRANSITION OF HEMATOPOIETIC STEM CELLS FROM THE FETAL LIVER TO THE ADULT BONE MARROW NICHE.

Pajcini, Kostandin V. and Shao, Lijian

[1]Pharmacology, Center for Stem Cells and Regenerative Medicine, University of Illinois at Chicago, IL, USA

Notch signaling is known to play important roles in hematopoietic development and differentiation. Notch1 is required for emergence of the definitive hematopoietic stem cell (HSC) from the hemogenic endothelium, and we have previously shown that Notch signaling is essential for survival and function of HSCs in the fetal liver. Activation of canonical Notch signaling requires direct cellular contact; thus, the identity of the ligand and the ligand-presenting cell during hematopoietic development would provide valuable information of the Notch signaling mechanism in HSCs as well as the identity of key niche cells that drive the expansion and cell fate decisions of embryonic HSCs. We have taken a comprehensive approach to determine the ligands and cells that initiate Notch signaling in the fetal liver. To this end, we have performed single-cell RT-qPCR for all Notch signaling proteins in E14.5 fetal HSCs and compared the findings to adult bone marrow HSCs. We also have analyzed fetal liver endothelial cells for surface expression of all Notch ligands. We determined that Jagged1 (Jag1) is highly expressed in both endothelial cells as well as in fetal HSCs. We have performed conditional loss-of-function analysis of Jag1 in fetal endothelial cells using Ve-cadherinCreERT2 as well as in fetal hematopoietic lineages using VavCre. Our results indicate that while loss of endothelial Jag1 has severe effects in embryonic vascular development, loss of hematopoietic Jag1 allows for normal fetal morphology, yet severely impedes the functional ability of fetal liver HSCs to expand and differentiate both in vitro and in vivo. Fetal to adult transplantation of VavCre+Jag1f/f HSCs indicated a defect in reconstitution potential of fetal HSCs that lack Jag1 expression. Furthermore, we have established a defect in occupancy of the early, post-natal bone marrow niche in VavCre+Jag1f/f pups. Our findings indicate that Notch ligand Jag1 is essential for fetal liver HSC development and for homing and reconstitution potential of fetal HSCs into the post-natal bone marrow microenvironment.

Funding source: NIH/NHLBI

Keywords: Hematopoietic development, Notch signaling, HSC niche

11:35 – 11:43

IDENTIFICATION OF POTENT HUMAN HEMATOPOIETIC STEM CELLS USING MITOCHONDRIAL PROFILE TO IMPROVE BONE MARROW TRANSPLANTATION

Qiu, Jiajing, Gjini, Jana, Lin, Miao, Arif, Tasleem and Ghaffari, Saghi

Cell, Developmental and Regenerative Biology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Bone marrow transplantation remains the only cure for many blood disorders. However, despite extensive research, identification of hematopoietic stem cells (HSCs) with high engraftment ratio and long-term repopulation capacity for bone marrow transplantation and immune reconstitution remains challenging. Using mitochondrial membrane potential (MMP) as an intrinsic functional marker of hematopoietic stem cell activity, we have recently identified a minor but greatly potent subpopulation of highly purified immunophenotypically-defined murine HSCs (Liang et al., Cell Stem Cell, 2020). We have now extended these studies to human HSCs. We found that lower mitochondrial activity is maintained in primitive human HSCs. Quiescence is the main property of the most potent HSCs. To our surprise, we found that even within highly primitive CD34+CD38-CD45-CD90+ HSCs that are mostly quiescent, HSCs with the lowest mitochondrial membrane potential (MMP) still differ in CDK6 expression and subcellular localization from the ones with high MMP upon cytokine exposure. Recurring cell cycle entrance diminishes stem cell potential. MMP-low HSCs showed delayed kinetics of cell cycle entry relative to MMP-high HSCs. Limiting dilution analysis of long-term culture initiating cell (LTC-IC) revealed that the frequency of LTC-IC in MMP-low HSCs is significantly greater than in MMP-high HSCs. Notably, these two populations were prominently distinct in their long-term repopulation capacity in vivo. At 5 months post transplantation, the engraftment ratio of MMP-low HSCs (CD34+CD38-CD45-CD90+) was 17-fold higher than that of MMP-high HSCs as determined by multilineage engraftment in immunodeficient NSG mice. Furthermore, morphological analysis showed that mitochondria are more fragmented in MMP-low as compared to MMP-high HSCs, suggesting lysosomal clearance of mitochondria might be implicated in maintaining HSCs' functional capacity. Our findings suggest that MMP-low HSCs are highly potent and likely maintained in a dormant state. They further indicate that by using mitochondrial activity in a single step flow cytometry approach highly potent HSCs can be identified for further human clinical applications.

Funding source: This work is supported by NYSTEM funds IIRP C32602GG, and NIH funds R01CA205975, R01HL136255.

Keywords: hematopoietic stem cell, mitochondria, bone marrow transplantation

11:45 – 11:53

REGULATION OF HEMATOPOIETIC STEM CELL FUNCTION BY MITOCHONDRIAL DYNAMICS

Yao, Yan[1], De Almeida, Mariana[2], Luchsinger, Larry[1] and Snoeck, Hans-Willem[1]

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Despite their established therapeutic potential, the biology of hematopoietic stem cells (HSCs), which sustain lifelong blood production, is poorly understood. In a recent focus on the role of metabolism, the field has shown that HSCs generate ATP predominantly through glycolysis and have low mitochondrial respiration activity. We previously showed however that HSCs have high mitochondrial mass and a fused mitochondrial network compared to more mature populations. Deletion of Mitofusin 2 (Mfn2), a protein involved in mitochondrial fusion and ER-mitochondria tethering, caused a partial loss of lymphoid capacity that was mediated by increased intracellular calcium and resulting NFAT activation, thus demonstrating ATP-independent functions of mitochondria in cell fate decisions of HSCs. To further uncover unique roles of mitochondria in maintaining identity and function of HSCs, we completely disrupted mitochondrial dynamics by conditional knockout of the two mediators of mitochondrial outer membrane fusion, Mitofusins (Mfn) 1 and 2, in hematopoietic system. The double mutant embryos (DKO) died in utero and were anemic. Fetal liver (FL) HSCs were expanded, but failed to reconstitute any lineages after transplantation. One allele of Mfn1 was sufficient to largely rescue Mfn1/2-DKO HSC function, whereas one allele of Mfn2 rescued only myeloid reconstitution, demonstrating the very distinct physiological roles of these highly homologous proteins in HSCs. Genome-wide expression analysis of purified HSCs showed general downregulation of transcripts associated with HSC or with lymphoid lineages and reciprocal upregulation of transcripts expressed in granulocytes, suggesting epigenetic regulation of fate decisions by mitochondrial dynamics at the level of the HSC. In conclusion, our findings highlight the importance and complexity of mitochondrial function and dynamics in HSCs and point to a novel role for mitochondria in lineage specification and HSC function.

Keywords: Hematopoiesis, Mitochondria, Mitofusins

11:55 – 12:03

15-PGDH INHIBITION POTENTIATES MURINE HEMATOPOIETIC REGENERATION VIA SPLENIC NICHE ACTIVATION

Smith, Julianne N., Christo, Kelsey, Jogasuria, Alvin, Udayakumar, Priyanka, Broncano, Alyssia, Cameron, Mark, Dawson, Dawn, Gerson, Stanton, Markowitz, Sanford and Desai, Amar

Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH, USA

Hematopoietic stem cell (HSC) transplantation (HST) is a curative treatment for hematologic malignancies and bone marrow (BM) disorders, but is limited by complications including a lengthy recovery period, severe infections and bleeding, and a high incidence of graft vs. host disease. We have previously demonstrated that inhibiting 15-hydroxyprostaglandin dehydrogenase (15-PGDH) using the small molecule SW033291 (PGDHi) in mice increases BM prostaglandin E2 (PGE2) levels, expands HSC numbers, and accelerates hematologic reconstitution following HST. Here we demonstrate that the splenic microenvironment, specifically 15-PGDH+ macrophages (MΦs), megakaryocytes (MKs), and mast cells (MCs), regulates steady-state hematopoiesis and potentiates recovery after HST. At steady-state, 15-PGDH was abundantly expressed in the spleen and PGDHi resulted in increased BrdU+ cells, total nucleated cells, and hematopoietic stem and progenitor cells per spleen, indicating that PGDHi induces non-pathologic extramedullary hematopoiesis. Notably, splenectomy prior to HST abrogated the impact of PGDHi on neutrophil, platelet, and HSC recovery. In the spleen, 15-PGDH localized to F4/80+ MΦs, CD61+ MK lineage cells, and FceR1 alpha+ MCs, identifying these cell types as likely direct responders that coordinate the impact of PGDHi on HSCs. To determine whether 15-PGDH+ splenic cells influence the survival or proliferation of BM cells, splenic MΦs, MKs, and MCs were individually added to BM co-cultures. Strikingly, the addition of 15-PGDH+ cell types conferred superior BM cell maintenance relative to control BM cultures. 15-PGDH similarly localized to human MΦs, MKs, and MCs, and these cell types also enhanced the maintenance of co-cultured autologous human BM cells. Our findings therefore establish that 15-PGDH expression marks novel niche cell types that regulate both murine and human BM cells. The splenic microenvironment is known to regulate HSC function, particularly during demand-adapted hematopoiesis, however practical strategies to enhance splenic support of transplanted HSCs have proven elusive. Our work provides a well-tolerated strategy to therapeutically target splenic niches, and specifically MΦs, MKs, and MCs, to pro-

mote hematopoietic regeneration and improve clinical outcomes of HST.

Funding source: This work was funded by NIH grants R35 CA197442 to S.D.M., K99 HL135740 to A.B.D., T32 EB005583 to J.N.P.S., and P30 CA043703 to support core facilities of the Case Comprehensive Cancer Center.

Keywords: Regenerative Medicine, Stem Cell Microenvironment, Prostaglandins

12:05 – 12:13
EMBRYONIC MULTIPOTENT PROGENITORS SPECIFIED DURING THE DEFINITIVE WAVE OF HEMATOPOIESIS REPRESENT A SIGNIFICANT SOURCE OF ADULT BLOOD

Patel, Sachin[1], Carmargo, Fernando[1], Christodoulou, Constantina[1], Daley, George[1], Lummertz da Rocha, Edroaldo[1], Osorio, Fernando[1], Pepe-Mooney, Brian[1], Weinreb, Caleb[2] and Yu, Qi[1]

[1]Stem Cell Program, Children's Hospital Boston, Boston, MA, USA, [2]Department of Systems Biology, Harvard University, Boston, MA, USA

It is generally thought that, at the clonal level, blood is constructed from a small number of embryonic-born hematopoietic stem cells (HSCs). These rare embryonic HSCs arise within the large arteries of the embryo as part of hematopoietic clusters derived from hemogenic endothelium. They then migrate to the fetal liver, where they mature and amplify, and, some time after, generate the progenitors that will establish and maintain the adult blood system. This view, however, is largely based on assays that measure lifespan and lineage output after embryonic disruption and cellular transplantation. How the hematopoietic system is clonally constructed and when progenitor specification occurs in the native state is unknown. Here, we use in situ transposon barcoding and fate mapping to assess the developmental and clonal origins of adult blood. Notably, our analysis reveals that a large fraction of adult blood production derives from a population of non-transplantable embryonic multipotent progenitors (eMPPs), independent of traditional adult HSCs. eMPPs predominantly drive early hematopoiesis in the young adult, have a decreasing yet life-long contribution over time, and are more competent in producing lymphoid progeny than adult HSCs. We find that putative eMPPs begin to be specified within intra-arterial hematopoietic clusters as early as embryonic day 10.5 and represent a distinct transcriptional program that diverges from traditional HSCs. Our results here implicate embryonic-born MPPs as significant contributors of lifelong hematopoiesis and suggest that adult blood is derived from both traditional HSC- and eMPP-dependent lineages.

Keywords: blood development, multipotent progenitors, clonal barcoding

12:15 – 12:30
GENOME EDITING TO CREATE SAFE AND EFFECTIVE STEM CELL BASED MEDICINES

Porteus, Matt

Stanford Medical School, Stanford, CA, USA

The genetic engineering of stem cells has the potential to make stem cell based therapies both more effective and safer. I will describe the development of a highly effective genome editing system using the ribonucleoprotein (RNP) delivery combined with AAV6 transduction to generate high frequencies of targeted integration of genes by homologous recombination. In a wide variety of cell types, the efficiency of targeted integration using this system ranges from 40-80% when the ex vivo stem cell biology culture conditions are optimized. We have applied this system in a variety of different ways including the direct correction of disease causing variants as a path towards genetically corrected autologous stem cell based therapies as well to combine with synthetic biology to create cells that can provide a therapeutic function after transplantation. I will discuss some of these applications during my presentation.

Keywords: genome editing, hematopoietic stem cells, mesenchymal stromal cells, human pluripotent cells

THURSDAY, JUNE 25, 16:00 – 18:00

PLENARY III: EMBRYOGENESIS AND DEVELOPMENT

16:05 – 16:20
GENETIC APPROACHES TO STUDY EARLY LINEAGE SPECIFICATION IN HUMAN EMBRYOS

Niakan, Kathy

The Francis Crick Institute, London, UK

During preimplantation development human embryos are comprised of pluripotent embryonic cells, which eventually form the fetus, and extra-embryonic cells, which contribute to the placenta and yolk sac. The central question we address is what are the molecular mechanisms that regulate these early cell fate choices in human embryos. We are using CRISPR/Cas9-mediated genome editing, TRIM-Away protein depletion, dominant negative mutations and small molecules to dissect the function of genes during human embryogenesis. These methods have enabled us to uncover that the first lineage specification event in human embryos is the initiation of a placental program. By integrating signaling insights from human blastocysts we have also defined human embryonic stem cell culture conditions that more closely recapitulate the embryonic niche. The molecular basis of these early cell lineage decisions are of fundamental importance and have wide-reaching clinical implications for infertility, miscar-

riages, developmental disorders and therapeutic applications of stem cells.

Keywords: Human, lineage specification, embryonic stem cells

16:25 – 16:40
PROSPECTS FOR CHANGING CELL FATE AT WILL

Zaret, Kenneth S.

University of Pennsylvania, Philadelphia, PA, USA

The incredible diversity of animals and plants depends upon embryonic cells to differentiate into various cell types and to stably retain such differentiation after development. In the future, we will be able to control cell fate at will by knowing how cells initiate fate changes and how to overcome chromatin barriers that normally promote differentiation stability. We have proposed that pioneer transcription factors are basis for initiating cell fate changes by virtue of their ability to scan nucleosomal DNA, recognize a partial motif on the nucleosome surface, and initiate cooperative binding events that impart a functional identity to otherwise naïve or silent chromatin. Yet we have found that large blocks of heterochromatin, marked by H3K9me3, can prevent pioneer factor binding in some cases and thereby serve as an impediment to cell fate changes. I will describe how we have identified many proteins embedded in H3K9me3 heterochromatin that function in groups to repress different types of heterochromatic genes and repeat elements, and whose activities can be overcome to enable new cell fates to be reprogrammed.

Keywords: cell fate; reprogramming; chromatin

16:45 – 17:00
CHROMATIN SAFEGUARD FOR CARDIAC MESODERM DIFFERENTIATION

Bruneau, Benoit

Gladstone Institutes, San Francisco, CA, USA

Haploinsufficiency of transcriptional regulators causes human congenital heart disease (CHD), predicting gene regulatory network (GRN) imbalances. The nature and impact of dosage-vulnerable GRNs in human CHDs are unknown. Here, we define transcriptional consequences from reduced dosage of the CHD transcription factor TBX5 during human cardiac differentiation from induced pluripotent stem (iPS) cells. Single cell RNAseq revealed discrete sub-populations of cardiomyocytes exhibiting dysregulation of distinct TBX5 dose-sensitive genes, related to cellular phenotypes and CHD genetics. Developmental trajectory inference revealed TBX5 dosage-dependent differentiation paths. GRN analysis of single cell RNAseq data identified vulnerable nodes enriched for CHD genes, indicating sensitivity to TBX5 dosage in cardiac network stability. A GRN-predicted genetic interaction between TBX5 and MEF2C validated in mouse, uncovering a highly dosage-sensitive CHD pathway. Our results demonstrate exquisite sensitivity to TBX5 dosage in discrete sub-populations of iPSC-derived cardiomyocytes and predict GRNs for human CHDs, with insights for quantitative transcriptional regulation in disease.

Keywords: heart, gene expression, iPS cells, congenital heart disease

17:05 – 17:13
REVEALING THE CRITICAL REGULATORS OF CELL IDENTITY IN HUMAN PRE-IMPLANTATION EMBRYOS AND HUMAN NAÏVE AND PRIMED PLURIPOTENT STEM CELLS USING SINGLE-CELL MULTI-OMICS

Pasque, Vincent[1], Chappell, Joel[1], Fernandez, Elia[2], Oberhuemer, Michael[1], Pham, Thi Xuan Ai[1], Sifrim, Alejandro[1] and Voet, Thierry[2]

[1]Department of Development and Regeneration, KU Leuven, Belgium, [2]Department of Human Genetics, KU Leuven, Belgium

Cellular identity during development is under the control of transcription factors (TFs) that form gene regulatory networks. However, the TFs and gene regulatory networks underlying human pre-implantation embryo development remain largely unexplored. Here, I will present new studies combining single-cell genome-plus-transcriptome sequencing and computational approaches to comprehensively construct the gene regulatory networks of human pre-implantation embryos, to identity key regulators of cell identity and to investigate the transcriptional effects of aneuploidies. I will show that a network of 248 TFs forms 7 major gene regulatory modules that characterize the distinct lineages of human pre-implantation embryos. I will present evidence that several elements of the network are shared between human and mouse and identify human-specific regulators. I will show that our approach identifies critical regulators of cell identity in human embryos. I will present evidence that link aneuploidies to changes in gene dosage and gene regulatory networks. I will also present our efforts to compare human naïve pluripotent stem cells in vivo and in vitro. Our results demonstrate how human TFs are likely to function in human pre-implantation embryos and in human naïve and primed pluripotency, providing a comprehensive consensus molecular regulatory atlas. We anticipate our analysis to be the starting point for a more sophisticated dissection of human embryogenesis. For example, the deletion and overexpression of individual or multiple TFs could be tested. Furthermore, TFs are major regulators of embryo development and are often perturbed in diseases, and a comprehensive understanding of how they work will be relevant in these areas.

Funding source: We are grateful to the FWO and KU Leuven for funding.

Keywords: human embryos, single-cell multi-omics, gene regulatory networks

17:15 – 17:23
PULSATION ACTIVATES PIEZO1 TO FORM LONG-TERM HUMAN HEMATOPOIETIC STEM CELLS

Scapin, Giorgia and Shah, Dhvanit

Center for Childhood Cancer and Blood Diseases, Nationwide Children's Hospital / The Ohio State University College of Medicine, Columbus, OH, USA

The origin of long-term, self-renewing hematopoietic stem cells (LTHSC) remain a mystery. The first set of LTHSCs is born from the hemogenic endothelial cells residing in the ventral wall of the dorsal aorta (DA) of the aorta-gonad-mesonephros region during embryonic development. However, it remains unknown why the ventral wall of the DA is the restricted site of HSC formation and how to make de novo engraftable human HSCs. Using single-particle tracking of pulsating DA, we demonstrate that the circumferential strain in the ventral wall, and not dorsal wall, is concurrent with and responsible for the magnitude, the site, and timing of the HSC formation. We extended our findings by developing a bioreactor to establish the functional link between DA pulsation and HSC formation. Using serial transplants, limiting dilution, and serial replating assays, we found that pulsation-mediated circumferential stretching of hemogenic endothelial cells or Piezo1 activation (Yoda1) yields 3-times higher amounts of LTHSC formation; which reconstitute normal multi-lineage adult blood. We further found that circumferential stretching or Piezo1 activation-derived HSCs reconstitute functional T and B cells, adult erythrocytes, and myeloid cells. Our Piezo1xScl-Cre conditional knockout further demonstrates that circumferential stretching of DA activates Piezo1; which enhances epigenetic regulator Dnmt3b expression to stimulate the endothelial-to-hematopoietic transition (EHT). To analyze the conserved role of PIEZO1-mediated mechanosensitive mechanisms in human hematopoiesis, we employed directed differentiation of human induced pluripotent stem cells (iPSCs) to hemogenic endothelial cells. We found that Yoda1-mediated PIEZO1 activation stimulated human EHT. In conclusion, pulsation-mediated circumferential strain activates Piezo1 to stimulate the EHT via the induction of Dnmt3b expression. This leads to the formation of LTHSCs, which can engraft and reconstitute multi-lineage, adult blood upon serial transplantations. We also establish its cross-talk with mechanosensitive and epigenetic mechanisms to produce functional, LTHSCs; which will yield cellular therapies for the treatment of human blood disorders.

Keywords: Long-Term Hematopoietic Stem Cells, Human iPSCs, Endothelial to Hematopoietic Transition

17:25 – 17:45
STEM CELLS: MAKING AND MAINTAINING TISSUES EVEN IN TIMES OF STRESS

Fuchs, Elaine

HHMI, The Rockefeller University, New York, NY, USA

Tissue stem cells form during embryogenesis, as progenitor cells confront increasingly complex surroundings and begin to assemble niches. Through intricate communication networks with their microenvironment, stem cells become progressively restricted as their neighbors instruct them what to do and when. Skin is an excellent system to interrogate stem cell: niche interactions. During embryogenesis, skin begins as a layer of progenitors that become specified to make and maintain either the epidermis or its hair follicle appendages. Both stem cell populations adhere to an underlying basement membrane rich in extracellular matrix and growth factors, and which separates epithelium and mesenchyme. However, the genes these stem cells express and the tasks they perform are influenced by their otherwise diverse microenvironments. Thus, basal epidermal progenitors generate a continuous flux of terminally differentiating cells that form and replenish the skin's barrier, while hair follicle progenitors periodically transition from a quiescent to activated state to fuel the cyclical regeneration of the hair follicle that produces the differentiated hair. The niche: stem cell interactions that dictate these orchestrated programs during homeostasis are still unfolding. At the surface of our skin, the epithelium is exposed to a variety of environmental stresses, including extremes in temperature and humidity as well as wounding, pathogens and inflammation. As the local environment changes, stem cells must be able to cope with these stresses. We use high throughput genetic and genomic approaches to learn at a molecular level how the stem cells' differentiation programs are primed to operate under environmental extremes and how stem cell interactions with their niches differ in homeostasis, wound repair and inflammation. Our global objective is to apply our knowledge of the basic science of epithelial stem cells to unfold new avenues for therapeutics.

Keywords: niche, inflammation, injury

THURSDAY, JUNE 25, 20:00 – 21:45

CONCURRENT - CLINICAL APPLICATIONS: HEMATOPOIETIC AND ENDOTHELIAL CELLS

20:05 – 20:20

IN VIVO CLONAL TRACKING AND LINEAGE MODELING IN HEMATOPOIETIC STEM CELL GENE THERAPY

Aiuti, Alessandro[1], Scala, Serena[2], Basso-Ricci, Luca[2], Quaranta, Pamela[3], Dionisio, Francesca[2], Omrani, Maryam[2], Naldini, Matteo Maria[4], Barcella, Matteo[2], Calabria, Andrea[2], Salerio, Federica Andrea[2], Monti, Ilaria[2], Giannelli, Stefania[2], Darin, Silvia[5], Migliavacca, Maddalena[5], Merelli, Ivan[2], Gattillo, Salvatore[5], Ostuni, Renato[2], Ciceri, Fabio[5], Gentner, Bernhard[2], Bernardo, Maria Ester[6], Ferrua, Francesca[6] and Cicalese, Maria Pia[7]

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Ex vivo gene therapy (GT) with engineered Hematopoietic Stem/Progenitor cells (HSPC) has emerged as an effective treatment for several monogenic disorders. Our group has been one of the pioneers in HSPC-GT clinical application. Since 2000, we have treated at SR-TIGET more than 100 patients affected by adenosine deaminase deficiency, metachromatic leukodystrophy, Wiskott-Aldrich Syndrome (WAS), Beta-thalassemia and recently Mucopolysaccharosidosis type I. HSPC-GT is based on infusion of gene-modified autologous HSPC isolated from bone marrow (BM) or mobilized peripheral blood (MPB), to correct inherited disorders. Upon gene correction each HSPC and its cell progeny become marked by a distinct integration site (IS), thus by analyzing IS we can trace in vivo the activity of gene corrected cells, providing a unique model to study the fate of infused HSPC in humans. We described the in vivo reconstitution dynamics of 7 HSPC subtypes including hematopoietic stem cells (HSC), multipotent progenitors (MPP) and lymphoid- or myeloid-committed precursors in 6 WAS patients up to 5 years after GT. Steady-state hematopoiesis was reached around 1-2 years post-GT. Mathematical modeling suggests that short-living MPP were more active in the early phases and were replenished around 1 year after GT by long-living HSC, which then remained in charge of the hematopoietic output. This implies that long-term HSC, that were activated in vitro, were capable of homing and resilience upon re-infusion. The analysis of CD34+ cell composition before transduction revealed that primitive HSC were present in both BM and MPB-HSPC sources with higher amounts of primitive and myeloid-committed progenitors in MPB. This latter finding, may explain the rapid neutrophil engraftment and platelet transfusion independence in MPB-GT, in line with the observations in transplanted patients. Moreover, the infusion of higher number of primitive HSPC subsets in MPB-GT resulted in higher BM chimerism 2 years after GT. Similar hematopoietic reconstitution was observed in the two groups from 1-year post-GT implying that both HSPC sources have similar long-term repopulating properties. Our work provides a global picture of the behavior of engineered HSPC during early engraftment and at steady state hematopoiesis in humans.

Keywords: Clonal tracking, hematopoietic stem cells, gene therapy

20:25 – 20:33

COUPLING EXPANSION AND TRANSDUCTION OF HUMAN HEMATOPOIETIC STEM AND PROGENITOR CELLS TO TREAT AUTOSOMAL RECESSIVE OSTEOPETROSIS

Villa, Anna[1], Capo, Valentina[2], Penna, Sara[2], Merelli, Ivan[3], Barcella, Matteo[2], Scala, Serena[2], Basso Ricci, Luca[2], Draghici, Elena[4], Sergi Sergi, Lucia[2], Palagano, Eleonora[5], Zonari, Erika[2], Desantis, Giacomo[2], Uva, Paolo[6], Cusano, Roberto[6], Aiuti, Alessandro[2], Ficara, Francesca[5], Sobacchi, Cristina[5] and Gentner, Bernhard[2]

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Autosomal recessive osteopetrosis (ARO) is a rare disease, affecting osteoclast differentiation or function. Most patients present mutations in TCIRG1 gene, encoding the V-ATPase proton pump, necessary for bone resorption. Symptoms include dense and brittle bones, limited bone marrow (BM) cavity, anaemia and progressive nerve compression, leading to death in the first decade of life. Standard treatment is allogeneic hematopoietic stem cell transplant, but availability of HLA-matched donor, toxicity of conditioning and sig-

nificant transplant-related morbidity limit applicability. Gene therapy (GT) may represent an alternative therapy, alleviating the need for recipient conditioning and abrogating graft-versus-host disease. Since patients show increased number of circulating CD34+ cells in peripheral blood (PB), these cells could be exploited as a novel cell source for autologous transplantation of gene-corrected hematopoietic stem and progenitor cells (HSPCs).Peripheral blood of 5 TCIRG1-deficient patients presented a cellular composition that resembled BM, enriched in progenitors such as erythroblasts and myeloblasts. Analysis of TCIRG1 CD34+ cell transcriptome indicated the expression of HSPC genes, similarly to conventional sources (cord blood and mobilized PB CD34+ cells). To reduce the burden of CD34+ collection in low-weight, severely affected children, we applied an UM171-based ex vivo expansion protocol of HSPCs coupled with gene transfer. We generated a corrective lentiviral vector (LV), driving TCIRG1 expression under the control of the PGK promoter. Circulating CD34+ cells from TCIRG1-defective patients were transduced with LV and expanded up to 27-fold. Expanded cells maintained long-term engraftment and multi-lineage repopulating potential when transplanted in vivo both in primary and secondary NSG recipients. When CD34+ cells were differentiated in vitro, bone resorption capacity, measured as collagen type-I fragment release in culture supernatants, was restored only in GT cells, at levels comparable to healthy donor cells. Overall, we provide evidence that expansion of circulating HSPCs coupled to GT could represent a feasible, readily available treatment for ARO patient, opening the way to future gene-based treatment of skeletal diseases caused by BM fibrosis.

Keywords: circulating Hematopoietic stem cells, gene therapy, bone

20:35 – 20:43
DEVELOPMENT OF IPSC-DERIVED EXTENDED CTL THERAPY FOR CERVICAL CANCER

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Cervical cancers harbor human papillomavirus (HPV) oncoproteins. Although HPV vaccines effectively prevent HPV infections, they have no effect on established cancers. Total hysterectomy for early stage cancers of course abolishes fertility. Metastatic cervical cancer is highly refractory to chemotherapy and prognosis is extremely poor. Development of an effective cervical cancer treatment is an urgent issue. Antigen-specific cytotoxic T lymphocytes (CTL) therapy can induce durable remission in selected tumors such as melanoma. As E6 and E7 are the key oncoproteins of cervical cancer, these would be ideal targets of T cell therapy. However, CTL continuously exposed to their target antigen become exhausted. CTL generated from induced pluripotent stem cells (iPSC) are functionally rejuvenated (rejT) (Cell Stem Cell 2013) and survive better in vivo than original CTL (Stem Cell Reports 2015, Haematologica 2019). HPV type16 (HPV16) E6-rejT sustainedly suppress HPV16-expressing cervical cancer (personal observations; manuscript in preparation). Generating rejT from individual patients is time-consuming. Although use of allogenic rejT can obviate this problem, HLA-mismatched immunorejection then becomes a major obstacle. A realistic solution is development of an HLA-edited "extended" rejT exploiting CRISPR/Cas9 technology. We used this approach first to knock out B2M in HPV16 E6-CTL-derived iPSC, thus ablating HLA class I, and next knocked in both HLA-A24, because the CTL epitope was HLA-A2402-restricted, and HLA-E, to avoid NK-cell activation. HLA-edited iPSC efficiently differentiated into HPV16 E6-rejT with high specificity and robust cytotoxicity against cervical cancer equal to those of wild type HPV16 E6-rejT. The cytotoxicity of NK cells against HLA-edited HPV16 E6-rejT was significantly less (18.0%, 9.4%, and 4.5%, E:T ratios 3:1, 1:1, and 1:3, respectively) than their cytotoxicity against a positive control, K562 cells that do not express class (34.6%, 17.7%, and 6.7%, respectively) with specific lysis in 51Cr release assays. We believe that extended HPV-rejT provide a promising approach to "off-the-shelf therapy" for cervical cancer.

Funding source: These studies were supported by a grant from JSPS/KAKENHI (Grant Number 18K07273) and by a grant from AMED (Grant Number JP19bm0404032)

Keywords: Extended CTL therapy, cervical cancer, rejuvenated CTL

20:45 – 20:53

DE NOVO MUTATIONS IN MITOCHONDRIAL DNA OF IPSCS PRODUCE IMMUNOGENIC NEOEPITOPES IN MICE AND HUMANS

Deuse, Tobias[1], Hu, Xiaomeng[1], Agbor-Enoh, Sean[2], Koch, Martina[3], Spitzer, Matthew[4], Gravina, Alessia[1], Alawi, Malik[5], Marshita, Argit[6], Peters, Bjoern[7], Kosaloglu-Yalcin, Zeynep[7], Yang, Yanqin[6], Rajalingam, Raja[8], Wang, Dong[1], Nashan, Bjoern[4], Kiefmann, Rainer[9], Reichenspurner, Hermann[10], Valantine, Hannah[5], Weissman, Irving[11] and Schrepfer, Sonja[1]

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The utility of autologous induced pluripotent stem cell (iPSC) therapies for tissue regeneration depends on reliable production of immunologically silent functional iPSC derivatives. However, rejection of autologous iPSC-derived cells has been reported, although the mechanism underlying rejection is largely unknown. The mutation rate for mitochondrial DNA (mtDNA) is 10- to 20-fold higher than that of nuclear DNA and both mutated or wild-type mtDNA can coexist in the same cell, a phenomenon called heteroplasmy. We have previously shown that individual single nucleotide polymorphisms (SNPs) are sufficient to create immunogenic neoantigens. We thus hypothesized that de novo mutations in mtDNA might produce neoantigens capable of eliciting immune recognition and rejection. Here we present evidence in mice and humans that nonsynonymous mtDNA mutations can arise and become enriched during the processes of reprogramming to the iPSC stage, long-term culture and differentiation into target cells. We observed the occurrence of neoantigens in mouse iPSCs after extended passaging in vitro. Mice that received autologous high-passage iPSC grafts elicited a stronger immune response than mice that received low-passage iPSCs, which correlated with diminish cell survival in vivo. This immune response was highly specific and dependent on the host major histocompatibility complex (MHC) genotype. Similarly, human iPSCs created from fibroblasts revealed an increased likelihood for de novo mutations and enrichment of rare SNPs with an increase in passages. When iPSCs were generated from healthy volunteers, differentiated into endothelial cells, and expanded in culture to passage 35, we occasionally detected neoantigenic mutations. Immune assays then confirmed that only neoantigen-carrying iPSC-derived progeny induced an immune response in vitro strong enough to cause cell killing. Although the compact, circular 16,569 bp human mtDNA only encodes for 13 proteins, it may contribute to almost 30% of total mRNA in certain tissues and generate a high amount of mutant peptide. Our results reveal that autologous iPSCs and their derivatives are not inherently immunologically inert for autologous transplantation and suggest that iPSC-derived products should be screened for mtDNA mutations.

Funding source: T.D. was funded by the Else Kröner Fondation (2012_EKES.04). S.S. received research grants from the Deutsche Forschungsgemeinschaft (DFG; 25 SCHR992/3-1, SCHR992/4-1) and the Fondation Leducq (CDA 2013-2015).

Keywords: pluripotent stem cells, immunology, antigenicity

20:55 – 21:03

ENHANCED SURVIVAL OF HUMAN IPSC-DERIVED ENDOTHELIAL CELLS ON NANOPATTERNED SCAFFOLDS FOR TREATMENT OF PERIPHERAL ARTERIAL DISEASE

Karaca, Esra[1], Yang, Guang[1], Alcazar, Cynthia[2], Hu, Caroline[2], Zaitseva, Tatiana[3] and Paukshto, Michael[3]

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Human induced pluripotent stem cell (iPSC)-derived endothelial cells (iPSC-ECs) are a promising cell type for treatment of peripheral arterial disease (PAD). This study aims to quantitatively assess the role of nanofibril size and alignment on iPSC-EC spatial organization and survival in a murine model of PAD. Aligned nanofibrillar collagen scaffolds of varying fibril diameters were prepared by altering the ionic strength (IS) of collagen. Human iPSC-ECs were seeded onto the aligned nanofibrillar scaffolds for quantification of cellular organization based on immunofluorescence staining of F-actin. The regenerative potential of iPSC-EC-seeded scaffolds was examined in a mouse hind limb ischemia model. Scaffold formulation with varying IS resulted in different fibril diameter, regardless of the degree of crosslinking, based on atomic force microscopy. Aligned scaffolds with low IS were characterized by 100 nm fibril diameter, whereas the high IS resulted in scaffolds with 200 nm fibril diameters. Scaffolds with randomly oriented nanofibril orientation showed

non-organized fibril assembly. Similarly, iPSC-ECs seeded onto the aligned scaffolds at high and low IS showed similar reorganization of cytoskeleton along the direction of nanofibrils, whereas cells on randomly oriented scaffolds depicted a random orientation. Bioluminescent iPSC-ECs-seeded scaffolds were implanted acutely to the site of the excised femoral artery. Bioluminescence imaging demonstrated markedly higher cell survival in the ischemic limb when seeded on aligned nanofibrillar scaffold of high IS, compared to on randomly oriented scaffold or when delivered in saline. In conclusion, aligned collagen nanofibrillar scaffolds with high IS promote iPSC-EC survival and alignment along the fibril direction. This study provides new insight into the role of biophysical microenvironment in the survival of iPSC-ECs, and has important implications in the delivery of therapeutic cells for treatment of PAD.

Keywords: hindlimb ischemia, biomaterials, cell survival

21:05 – 21:13
TARGETING HUMAN OVARIAN CANCER WITH IMMUNE CELLS DERIVED FROM THE PATIENT OR FROM HOMOZYGOUS HLA HAPLOTYPE IPS CELLS

Trounson, Alan O.[1], Boyd, Richard[2], Shu, Roland[2], Evtimov, Vera[2], Boyd, Nicholas[2], Nguyen, Nhu-Y[2], Nguyen, Thao[2], Cartledge, Kellie[2], Cao, Madeline[2], Tiedemann, Mathew[2], Junli, Zhuang[2], Hammett, Maree[2], Docherty, Callum[2], Howard, Maureen[2], Hudson, Peter[2] and Nisbet, Ian[2]

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Chimeric antigen receptors (CARs) have been used for efficient destruction of B cell tumors but have been relatively ineffective as therapy for solid tumors. Ovarian cancer is a difficult cancer to treat, with five-year survival rates commonly around 25% of patients at diagnosis. The oncofetal glycoprotein antigen TAG-72 is commonly expressed on adenocarcinomas and is present on >95% of serous and >85% of clear cell ovarian cancers. We designed a novel TAG-72 CAR to increase antigen binding and lower antigenicity over other TAG-72 CARs. Our TAG-72 CAR-T cells are extremely active in killing TAG-72 expressing human ovarian cancer cells in vitro and in vivo (NOD-SCID gamma (NSG) mice xenografted human ovarian cancer cells – e.g. Ovcar3) but were less effective against low TAG-72 expressing tumour cells (e.g. Mesov). In vivo relapse in mice was often observed after 30-40 days of CAR-T cell treatment. Anticipating that treatment of solid tumors will require more than just a conventional CAR-T cell, we investigated a panel of key genes purportedly linked to immune suppression. By deleting them individually by gene editing in TAG-72 CAR-T cells, we identified potent novel genes as prime candidates for boosting CAR-T cell function and longevity. When the TAG-72 CAR-T cells with these gene knockouts (KO) were tested in our NSG mouse model the ovarian cancers were eradicated. At 100 days post treatment with TAG-72+gene KO CAR-T cells, human CD3+ T cells were still present at the site of the tumor, in contrast to their absence in non-gene edited human TAG-72 CAR-T cells. We have also explored the generation of TAG-72 CAR cells from induced pluripotent stem (iPS) cells. Homozygous HLA haplotype iPS cells differentiated into natural killer cells (iNK cells) are able to kill human ovarian cancer in vitro and in vivo. iNK cells gene edited to knock-in TAG-72 CAR, killed ovarian cancer cells more efficiently than the iNK cells without a CAR. The TAG-72 CAR-iNK cells kill both high and low expressing TAG-72+ human ovarian cancers. The redesign of TAG-72 CAR-T cells and the gene edited KO produces a very effective therapeutic for eradication of ovarian cancer in mice. The development of CAR-iNK cells with enhanced cancer killing capacity and potential longevity may be an important development in stem cell based immune cancer therapies.

Funding source: Funded in part by the Australian Department of Industry, Innovation and Science as a CRC-P grant

Keywords: immunotherapy, iPSC, ovarian cancer

21:15 – 21:30
BLOOD STEM CELL TRANSPLANTATION WITHOUT GENOTOXICITY: TRANSLATION FROM MICE TO HUMAN TRIALS OF ANTI-CD117 ANTIBODY CONDITIONING

Shizuru, Judith

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Replacement of diseased hematopoietic stem cells (HSC) by hematopoietic cell transplantation (HCT) is effective therapy for many otherwise incurable disorders such as genetic disorders of blood formation, blood cancers, and autoimmune diseases. While the therapeutic potential of HCT is immense, its use has been limited by the toxicities of the procedure. DNA-damaging chemotherapy or radiation “conditioning” are the current treatments patients receive to vacate recipient HSC niches and permit transplanted HSC to engraft. We have pursued a non-genotoxic approach to target and deplete HSC using a humanized monoclonal antibody, JSP191 (formerly AMG 191), that binds human CD117 (c-Kit). CD117 is a receptor tyrosine kinase expressed on HSC and progenitors. We showed that JSP191 suppresses human hematopoiesis in vitro, depletes human HSC in xenografted mice, and safely depletes HSC of non-human primates. These studies have led to a clinical trial testing JSP191 as the sole conditioning agent in patients undergoing HCT for severe combined immunodeficiency (SCID) (ClinicalTrials.gov:

NCT02963064). SCID is a severe genetic immune disorder curable only by HCT. Data to date show JSP191 is safe and efficacious in achieving HSC engraftment. We further showed that anti-CD117 antibodies can target and eliminate disease initiating HSC that cause the clonal disorders, myelodysplastic syndrome (MDS) and secondary acute myeloid leukemia (AML). A clinical HCT trial testing the safety and efficacy of JSP191 in MDS/AML will open soon. By combining anti-CD117 antibodies with other therapeutic modalities, more potent HSC depletion can be achieved. This combination strategy makes anti-CD117 antibodies useful across a broad spectrum of diseases. These studies have opened the path to the clinical use of biologic agents to replace DNA-damaging therapies and expand the ability of the field to treat the vast number of patients who could benefit from this life-saving stem cell therapy.

Keywords: hematopoietic stem cell, transplantation, non-toxic, SCID, engraftment

THURSDAY, JUNE 25, 20:00 – 21:45

CONCURRENT - CLINICAL APPLICATIONS: NEURAL

20:05 – 20:20
DEVELOPING A STEM CELL BASED THERAPY FOR PARKINON'S DISEASE

Parmar, Malin

Lund University, Lund, Sweden

Cell based transplantation aimed at the replacement of lost dopamine (DA) neurons holds great potential for the treatment of Parkinson's disease (PD). Considerable progress has been made in generating fully functional and transplantable dopamine (DA) neurons from human pluripotent stem cells (hPSCs) and the field is rapidly developing. We have developed robust and efficient differentiation protocols resulting in the formation of authentic and functional DA neurons from human embryonic stem cells (hESCs). Here, I will summarize the translational trajectory and describe the GMP manufacturing, pre-clinical validation and regulatory process embarked on to bring this hESC-derived dopamine cell product to clinical trial for PD.

Keywords: human embryonic stem cells, Dopamine neurons, Parkinson's disease

20:25 – 20:33
SEIZURE RELIEF IN EPILEPTIC MICE THROUGH GABAERGIC NEURONS DERIVED FROM LINEAGE REPROGRAMMING OF GLIAL CELLS

Heinrich, Christophe[1], Berninger, Benedikt[2], Conzelmann, Karl-Klaus[3], D'Orange, Marie[4], Lentini, Célia[4], Trottmann, Marie[4] and Vignoles, Rory[4]

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Injury to the human central nervous system (CNS) is devastating because the adult mammalian CNS lacks intrinsic regenerative capacity to replace lost neurons and induce functional recovery. The goal of regenerative medicine is to replace lost neurons in order to restore lost functions. An exciting approach towards achieving this goal is to induce direct fate conversion of brain-resident glial cells into induced neurons (iNs) by direct lineage reprogramming. Considerable progress has been made in converting various source cell types of mouse and human origin into clinically-relevant iNs. However, it remains a fundamental question whether glia-derived iNs are endowed with the capability of promoting effective functional recovery in pathological contexts. Here we aimed to answer this question in the context of Mesial-Temporal Lobe Epilepsy (MTLE), the most common form of drug-resistant epilepsy that is characterized by hippocampal recurrent seizures and GABAergic neuron loss suggested to promote the epileptic state. Using a predictive MTLE mouse model, we show that glial cells can be efficiently reprogrammed by Ascl1 and Dlx genes within the adult MTLE hippocampus to generate GABAergic iNs showing specification into diverse interneuron subtypes known to degenerate in MTLE. After long-term survival of several months, glia-derived iNs display a sustained and considerable level of synaptic integration within epileptic networks, receiving synaptic contacts from local granule cells but also from long-range projection neurons, as evidenced by rabies virus-mediated transsynaptic tracing. Importantly, iNs also send efferent projections impinging on mature granule cells primarily involved in hippocampal seizures, hence representing appropriate target cells for GABA iNs to control epilepsy. Finally, by manipulating iNs using a chemogenetic approach, we demonstrate that glia-derived GABAergic iNs are endowed with the capability of suppressing spontaneous chronic seizures in MTLE mice. Thus, our study provides compelling evidence that glia-derived GABA iNs can promote functional recovery in a pathological context, and uncovers glia-to-neuron reprogramming as a

potential cell-replacement strategy to control seizures in patients with intractable epilepsy.

Funding source: Agence Nationale de la Recherche (ANR, ReprogramEpi, ANR-14-CE13-0001), LabEx CORTEX (ANR-11-LABX-0042), FRC, FFRE, LFCE, CURE (Award ID: 262178) to C.H.; Wellcome Trust (206410/Z/17/Z) and DFG (BE 4182/8-1) to B.B..

Keywords: Direct glia-to-neuron lineage reprogramming, Functional rescue, Epilepsy

20:35 – 20:43
OLIGODENDROCYTE PRECURSOR CELL TRANSPLANTATION PROMOTES OLIGODENDROGENESIS AND SYNAPTOGENSIS IN MICE AFTER CEREBRAL ISCHEMIA

Li, Wanlu[1], He, Tingting[2], Shi, Rubing[1], Song, Yaying[2], Tang, Yaohui[1], Yang, Guo-Yuan[1] and Wang, Yongting[1]

[1]School of Biomedical Engineering and Med-X Research Institute, Shanghai Jiao Tong University, Shanghai, China, [2]Department of Neurology, Ruijin Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

Ischemic stroke induces injury to the white matter, which is exquisitely vulnerable to ischemia and often more severely injured than gray matter. Insufficient recruitment of oligodendrocyte precursor cells (OPCs) to the demyelinated area and the lack of differentiated oligodendrocytes are associated with worsened outcomes of stroke. OPCs transplantation showed elicit extensive and robust myelination in congenitally dysmyelinated brain. However, whether and how OPCs transplantation affect ischemic stroke is unclear. In this work, we investigated the effects of OPC transplantation at the sub-acute phase after ischemic stroke using a mouse model of transient middle cerebral artery occlusion (tMCAO).OPC transplantation reduced brain atrophy at 5 weeks after tMCAO. Mice received OPC transplantation performed better in rotarod test, passive avoidance and T-maze test at 5 weeks after ischemic stroke, as well as upregulated the expression of CXCL12. Administration of CXCR4 antagonist AMD3100 eliminated the beneficial effect of OPCs. Our immunostaining results showed that OPC treatment improved synaptogenesis in vivo and in vitro. The mechanistic study further demonstrated that siRNA knockdown of DCC, a receptor of the axon-guiding protein Netrin-1, abolished OPC-induced neurite growth and synaptogenesis of neurons in the co-culture system. Our work showed that OPC transplantation reduced brain atrophy and promoted oligodendrogenesis and synaptogenesis in the sub-acute phase after ischemic injury via CXCL12/CXCR4 and Netrin-1/DCC axis, suggesting a new avenue for treating ischemic stroke.

Keywords: Cerebral ischemia, Oligodendrocyte precursor cells, Oligodendrogenesis and synaptogenesis

20:45 – 20:53
HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED GABAERGIC INTERNEURON TRANSPLANTS ATTENUATE NEUROPATHIC PAIN

Caron, Leslie, Manion, John, Clark, Teleri, Muralidharan, Arjun, Ruan, Travis, Loo, Lipin and Neely, Greg

The Charles Perkins Centre, The University of Sydney, Australia

Neuropathic pain causes severe suffering, and most patients are resistant to current therapies. A core element of neuropathic pain is the loss of inhibitory tone in the spinal cord. Previous studies have shown that foetal GABAergic neuron precursors can provide relief from pain. However, the source of these precursor cells makes them unsuitable for therapeutic use. Here, we extend these findings by showing that spinally transplanted, terminally differentiated human induced pluripotent stem cell-derived GABAergic (iGABAergic) neurons provide significant, long-term, and safe relief from neuropathic pain induced by peripheral nerve injury in mice. We show that parvalbumin (PAVB)-enriched GABAergic neuron populations have a greater analgesic efficiency than Somatostatin (SST) GABAergic neurons. Furthermore, iGABAergic neuron transplants survive long term in the injured mouse spinal cord and show evidence of synaptic integration. Finally, our hiPSC-based analgesic therapy can also relieve breast cancer-induced bone pain in rats. Together, this provides the proof in principle for the first viable GABAergic transplants to treat human neuropathic pain patients.

Keywords: Human Pluripotent Stem Cells, Neuropathic Pain, Stem Cell Therapy

20:55 – 21:03
GROWTH FACTOR ENHANCED MOUSE IPSC-DERIVED RETINAL ORGANOIDS AS CELL SOURCE FOR RETINAL CELL REPLACEMENT

Oswald, Julia[1], Masland, John[2], Pernstich, Chris[3] and Baranov, Petr[2]

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Enabled by the ability to differentiate neurons from stem cells, functional cell replacement has become a new therapeutic frontier. In ophthalmology, first clinical trials and multiple, prior research studies show the potential of both retinal pigment epithelium (RPE) and photoreceptor replacement, to halt visual decline within patients affected by Age-related macular degeneration (AMD) and cone/rod dystrophies. Like AMD, Glaucoma is a progressive neurodegenerative disease, for

which cell replacement promises to prevent or recover vision loss. While uniform cell populations like the RPE are easy to differentiate in-vitro, Glaucoma affects a highly diverse class of neurons - retinal ganglion cells (RGCs). Classified into 40 subtypes by morphology, differential gene expression and functional diversity, RGCs are integral to functional vision. While the biological significance of RGC diversity is not understood, it is essential to consider when assessing donor cell populations for cell replacement.Over the past 2 years we have derived a slow-release growth factor protocol, using BDNF,GDNF and CNTF, for the generation of 3D retinal organoids from iPSC with up to 5% total RGC yield, equal to a 46% increase in RBPMS+ RGCs as compared to the standard Sasai protocol. Amongst others, organoid-derived RGCs expressed markers including Spp1, Kcng4 (α-RGCs) and Melanopsin (intrinsically photosensitive RGCs), representing all major RGC subclasses in-vivo, within 3-weeks of culture. While the growth factor treatment increased proliferation in other retinal cell types within the organoids, RGC yields were enhanced due to prolonged survival of mature RGCs in-vitro as confirmed by EdU pulse experiments and Caspase staining. Following isolation at 3 weeks of culture, RGCs derived from our protocol were able to survive up to 12 months within healthy and diseased host retinas, following intravitreal transplantation at an initial transplant success rate of about 80% in healthy hosts. Able to extend axonal projections of 250um length within 3 weeks post-transplant, reaching into the host optic nerve and displaying spontaneous spiking when cultured on multi-electrode arrays, RGCs derived from our protocol have the potential to eventually rewire the retina-to-brain connection.

Funding source: This work was supported by the HMS Department of Ophthalmology, NIH/NEI U24 grant EY029893, BrightFocus Foundation, Gilbert Family Foundation and NIH National Eye Institute core grant P30EY003790.

Keywords: Retinal Organoid, Cell Replacement, Glaucoma

21:05 – 21:13
FIRST TRANSPLANTATION TRIAL OF A POLARIZED IPSC-DERIVED RETINAL SHEET IN A NON-HUMAN PRIMATE MODEL FOR THE TREATMENT OF MACULAR DEGENERATIONS

Barabino, Andrea[1], Griffith, May[2], Hanna, Roy[3], Cécyre, Bruno[3], Kalevar, Ananda[4], Qian, Cynthia[5], Rezende, Flavio[6] and Bernier, Gilbert[7]

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Macular degenerations (MDs) are retinal disorders affecting millions of people worldwide. Photoreceptors (PRs), the specialized neurons involved in phototransduction, progressively degenerate leading to vision loss. Currently, there is no treatment to delay, stop or reverse these diseases, making MDs incurable. Transplantation of dissociated retinal progenitor cells (RPCs) derived from induced pluripotent stem cells (iPSC) in rodent and non-human primate models have recently been reported. Although their ability to maturate, integrate and form connections with the underlying bipolar cells, RPCs cannot migrate and polarize properly, thus resulting in an unfunctional tissue. Hence, the idea of grafting a polarized outer nuclear layer-like tissue, composed mostly by cone PRs, aiming at the restoration of visual functions. In this study, we describe the generation and characterization of retinal sheets (RSs) differentiated from human iPSC carrying a fluorescent reporter. We also developed a non-human primate model of MD by cobalt chloride injection in the subretinal space, which allows the complete disruption of the outer nuclear layer while keeping intact all other layers. Finally, we revealed the preliminary results of our first transplantation of polarized RSs in this non-human pri-

mate model. Taken together, these results unveil new insights towards the cure of MDs.

Funding source: Foundation Fighting Blindness (FFB) Canada, and Maisonneuve-Rosemont Hospital Foundation, Université de Montréal, StemAxon, Healios

Keywords: Retina regeneration iPSc organogenesis, preclinical trial photoreceptors cone, macula degeneration vision

21:15 – 21:30
STEM CELL MIRACLE OR RUSSIAN ROULETTE?: EXPLORING CHOICE IN THE CONTEXT OF MS

Munsie, Megan

Anatomy and Neuroscience, University of Melbourne, Australia

More than 2.3 million people across the globe live with multiple sclerosis (MS). New treatments are improving lives for some, but there remains no cure. Understandably, many living with MS are frustrated by the lack of options and seek reputed treatment, including autologous haematopoietic stem cell transplantation (AHSCT), in an effort to make a significant difference to their health. Such a choice often requires travel far away from home and may involve considerable personal cost and the potential for serious harm. While AHSCT has a role in current clinical care, it is very unlikely to be right for everyone. Clinical research is underway to identify when ASCT should be recommended. Making the decision to pursue stem cell treatment, or not, is not easy. It requires careful consideration in relation to each individual's personal circumstances and ideally should be made in consultation with their current healthcare team. This paper will explore the experience and expectations of Australians with MS who have undertaken 'stem cell' treatment and how such a 'choice' is framed and mediated by digital media and the portrayal of hope. The use of registries to collect clinical data and its value will also be considered. While the notion of nothing ventured, nothing gained is an understandable driver, how to raise and talk about issues of risk and benefit for those with MS, and many others affected by neurological disorders, is an ongoing challenge for the field.

Keywords: access, patient choice, unproven therapies

THURSDAY, JUNE 25, 20:00 – 21:45

CONCURRENT - MODELING DEVELOPMENT AND DISEASE: CARDIAC AND MUSCLE

20:05 – 20:20
DEVELOPING AN OPTIMIZED GENE THERAPY FOR DIRECT CARDIAC REPROGRAMMING

Hoey, Tim

Tenaya Therapeutics, South San Francisco, CA, USA

Heart failure is a major cause of death, with an estimated prevalence of 38 million patients worldwide. Although many treatment strategies have been investigated, the inability of cardiomyocytes to regenerate has been a significant roadblock for generating improved cardiac function. Direct cardiac reprogramming has the ability to convert non-myocytes into functioning myocardial cells at the site of injury. Cardiac reprogramming has been shown to improve cardiac function after myocardial infarction (MI) in rodents, however reprogramming of human cells has proven much more complex. We have used multiple cell-based and in vivo models to develop a first-in-class AAV vector mediating direct cardiac reprogramming using an optimized combination of three factors, cis-regulatory sequences, and viral capsid. We identified reprogramming factors by multiple rounds of screening and cassette engineering to maximize the conversion of primary human cardiac fibroblasts (hCFs) into cardiomyocytes (CMs), in vitro. Our lead candidate efficiently reprograms hCFs, in vitro, yielding cells that exhibit calcium transients and express CM-specific genes. Critically, our lead candidate also infects and converts resident fibroblasts into CMs in vivo, as demonstrated by lineage tracing, and improves cardiac function in rat myocardial infarction models. We have also demonstrated efficacy in the setting of MI in a large animal model using Yucatan swine. Future studies will assess the optimal timing of viral vector delivery, route of administration, and dose-dependent activity in the rat and pig animal models as we advance this therapy to clinical testing.

Keywords: reprogramming, gene therapy, cardiomyocyte

20:25 – 20:33

IPSC MODEL OF HYPOPLASTIC LEFT HEART SYNDROME REVEALS METABOLIC AND CELL STRESS PATHWAYS LINKED TO CLINICAL OUTCOME

Xu, Xinxiu[1], Adams, Phillip[2], Beutner, Gisela[3], Criscione, Joseph[4], Zhu, Wenjuan[5], Feinstein, Timothy[1], Gabriel, George[1], Ganapathiraju, Madhavi[6], Kuo, Catherine[4], Lin, Jiuann-Huey[7], Nguyen, Phong[4], Porter, George[3], Shiva, Sruti[8], Tsang, Michael[1], Tan, Tuantuan[6], DeMoya, Ricardo[1], Yagi, Hisato[1] and Bais, Abha[1]

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Hypoplastic left heart syndrome (HLHS) is a severe congenital heart disease with hypoplasia of the left ventricle, mitral valve, and aorta/aortic valve. While some cases can survive with surgery to convert the right ventricle into the systemic pumping chamber, most cannot because of the high risk of heart failure (HF) in HLHS patients. We have developed the first HLHS mouse model and demonstrated that HLHS mutant mice display decreased cell proliferation, increased apoptosis, and cardiomyocyte differentiation defects in conjunction with metabolic disturbance. Here we further modeled HLHS using induced pluripotent stem cell derived cardiomyocytes (iPSC-CM) and show iPSC-CM from the HLHS mice replicated the defects seen in vivo, indicating they are not hemodynamic in origin. Furthermore, we generated iPSC-CM from 10 HLHS patients. In these patients, 6 patients survived for more than 5 years without heart transplantation (Group I) and 4 patients had poor outcome; defined as death or cardiac death/heart transplant (Group II). While there was no significant difference between iPSC-CM from 3 controls and 6 Group I HLHS patients, iPSC-CM from Group II patients had reduced proliferation, increased cell death, mitochondrial respiration deficit with transition pore closure defect, and oxidative stress with increased reactive oxygen species. Interestingly, protein synthesis overload with endoplasmic reticulum (ER) stress and the unfolded protein response (UPR) were upregulated in the iPSC-CM from Group II based on single cell RNA sequencing analysis. Drug screening also showed compounds that restored mitochondrial transition pore closure rescued the iPSC-CM respiration defect, restored cell proliferation and suppressed apoptosis. Our work reveals mitochondrial transition pore defect and ER stress may underlie HF risk in HLHS, suggesting the possibility of developing therapies that target these processes.

Funding source: This work is supported by funding from HL142788, University of Pittsburgh School of Medicine, and postdoctoral fellowship from American Heart Association and The Children's Heart Foundation.

Keywords: Hypoplastic left heart syndrome, metabolic disturbance, single cell RNA sequencing

20:35 – 20:43

A METASTABLE ATRIAL STATE UNDERLIES THE PRIMARY GENETIC SUBSTRATE FOR MYL4 MUTATION-ASSOCIATED ATRIAL FIBRILLATION

Ghazizadeh, Zaniar[1], Olafsson, Sigurast[2], Kiviniemi, Tuomas[2], Plotnick, David[2], Beerens, Manu[2], Zhang, Kun[2], Gillon, Leah[2], Steinbaugh, Mike[3,4], Barrera, Victor[3,4], Ho Sui, Shannan[4], Werdich, Andreas[2], Kapur, Sunil[2], Eranti, Antti[5], Gunn, Jarmo[5], Jalkanen, Juho[5], Airaksinen, Juhani[5], Kleber, Andre[3,6], Hollmén, Maija[7] and MacRae, Calum[2]

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Atrial fibrillation (AF) is the most common clinical arrhythmia and is associated with heart failure, stroke and increased mortality. The myocardial substrate for AF is poorly understood due to limited access to primary human tissue and mechanistic questions around existing in vitro or in vivo models. Using an MYH6:mCherry knock-in reporter line we developed a protocol to generate and highly purify human pluripotent stem cell-derived cardiomyocytes displaying physiological and molecular characteristics of atrial cells (hESC-atrial cells). We modeled human MYL4 mutants, one of the few definitive genetic causes of AF. To explore non cell-autonomous components of AF substrate, we also created a zebrafish Myl4 KO model, which exhibited molecular, cellular and physiologic abnormalities that parallel those in humans bearing the cognate mutations. There was evidence of increased retinoic acid signaling in both hESC and zebrafish mutant models, as well as abnormal expression and localization of cytoskeletal proteins, and loss of intracellular NAD and NADH. To identify potentially druggable proximate mechanisms,

we performed a chemical suppressor screen integrating multiple human cellular and zebrafish in vivo endpoints. This screen identified connexin 43 hemichannel (HC) blockade, as a robust suppressor of the abnormal phenotypes in both models of MYL4-related atrial cardiomyopathy. Immunofluorescence and co-immunoprecipitation studies revealed an interaction between MYL4 and Cx43 with altered localization of Cx43 HCs to the lateral membrane in MYL4 mutants, as well as in atrial biopsies from unselected forms of human AF. The membrane fraction from MYL4-/- hESC-atrial cells demonstrated increased phospho-Cx43 which was further accentuated by retinoic acid (RA) treatment and by the presence of risk alleles at the Pitx2 locus. Protein kinase C was induced by RA, and PKC inhibition also rescued the abnormal phenotypes in the atrial cardiomyopathy models. These data establish a mechanistic link between the transcriptional, metabolic and electrical pathways previously implicated in AF substrate and suggest novel avenues for the prevention or therapy of this common arrhythmia.

Keywords: Atrial fibrillation, High-throughput screening, Zebrafish

20:45 – 20:53
GENERATION OF SKELETAL MUSCLE FIBERS FROM HUMAN PLURIPOTENT STEM CELLS RECAPITULATING A FATIGUE SYMPTOM OF DUCHENNE MUSCULAR DYSTROPHY IN VITRO

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Duchenne muscular dystrophy (DMD) is a progressive muscle degenerating disease caused by a mutation of dystrophin gene, and currently no optimal therapies are available. Although DMD symptoms include muscle destruction, reduced maximal contractile force and fatigues leading to functional difficulties, none of them have been rarely recapitulated in vitro. Thus, identifying a key molecule or target preventing the initiation of DMD remain quite difficult. Currently, there are two major limitations to recapitulate such as symptoms in vitro: one is that myofibers differentiated in vitro are not functionally mature and second is that a functional assessment of contractile myofibers in long-term culture has not been done. In this study, we first established a combinational myogenic culture system using hiPSCs with electrical-field stimulation and collagen gel. Myofibers cultured in this system showed a progressed myogenic maturation characterized by increased cell fusions, expression of slow/fast muscle markers and sarcomere formation, and a functional maturation characterized by an excitation-contraction coupling. Using the system, we developed a way to assess contraction in a real-time and quantitative manner and investigated a dystrophic dysfunction of DMD iPSC-muscle fibers. Results indicate that dystrophin deficiency didn't affect a functional maturation and maximal contractile performance. However, while control fibers maintained their performance, dystrophic fibers showed a progressive decline of performance for 2 weeks without any damages of fibers or cell death. These data indicate that dystrophin is indispensable for fibers to maintain their functions and represent a fatigue of DMD patients. Furthermore, a cellular analysis revealed that dystrophic fibers had a reduced mitochondrial membrane potential and increased mitochondrial ROS implying a possibility that mitochondrial and muscle dysfunctions are linked under the dystrophic condition. As a conclusion, we established culturing contractile skeletal muscle fibers and recapitulated a fatigue-like phenotype using patient-derived iPSCs in vitro. Further investigations will reveal how muscle weakness and degeneration are initiated and progressed under the dystrophic condition and identify the target to prevent DMD.

Keywords: Duchenne muscular dystrophy, human induced pluripotent stem cells, Disease modeling

20:55 – 21:03
RECAPITULATING EARLY EMBRYONIC HEART DEVELOPMENT WITH HUMAN PLURIPOTENT STEM CELLS

Drakhlis, Lika[1], Farr, Clara-Milena[1], Sklarek, Jana[1], Ritzenhoff, Katharina[1], Franke, Annika[1], Manstein, Felix[1], Bolesani, Emiliano[1], Kempf, Henning[1], Hegermann, Jan[2], Nolte, Lena[3], Meyer, Heiko[3], Martin, Ulrich[1] and Zweigerdt, Robert[1]

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In response to proper stimuli, human pluripotent stem cells (hPSCs) self-organize into embryo-like tissue and organ patterns in vitro. To trigger tissue-specific spatiotemporal differentiation, (bio-)chemical stimuli are typically combined with either two-dimensional (2D) geometric confinement or matrix encapsulation of hPSCs to form 3D "organoids". Despite substantial progress in other organs, including small intestine, kidney and brain, proper in vitro models of cardiogenesis are not available. Here, we direct hPSC differentiation into complex, highly structured "heart-forming organoids" (HFOs). HFOs are composed of a myo- and endocardial cell layer surrounded by proepicardial cells, framed by distinct foregut endoderm tissues and pervaded by a vascular network. The overall architecture of HFOs closely resembles embryonic heart anlagen prior to heart tube formation, which occurs in an established interplay with foregut endoderm development. We subsequently show the utility of HFOs to model aspects of congenital heart disease. Compared to het-

erozygous controls, NKX2.5 knock-out HFOs show a myocardial noncompaction and hypertrophy phenotype, which reflects cardiac malformations known from in vivo studies in mice and observations of human patients. Together, an advanced model of early human cardiogenesis is presented opening new perspectives to study mechanisms of human heart development and disease in a dish.

Keywords: Organoids, Heart development, Human pluripotent stem cells

21:05 – 21:13
INFLUENCE OF PERFUSABLE MICROVASCULATURE ON EXCITATION-CONTRACTION COUPLING IN IPSC-DERIVED MYOCARDIUM

King, Oisín[1], Cruz-Moreira, Daniela[2], Kit-Anan, Sam Worrapong[1], Sayed, Alaa[1], Wang, Brian[1], Fourre, Jerome[1], Randi, Anna[1], Rasponi, Marco[2] and Terracciano, Cesare[1]

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The myocardium is one of the most densely vascularised tissues in the body, with dynamic metabolic demand from beating cardiomyocytes (CM) necessitating an intimate relationship with microvasculature. Endothelial cells (EC) produce a diverse array of cardio-active factors which acutely and chronically modulate myocardial phenotype. Disruption of CM-EC signalling results in pathological remodelling, and ultimately organ failure. However, as physiologically relevant recapitulation of CM-EC interaction has been difficult to achieve in vitro, many molecular mechanisms governing their interaction remain poorly understood. To induce cardiac vasculogenesis in vitro, we have developed microfluidic chips which subject 3D hydrogel cultures to precisely controlled flow. We then co-cultured human cardiac microvascular ECs, human left ventricular fibroblasts (FB), and human induced pluripotent stem cell-derived cardiomyocytes for 5 days under a pro-vasculogenic protocol (0.5 ul/min flow rate, 50ng/ml VEGF, 100ng/ml Ang-1). Via live and fixed immunofluorescence microscopy, we observed spontaneous formation of a microvasculature network with a continuously open lumen embedded within beating myocardium. Simultaneous quantification of iPSC-CM contractility and perfused red blood cell velocity reveals biomimetic pulsatile flow profile within the microvasculature. To evaluate the influence of microvasculature on CM function, we incorporated CMs differentiated from stem cells with the genetically encoded calcium biosensor GCaMP6F. Compared to CM only control, vascularised preparations demonstrate significantly faster calcium transient time to peak (-11.5%, p=0.007) and time to 50% relaxation (-15%, p=0.01). Under static conditions and 1Hz electrical stimulation, presence of EC was associated with reduced iPSC-CM arrhythmia at baseline ($p<0.0001$) and during 1uM isoprenaline treatment (p=0.0003), while maintaining isoprenaline induced Ca2+ handling quickening. To the best of our knowledge, this work represents the first fully perfusable model of the myocardial microvasculature, and highlights the importance of EC regulatory influence on CM function. Further work aims to investigate underlying molecular mechanisms to provide therapeutically relevant insight into cardiac biology.

Funding source: British Heart Foundation

Keywords: Electrophysiology, Vasculature, Microfluidics

21:15 – 21:30
MODELING DEVELOPMENT AND FUNCTION OF THE CARDIAC CONDUCTION SYSTEM FOR BIOLOGICAL PACEMAKER APPLICATIONS

Protze, Stephanie[1], Lohbihler, Michelle[1], Sarao, Renu[1], Masse, Stephan[2], Backx, Peter[3], Vasconcelos, Sara[2], Nanthakumar, Kumaraswamy[2] and Laflamme, Michael[1]

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The conduction system of the heart consists of the primary pacemaker (sinoatrial node, SAN), the secondary pacemaker (atrioventricular node, AVN) and specialized conduction fibers (His-purkinje system) that regulate the heartbeat throughout life. Failure of any part of the conduction system results in an irregular and/or slow heart rate, a condition treated by implantation of an electronic pacemaker (EPM). This current standard of treatment is associated with a relatively high rate of complications, including lead dislocation and infections. In addition, EPM's lack the ability to respond to the autonomic nervous system, require recurrent surgical battery replacements and induce remodeling that can cause heart failure, which is especially challenging for pediatric and adolescent patients. Using pluripotent stem cell-derived biological pacemakers (BioPMs), to replace the failing parts of the conduction system are a promising alternative. We have previously established a developmental biology-based protocol for the differentiation of human pluripotent stem cells (hPSCs) to SAN-like pacemaker cells (SANLPCs) and showed that these cells can function as a BioPM when transplanted into the apex of a rat heart. We are currently establishing a pre-clinical pig model to test the long-term reliability and safety of SANLPC-comprised biological pacemakers. We are also developing protocols for the differentiation of hPSCs to AVN-like pacemaker cells (AVNLPCs). To this end we generated a NKX2-5eGFP/w TBX3tdTomato/w double reporter hPSC line that allows us to identify NKX2-5+TBX3+ AVNLPCs in the

differentiation cultures. These AVNLPCs express AVN genes (TBX3, TBX2, MSX2, BMP2), display pacemaker action potentials and beat at 65+/-5 bpm, comparable to the rate of the human AVN. We are currently generating 3D tissues containing AVNLPCs that will allow us to analyze their conducting properties. Taken together, we are developing BioPMs that represent a promising, novel approach for patients with cardiac conduction system diseases.

Keywords: cardiac conduction system, pluripotent stem cells, biological pacemaker,

THURSDAY, JUNE 25, 20:00 – 21:45

CONCURRENT - TISSUE STEM CELLS AND REGENERATION: EARLY DEVELOPMENT AND PLURIPOTENCY

20:05 – 20:20
DECODING GENE REGULATION NETWORK IN HUMAN GERMLINE CELLS BY SINGLE-CELL FUNCTIONAL GENOMICS APPROACHES

Tang, Fuchou

Peking University, Beijing, China

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

Keywords: single-cell sequencing; human germline cell development; epigenetics; pluripotent stem cells

20:25 – 20:33
IN VITRO CAPTURE AND CHARACTERIZATION OF EMBRYONIC ROSETTE-STAGE PLURIPOTENCY, A NOVEL NAIVE-PRIMED PLURIPOTENCY INTERMEDIATE WITH A UNIQUE EPIGENOME

Ten Berge, Derk[1], Neagu, Alex[1], Van Genderen, Emiel[1], Escudero, Irene[1], Verwegen, Lucas[1], Baart, Esther[2] and Marks, Hendrik[3]

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Pluripotent cells generate all embryonic tissues and in the mouse arise in an immature naive state in the blastocyst. Naive cells become responsive to lineage-inductive signals only after maturation to the transcriptionally and epigenetically different primed pluripotent state of the egg cylinder epiblast. During this process, the blastocyst implants while the naive cells polarize and arrange into a rosette, which subsequently undergoes lumenogenesis to form the egg cylinder. Pluripotency therefore progresses through several developmental intermediates before acquiring its true potential. How these transitions are regulated is unclear, and the existence of discrete intermediate pluripotent states is controversial. Here, we demonstrate a novel pluripotent state in the embryonic rosette, defined by co-expression of naive factors with transcription factor OTX2. Downregulation of WNT signals in the blastocyst drives transition into rosette pluripotency by inducing OTX2. The rosette then activates MEK signals that induce lumenogenesis and drive progression to primed pluripotency. Importantly, naive cells acquire MEK sensitivity only after progressing to rosette-stage pluripotency. Consequently, combined WNT and MEK inhibition supports rosette-like stem cells (RSCs), a self-renewing naive-primed intermediate. RSCs gain a unique epigenome that includes remodelling of constitutive heterochromatin and bivalent marking of primed pluripotency genes. Notwithstanding this primed chromatin landscape, WNT induces reversion to naive pluripotency. The rosette is therefore a reversible pluripotent intermediate where control over pluripotency progression and morphogenesis pivots from WNT to MEK signals. Our findings delineate the mechanisms and checkpoints coordinating peri-implantation morphogenesis with pluripotency progression, and may advance our understanding of cell fate commitment and barriers to cell reprogramming (Nature Cell Biology, accepted for publication pending minor revision).

Keywords: epiblast, implantation, self-renewal

20:35 – 20:43

DERIVATION OF EMBRYONIC STEM CELLS FROM MULTIPLE AVIAN SPECIES

Chen, Xi[1], Guo, Zheng[1], Tong, Xinyi[1], Wang, Xizi[1], Liu, Xugeng[1], Huss, David[2], Tran, Martin[3], Dave, Mohit[2], Wu, Christina[1], Huang, Yixin[1], Cao, Lin[1], Lansford, Rusty[2] and Ying, Qi-Long[1]

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Germline-competent embryonic stem cells (ESCs) have been successfully derived from mice and rats, but not from any other species. Failure to establish ESCs from other species has restricted the application of ESCs beyond rodents. In this project, we aim to derive authentic ESCs from avian species. Our initial screening identified a small molecule cocktail that can maintain pluripotent stem cells in the dish from freshly laid chicken blastoderm at EGK.X stage. The culture conditions were then optimized to enhance the long-term maintenance of chicken ESCs (>80 passages), and to also facilitate the derivation of ESCs from quail (>50 passages), pheasant, duck, turkey, goose, and ostrich. Here, we also demonstrated that ESC lines can be established from individual eggs. ESCs derived from different avian species express key pluripotent genes such as Nanog and Pou5f3, and can be induced to differentiate into the three somatic lineages and germ cells in vitro. More importantly, long-term expanded chicken ESCs expressing the GFP transgene can efficiently integrate into chicken embryonic development when injected into EGK.X stage embryos and give rise to cells of all somatic, extraembryonic and germ cell lineages. We further demonstrated that high-grade transgenic chimeras, shown by GFP prevalence and feather color, can be monitored, live-imaged, and hatched eventually without any noticeable developmental defects. Currently, we are raising the ESC-derived chimeric chickens to test the functional germline contribution to the next generation. The establishment of avian ESC lines will have a profound impact in developmental biology, agricultural practices and our understanding of pluripotency.

Keywords: embryonic stem cell, avian, pluripotency

20:45 – 20:53

COPS5 SAFEGUARDS GENOMIC STABILITY OF EMBRYONIC STEM CELLS THROUGH REGULATING CELLULAR METABOLISM AND DNA REPAIR

Li, Peng and Chen, Lingyi

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The highly conserved COP9 signalosome (CSN), composed of 8 subunits (Cops1 to Cops8), has been implicated in pluripotency maintenance of human embryonic stem cells (ESCs). Yet, the mechanism for the CSN to regulate pluripotency remains elusive. We previously showed that Cops2, independent of the CSN, is essential for the pluripotency maintenance of mouse ESCs. In this study, we set out to investigate how Cops5 and Cops8 regulate ESC differentiation, and tried to establish Cops5 and Cops8 knockout (KO) ESC lines by CRISPR/Cas9. To our surprise, no Cops5 KO ESC clones were identified out of 127 clones, while three Cops8 KO ESC lines were established out of 70 clones. We then constructed an inducible Cops5 KO ESC line. Cops5 KO leads to decreased expression of pluripotency marker Nanog, proliferation defect, G2/M cell cycle arrest, and apoptosis of ESCs. Further analysis revealed dual roles of Cops5 in maintaining genomic stability of ESCs. On one hand, Cops5 suppresses the autophagic degradation of Mtch2 to direct cellular metabolism toward glycolysis and minimize reactive oxygen species (ROS) production, hence reducing endogenous DNA damage. On the other hand, Cops5 is required for high DNA damage repair (DDR) activities in ESCs. Without Cops5, elevated ROS and reduced DDR activities leads to DNA damage accumulation in ESCs. Subsequently, p53 is activated to trigger G2/M arrest and apoptosis. Altogether, our studies revealed an essential role of Cops5 in maintaining genome integrity and self-renewal of ESCs, by regulating cellular metabolism and DDR pathways.

Funding source: The National Natural Science Foundation of China (Grant No. 31622038, 31671497 and 31871485) The Natural Science Foundation of Tianjin (Grant No. 18JCJQJC48400), the 111 Project Grant (B08011)

Keywords: Cops5, genomic stability, embryonic stem cells

20:55 – 21:03

DERIVATION OF TROPHOBLAST STEM CELLS FROM NAÏVE HUMAN PLURIPOTENT STEM CELLS

Dong, Chen[1], Beltcheva, Mariana[1], Gontarz, Paul[1], Zhang, Bo[1], Popli, Pooja[2], Fischer, Laura[1], Khan, Shafqat[1], Park, Kyoung-mi[1], Yoon, Eun-Ja[1], Xing, Xiaoyun[3], Kommagani, Ramakrishna[2], Wang, Ting[4], Solnica-Krezel, Lilianna[1] and Theunissen, Thorold[1]

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Naïve human pluripotent stem cells (hPSCs) provide a unique experimental platform of cell fate decisions during pre-implantation development, but their lineage

potential remains incompletely characterized. As naïve hPSCs share transcriptional and epigenomic signatures with trophoblast cells, it has been proposed that the naïve state may have enhanced predisposition for differentiation along this extraembryonic lineage. Here we examined the trophoblast potential of isogenic naïve and primed hPSCs following direct culture in human trophoblast stem cell (hTSC) medium. Using a wide range of established morphological and molecular criteria, we demonstrate that naïve hPSCs can readily give rise to hTSCs. Additionally, naïve hPSC-derived hTSCs (naïve hTSCs) are bipotent and capable of undergoing terminal differentiation into both extravillous and syncytiotrophoblast. In contrast, primed hPSCs do not support hTSC derivation. Global transcriptomic analyses indicate that naïve hTSCs acquire a post-implantation trophectoderm identity, and are highly similar to bona fide hTSCs. We also assayed the chromatin accessibility landscape of both primary and naïve hTSCs for the first time, which exhibit a high degree of similarity. The results highlight the role of distal regulatory elements in hTSC specification, and suggest that the Hippo signaling pathway may be important for both the maintenance of naïve hPSCs as well as their transition into hTSCs. The derivation of hTSCs from naïve hPSCs presents the first functional evidence that the naïve state of human pluripotency possesses enhanced trophoblast potential. It now provides us with a novel experimental paradigm to study early mechanisms governing human trophoblast specification, as well as a robust methodology for a renewable, patient-specific source of hTSCs. Such advances may provide a pathway to uncover the origins of common pathologies afflicting the trophoblast lineage, such as miscarriage, pre-eclampsia, and fetal growth restriction.

Funding source: NIH Director's New Innovator Award (DP2 GM137418) Shipley Foundation Program for Innovation in Stem Cell Science Edward Mallinckrodt, Jr. Foundation Washington University Children's Discovery Institute

Keywords: Naive pluripotency, Primed pluripotency, Trophoblast stem cells

21:05 – 21:13

ERK SIGNALING BIFURCATION AT MITOTIC EXIT PATTERNS THE MOUSE PREIMPLANTATION EMBRYO

Pokrass, Michael[1], Ryan, Kathleen[1], Xin, Tianchi[2], Pielstick, Brittany[3], Timp, Winston[3], Greco, Valentina[2] and Regot, Sergi[1]

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Despite the noisy nature of single cells, multicellular organisms robustly generate different cell types from one zygote. This process involves dynamic cross regulation between signaling and gene expression that is difficult to capture with fixed-cell approaches. In order to study signaling dynamics and cell fate specification during mammalian preimplantation development, we generated two transgenic mouse lines expressing Kinase Translocation Reporters (KTRs) to measure ERK and JNK activities in single cells of live, developing embryos. Our results show that JNK activity is restricted to the trophectoderm (TE) in blastocysts, while ERK is predominantly active within the inner cell mass (ICM) due to FGF signaling. Strikingly, a subset of cell divisions result in a brief pulse of ERK inactivity in both daughter cells that correlates with increased NANOG expression. Conversely, cells that exhibit high ERK activation at mitotic exit express elevated GATA6 and decreased NANOG levels. Interestingly, these dynamics depend on maternal cell signaling history and are sensitive to perturbation of Anaphase Promoting Complex (APC) activity, indicating that the mitotic machinery helps instruct ERK activity at mitotic exit to specify ICM cells to the epiblast and primitive endoderm lineages. Moreover, using a dual fluorescent protein reporter inserted at the Nanog endogenous locus in embryonic stem (ES) cells, we show that ERK inactivity after mitosis promotes rapid posttranslational stabilization of NANOG. Our data show that cell cycle, signaling, and differentiation are coordinated during mammalian preimplantation development and ultimately highlight the importance of dynamics to understand and eventually control stem cells.

Keywords: ERK, Blastocyst, Cell Fate

21:15 – 21:30

DEFINING TOTIPOTENCY IN MOUSE STEM CELLS USING CRITERIA OF INCREASING STRINGENCY

Rossant, Janet[1], Posfai, Eszter[2], Schell, John Paul[3], Janiszewski, Adrian[4], Rovic, Isidora[5], Murray, Alexander[2], Bradshaw, Brian[2], Pardon, Tine[6], El Bakkali, Mouna[6], Talon, Irene[6], Geest, Natalie[6], Kumar, Pankaj[3], To, San Kit[6], Petropoulos, Sophie[3], Jurisicova, Andrea[5], Pasque, Vincent[6] and Lanner, Fredrik[3]

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Totipotency is the ability of a single cell to give rise to all the differentiated cells that build the conceptus, yet how to capture this property in vitro remains incompletely understood. Defining totipotency relies upon a variety of assays of variable stringency. Here we describe criteria to define totipotency. We illustrate how distinct criteria of increasing stringency can be used to judge totipotency by evaluating candidate totipotent cell types in the mouse, including early blastomeres and expanded or extended pluripotent stem cells. Our data challenge the notion that expanded or extended pluripotent states harbor fully totipotent potential relative to conventional embryonic stem cells under in vivo conditions. By contrast there is increasing evidence that human pluripotent stem cells are more readily able to generate trophoblast lineage cells than their mouse equivalents, although the stringent in vivo criteria applied in the mouse cannot be applied. This correlates with evidence for the later restriction of lineage potential in the human blastocyst and emphasizes the importance of understanding the embryonic comparators of different pluripotent stem cell types.

Keywords: Totipotency, pluripotency, mouse

THURSDAY, JUNE 25, 20:00 – 21:45

CONCURRENT - TISSUE STEM CELLS AND REGENERATION: EPITHELIAL STEM CELLS

20:05 – 20:20
FEEDBACK BETWEEN MORPHOGENESIS AND CELL FATE DURING EPITHELIAL PATTERNING

Devenport, Danelle

Princeton University, Princeton, NJ, USA

How cells assemble into elaborate forms that perform specialized functions is a central question in developmental biology and regenerative medicine. Although stem cells in culture have a remarkable capacity for self-organization, the organism-level information that ensures organs form with the proper size and higher-order architecture are poorly understood. As the largest organ in the body and arguably the first organoid to be grown in a dish, the skin is an ideal model to investigate how massive collections of cells assemble into higher order patterns and forms. Using long-term live imaging of murine skin development, we have tracked the divisions and fates of embryonic epidermal stem cells as they build the outer skin layers and have followed the elaborate cell movements that drive the formation, patterning, and polarization of hair follicles. I will present our recent findings demonstrating how morphogenesis feeds back on cell fate specification to generate robust spatial patterning and tissue architecture.

Keywords: epithelial stem cells, epidermis, hair follicle

20:25 – 20:33
LINEAGE TRACING OF ZONAL POPULATIONS REVEALS THAT MID-LOBULAR HEPATOCYTES PREFERENTIALLY REPOPULATE THE LIVER

Wei, Yonglong[1], Yoon, Jung[1], Wang, Yunguan[2], Zhang, Yu[1], Zhu, Min[1], Sharma, Tripti[1], Lin, Yu-Hsuan[1], Li, Lin[1], Zhang, Shuyuan[1], Jia, Yuemeng[1], Wang, Tao[2] and Zhu, Hao[1]

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The source of new hepatocytes in the liver remains unclear. The lobule is the histologic unit iterated throughout the liver, and cross-sectioned lobules have been divided into three arbitrarily defined concentric rings, or zones, extending from the portal vein to the central vein. Venous blood from the gut mixes with oxygenated arterial blood in the portal triads (zone 1), then travels through the sinusoids through the mid-lobule (zone 2), before draining into central veins (zone 3) and returning to the heart. Whether hepatocytes in different zones differ in regenerative activity is unknown, in part because fate-mapping has only been performed on a few hepatocyte subsets. Single cell RNA-sequencing has provided a higher resolution picture of the spatial heterogeneity among hepatocytes along with additional markers for hepatocyte subtypes. However, the experimental tools to interrogate these cell types have not been available. We developed eleven new CreER strains to systematically compare the regenerative activities of distinct subsets of hepatocytes during homeostasis and after injury. During homeostasis, this panel of CreER lines showed that cells from periportal zone 1 contracted in number, cells from pericentral zone 3 contracted in number, and Glutamine Synthetase positive cells in zone 3 remained constant in number. In agreement with zone 1 and 3 contraction, cells from mid-lobular zone 2 labeled by Hamp2-CreER or Mup3-CreER mice expanded in number. Sparse labeling and clonal analysis of zone 2 hepatocytes showed that most proliferated, indicating broad contributions by mid-lobular hepatocytes to liver homeostasis. Zone 2 cells also contributed to regeneration after pericentral and periportal injuries, in part because they represent a reserve population sheltered from common injuries. Hepatocytes in different regions of the liver thus exhibit significant differences in turnover and zone 2

is an important source of new hepatocytes during homeostasis and regeneration.

Keywords: Hepatocyte homeostasis, Liver zonation, Lineage tracing

20:35 – 20:43

VASCULAR SMOOTH MUSCLE-DERIVED PROGENITORS CONTRIBUTE TO BROWN ADIPOCYTE DEVELOPMENT

Shamsi, Farnaz[1], Lynes, Matthew[1], Piper, Mary[2], Ho, Li-Lun[3], Huang, TianLian[1] and Tseng, Yu-Hua[1]

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Brown adipose tissue (BAT) functions in energy expenditure in part due its role in temperature regulation. The thermogenic activity of BAT is inversely correlated to body mass index in humans making it an attractive target for anti-obesity therapies. BAT is distributed in multiple depots throughout the body. Other than mature adipocytes, several other cell types form the adipocytes niche and play important roles in BAT function and remodeling. Prolonged cold exposure increases BAT mass and activity partially through de novo recruitment of brown adipocytes as well as coordinated expansion of other cells within the adipose niche to enable maximal thermogenic activity. However, the source of cold-induced brown adipocytes and the molecular mechanism regulating BAT expansion is not known. Adipocytes are thought to arise from a pool of mesenchymal progenitor cells that have been previously characterized as Pdgfra, Pdgfrb, Cd34, Cd29, or Sca-1 expressing cells. Lineage tracing experiments have demonstrated that interscapular BAT arises from a Myf5 positive lineage shared with skeletal muscle and distinct from the majority of white adipose tissue precursors. In this study, we sought to investigate the cellular origin of brown adipocytes using single cell RNA sequencing. The unsupervised clustering identified different cell types in BAT, including several sub-populations of adipose progenitors, endothelial cells, vascular smooth muscles, and Schwann cells. Our analysis identified two distinct types of adipocyte progenitors: the putative Pdgfra-expressing mesenchymal progenitors and a vascular smooth muscle-derived adipocyte progenitor population that contribute to de novo recruitment of thermogenic adipocytes in cold. Using flow cytometry, we confirmed that this new population of adipocyte progenitors was indeed distinct from the previously identified Pdgfra positive adipocyte progenitors. Using lineage tracing, we showed that the novel population of adipocyte progenitors contribute to the mature adipocyte pool in vivo. Together, these findings suggest a new model for the development of BAT that could be critical in designing strategies to increase the number of brown adipocytes in humans.

Funding source: Sources of Research Support: NIH (R01DK077097, R01DK102898, P30DK036836, K01DK111714), ADA grant #1-18-PDF-169, Harvard Stem Cell Institute.

Keywords: Brown Adipose Tissue, Vascular Smooth Muscle, Single cell RNA-sequencing

20:45 – 20:53

AGE AND INJURY-INDUCED STROMAL ASPORIN MODULATES REGENERATION OF THE SMALL INTESTINAL EPITHELIUM

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Extracellular matrix (ECM) is one component of the stem cell niche with potential to modulate stem cell behavior. While it is known that ECM composition changes during ageing, it is unclear whether such ECM alterations contribute to the stem cell attrition that underlies aging. By culturing intestinal epithelium on decellularized small intestinal matrix (iECM), we show that aged ECM reduces the regenerative capacity of young intestinal epithelium by increasing TGF-beta signaling and suppressing stem cell signature genes. This is caused by age-induced expression of ASPN, a secreted Leucine-rich repeat protein produced by the pericryptal myofibroblasts. Extracellular ASPN potentiates epithelial TGF-beta signaling via transmembrane receptor CD44, and antibodies blocking the binding of ASPN to CD44 mitigate the effects of ASPN on epithelial regeneration. Interestingly, in contrast to sustained high expression of ASPN in ageing, ASPN is transiently induced upon chemotherapy-mediated damage in young mice and a short pulse of exogenous ASPN boosts intestinal regeneration in vitro. Consistently, fibroblast specific deletion of ASPN impairs chemotherapy-induced intestinal regeneration demonstrating the essential roles of ASPN in tissue repair. In summary, intestinal epithelial culture on iECM allows interrogation of ECM-epithelium interactions in homeostasis and diseases, and our findings highlight the role of stromal ASPN in small intestinal ageing and regeneration.

Keywords: Stem Cell, Ageing, TGF-beta

20:55 – 21:03

INJECTIONS OF NUCLEOSIDE MODIFIED MRNA ENCODING VEGF-A INDUCE CHOLANGIOCYTE-DRIVEN LIVER REGENERATION IN MOUSE MODELS OF ACUTE AND CHRONIC LIVER INJURIES

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Injured livers possess a remarkable property to quickly regenerate, however the overwhelming proliferative capacity of hepatocytes becomes severely compromised in case of massive hepatocyte death or chronic liver injury. Histologic examinations of human diseased livers indicate a clear association between the severity of the liver disease and expansion of liver progenitor cells (LPCs) that are believed to be a subset of specialized cholangiocytes. This association suggests an alternative liver repair via LPCs, and raises the exciting possibility to force the intrinsic regenerative potential of LPCs by discovering druggable pathways that trigger this cell conversion to treat liver diseases. Here, we tested the hypothesis that VEGFR2 defines a population of LPCs among cholangiocytes in injured mouse livers, and that activation of VEGFR2 on LPCs with VEGF-A triggers LPC conversion into hepatocytes. Liver injury was induced either by a single dose of acetaminophen to recapitulate an acute damage, or with a 3 weeks CDD diet along with Ethionine to mimic the chronic non-alcoholic fatty liver disease-like injury. AAV8-p21 viruses were administered IV prior to liver injury to model most acute and chronic human liver diseases in which hepatocyte proliferation is exhausted via expression of the senescent gene p21. While VEGFR2 expression is absent on cholangiocytes in healthy livers, injury induces its expression on a subset of cholangiocytes. As previously reported, compromising hepatocyte proliferation with delivery of AAV8-p21 in the cholangiocyte lineage tracing mouse model Krt19-CreERT2,R26-STOPFl/Fl-tdTomato induces emergence of tdTomato positive hepatocytes generated from cholangiocytes. Importantly, we provide key evidence that transient activation of VEGFR2 in vivo by expression in the liver of VEGFA, via the non-integrative nucleoside modified mRNA complexed with lipid nanoparticles (mRNA-LNP), promotes robust cholangiocyte conversion to hepatocytes in both acute and chronic injury models as well as reversion of steatosis and decreased fibrosis following chronic liver injury. Altogether, this study pioneers a therapeutic strategy to force activation of LPCs to treat liver diseases by delivering VEGFA in the liver via the safe and clinically relevant mRNA-LNPs.

Keywords: liver regeneration, liver progenitor cells, cholangiocyte-driven liver regeneration

21:05 – 21:13

STRESS HORMONE CORTICOSTERONE GOVERNS HAIR FOLLICLE STEM CELL QUIESCENCE BY SUPPRESSING A DERMAL NICHE ACTIVATOR GAS6

Hsu, Ya-Chieh[1], Choi, Sekyu[1], Gonzalez-Celeiro, Meryem[1], Stein, Daniel[1], Zhang, Bing[1], Besnard, Antione[3] and Sahay, Amar[4]

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Chronic, sustained exposure to stressors can profoundly impact tissue homeostasis and regeneration. However, how stress leads to tissue changes remain largely elusive. Here, we report that the adrenal gland-derived stress hormone corticosterone (the cortisol equivalent in rodents) enforces hair follicle stem cell quiescence in mice. Without corticosterone, hair follicle stem cells lose quiescence and enter continuous rounds of regeneration cycles throughout life with no signs of exhaustion. Conversely, under chronic stress, elevated corticosterone levels prolong hair follicle stem cell quiescence and inhibit hair follicle regeneration. Mechanistically, corticosterone acts on the dermal niche to suppress the expression of Growth Arrest Specific 6 (Gas6), a secreted factor that stimulates hair follicle stem cell activation. Of significance, restoring Gas6 expression levels is sufficient to overcome the stress-induced regeneration block on hair follicle stem cells. Our findings delineate a cellular and molecular mechanism by which stress leads to defects in tissue regeneration. Moreover, we identify corticosterone as a potent systemic inhibitor of hair follicle stem cell activity via its impact on the niche, and demonstrate that removal of such inhibition drives hair follicle stem cells into continuous regeneration cycles without losing stem cell potential.

Funding source: The Pew Scholars, Pew Charitable Trusts New York Stem Cell Foundation— Robertson Stem Cell Investigator Award

Keywords: Stress, Hair follicle stem cells, Stem cell niche

21:15 – 21:30

CELL COMPETITION IN EPITHELIAL REPAIR

Piddini, Eugenia

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Cell competition is a type of cell interaction that causes the elimination of relatively less fit cells by fitter cells, which take over the tissue in their stead. This interaction shapes tissue colonization in a variety of tissues and organs, both in physiological conditions and in pathologies like cancer. My group investigates the mechanisms of cell competition and how it modulates cell colonization in tissues and organs, focusing primarily on epithelia. We have shown that epithelial cells can compete using mechanical insults. In mechanical cell competition, loser cells have intrinsic hypersensitivity to cell density and crowding from winner cells kills them by apoptosis. A key player in mechanical cell competition is p53, which is both necessary and sufficient for cells to become hypersensitive to cell compaction and behave as mechanical losers. Our recent unpublished work has identified the first physiological context where mechanical cell competition may play a role, i.e. in collective cell migration in damaged epithelia. When epithelia are damaged by injury, cells migrate across wounds to seal the gap. In some epithelia, leader cells at the front of migrating sheets drive this migratory process. However, it is unclear how leaders emerge from an apparently homogeneous epithelial cell population. Our work shows that p53 activates the leader cell program, driving cell migration. However, p53 also induces leader cells to become hypersensitive to crowding such that, upon wound closure, leaders are eliminated by cell competition. Thus, mechanical stress-induced p53 activation directs the emergence of a transient population of leader cells, which drive migration, and ensures leaders are eliminated on wound closure.

Keywords: Cell competition, cell migration, p53

FRIDAY, JUNE 26, 11:00 – 12:45

CONCURRENT - CELLULAR IDENTITY: HEMATOPOIETIC AND ENDOTHELIAL CELLS

11:05 – 11:20
ADAPTABLE AND HEMODYNAMIC HUMAN VASCULOGENIC ENDOTHELIAL CELLS FOR ORGANOGENESIS AND TUMORIGENESIS

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During embryogenesis, endothelial cells (ECs) interact with host tissues and adopt tissue-specific transcriptional and functional properties to instruct organ development. This adaptability is lost in adult ECs and they fail to vascularize organoids or decellularized scaffolds in a tissue-specific manner. Here, we show that reactivation of embryonic-restricted ETS variant 2-transcription factor (ETV2) in mature human ECs in three-dimensional (3D) Laminin-Entactin-CollagenIV (L.E.C) matrix "Resets" these stringent ECs into compliant Vascular ECs (R-VECs), forming durable, perfusable and adaptable vascular plexi. ETV2 resets the vascular memory of mature ECs that through chromatin remodeling induces biophysical sensors and tubulogenic pathways, including Rap1 activation promoting durable lumen formation. In 3D matrices, without the constraints of synthetic bioprinted scaffolds, R-VECs self-assemble into an interconnected multi-layered stable vascular network within large-volume microfluidic chambers capable of transporting human blood. In vivo, implanted R-VEC vessels anastomose to host circulation, are non-leaky, accommodate perivascular cells, and manifest long-lasting organized patterning, without vascular malformations or angiomas. R-VECs physiologically perfuse human pancreatic islets, coordinating glucose-induced insulin production. R-VECs vascularize decellularized intestinal tissue, as well as avidly arborize and interact with normal and tumor organoids, choreographing the survival and growth of epithelial cells. Molecular and epigenetic profiling, demonstrate R-VECs establish an adaptive vascular niche, which differentially respond and conform to organoids and tumoroids. Deciphering the cross-talk between R-VECs and non-vascular cells facilitates identification of factors that determine organotypic-EC heterogeneity, enables physiologic chemical screens and sets the stage for organ repair and tumor targeting.

Keywords: Stem cells, organoids, regeneration, vascular niche, angiocrine factors

11:25 – 11:33
SUPPRESSING METHYLATION AT H3K9 AND H3K36 REVEALS DISTINCT ROLES FOR HISTONE MODIFICATIONS DURING DIFFERENTIATION AND HOMEOSTASIS

Brumbaugh, Justin[1], Kim, Ik Soo[2], Ji, Fei[3], Huebner, Aaron[3], Di Stefano, Bruno[3], Schwarz, Benjamin[3], Charlton, Jocelyn[4], Coffey, Amy[3], Choi, Jiho[3], Walsh, Ryan[3], Schindler, Jeffrey[5], Anselmo, Anthony[3], Meissner, Alexander[4], Sadreyev, Ruslan[3], Bernstein, Bradley[2], Hanno, Hock[5] and Hochedlinger, Konrad[3]

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Development and differentiation are associated with profound changes to histone modifications. However, establishing a functional link between a single histone mark and a biological process (e.g., differentiation) has been difficult due to the paucity of tools that target individual modifications. We have developed transgenic systems to specifically suppress the methylation of target histone residues in pluripotent stem cells and mice. Our systems take advantage of lysine (K) to methionine (M) mutants of histone H3, which function as dominant negative inhibitors of methylation at their respective sites. We focused on two mutants that suppress methylation at either H3K9 (H3K9M) or H3K36 (H3K36M). When expressed in embryonic stem cells, these mutants induced widespread changes to the chromatin landscape and a corresponding block in differentiation. Upon induction of H3K9M or H3K36M in adult mice, we observed potent differentiation defects in a variety of stem/progenitor cells and regenerative tissues. In many cases, stem and progenitor cells accumulated at the expense of mature cell types, revealing precise stages of differentiation that depend upon specific histone modifications. Focusing on hematopoiesis, H3K36M expression led to severe anemia due to a block in erythropoiesis. H3K36M mice also exhibited lymphoid, megakaryocyte, and hematopoietic stem cell defects. Mice expressing H3K9M exhibited distinct phenotypes including an expansion of multipotent progenitors and a block in proB cell differentiation. Finally, we found that discontinuation of mutant histone expression largely restored differentiation programs, suggesting that the effects of methylation loss are reversible. Collectively, our work provides evidence that individual chromatin modifications are required at multiple stages of differentiation and offers powerful

tools to interrogate the physiological consequences of these modifications in vivo.

Keywords: Chromatin, Epigenetics, Cell fate

11:35 – 11:43
MOLECULAR SIGNATURES DISTINGUISH FUNCTIONAL HETEROGENEITY OF MOUSE HEMATOPOIETIC STEM CELLS

Lu, Rong, Jiang, Du, Contreras, Jorge and Chowdhury, Adnan

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Single cell transcriptomics have shown that the gene expressions of cells performing an identical function can vary considerably. In unicellular organisms, this variation may arise solely from stochastic transcriptional events. But in higher-level organisms, they may also reflect regulatory mechanisms that fine-tune cellular function. By simultaneously measuring the transcriptomes and in vivo cellular activities of individual hematopoietic stem cells (HSCs), we present evidence that intercellular variations in gene expression directly correlate with intercellular variations in stem cell function in mice. We identified genes whose expressions are significantly associated with specific self-renewal and differentiation activities of HSCs, and verified their functions using CRISPR/Cas9 and HSC transplantation assays. Our data reveal striking quantitative association patterns between gene expression and stem cell activities across individual cells. We also uncovered specific genes that implement each association pattern. These association patterns reflect elemental regulatory modes across molecular and cellular levels and provide a new perspective for understanding complex gene regulatory networks. In addition, our data uncovered an unexpected but systematic and predictable transformation in the differentiation of individual HSCs during transplantation. And we have identified potential molecular players mediating this transformation. Lastly, our data show that lineage balanced HSCs may play a key role in modulating the blood production balance between different lineages. Our intercellular variation analyses provide a new approach to identifying the cellular and molecular mechanisms underlying tissue regeneration. It can be readily applied to other types of stem and progenitor cells.

Funding source: This work is supported by NIH-R00-HL113104, R01HL135292 and R01HL138225. R. Lu is a Scholar of the Leukemia & Lymphoma Society and a Richard N. Merkin Assistant Professor.

Keywords: CELLULAR HETEROGENEITY, SINGLE CELL TRANSCRIPTOME, HEMATOPOIETIC STEM CELLS

11:45 – 11:53
GENERATING HUMAN TYPE 1 DENDRITIC CELLS BY DIRECT CELL REPROGRAMMING

Pereira, Carlos-Filipe[1], Rosa, Fabio[2], Pires, Cristiana[2], Kurochkin, Ilia[3] and Zimmermannova, Olga[2]

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Dendritic cells (DCs) are professional antigen presenting cells specialized in the recognition, processing and presentation of antigens to T cells, inducing adaptive immune responses. DC vaccines are emerging as promising immunotherapies but are limited by the rarity of antigen cross-presenting type 1 DCs (DC1s). We have previously identified PU.1, IRF8 and BATF3 as sufficient and necessary to induce a DC1 program in mouse fibroblasts. Importantly, induced DCs (iDCs) were able to present and cross-present antigens to T cells, demonstrating that antigen presentation can be dynamically instructed by cell reprogramming. Here, the ability to derive DC1s from unrelated human cell types was investigated by combined expression of PU.1, IRF8 and BATF3. iDCs generated from human embryonic fibroblasts acquire DC morphology, activate the expression of DC1 markers including CLEC9A, CD141 and the co-stimulatory molecules CD40, CD80 and CD86. Moreover, iDCs establish a step-wise DC1 transcriptional signature at the single cell level as assessed by droplet based single-cell RNA-seq. During this process antigen presentation signatures are upregulated and cell cycle gene signatures downregulated. Interestingly, only DC1s but not other DC subsets could be detected during reprogramming. Single cell trajectory analysis revealed gene modules associated with successful and unsuccessful reprogramming as well as an interferon primed DC state. Remarkably, human DC1 reprogramming efficiency can be enhanced 190-fold by combining inflammatory cytokine signaling and constitutive overexpression of PU.1, IRF8 and BATF3. This improved DC reprogramming protocol allows reprogramming of human adult cells including dermal fibroblasts, mesenchymal stem cells and monocytes. These findings provide insights into the establishment of antigen presenting machinery and human DC1 specification. Moreover, it represents a platform for generating patient-specific DC1s, the most attractive DC subset for cancer immunotherapy.

Funding source: The Knut and Alice Wallenberg foundation, the Medical Faculty at Lund University and Region Skåne are acknowledged for generous financial support. This project was funded by FCT, Cancerfonden and the Swedish Research Council.

Keywords: Cell Reprogramming, Dendritic Cells, Cancer Immunotherapy

11:55 – 12:03

TRANSCRIPTIONAL REGULATION OF MITOCHONDRIAL METABOLISM BY TIF1GAMMA DRIVES ERYTHROID PROGENITOR DIFFERENTIATION

Rossmann, Marlies P.[1], Hoi, Karen[2], Chan, Victoria[2], Perlin, Julie[3], Hagedorn, Elliott[3], Abraham, Brian[4], Yang, Song[3], Vyas, Sejal[5], Nag, Partha[6], Sullivan, Lucas[7], Warren, Curtis[8], Dorjsuren, Bilguujin[2], Custo-Creig, Eugenia[2], Adatto, Isaac[2], Cowan, Chad[8], Schreiber, Stuart[6], Young, Richard[9], Haigis, Marcia[5] and Zon, Leonard[3]

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Understanding in-vivo mechanisms of hematopoiesis is critical for developing directed blood differentiation approaches. Zebrafish moonshine (mon) mutant embryos defective for the conserved transcriptional intermediary factor 1 gamma (tif1γ) do not specify enough erythroid progenitors due to a transcription elongation block. To elucidate the TIF1γ-mediated mechanisms in erythroid differentiation, we performed a chemical suppressor screen using 3,100 compounds and identified leflunomide, which inhibits the essential mitochondrial pyrimidine synthesis enzyme dihydroorotate dehydrogenase (DHODH). DHODH needs to be inhibited at the end of gastrulation, when the first hematopoietic progenitors are specified from mesoderm. Leflunomide as well as the structurally unrelated DHODH inhibitor brequinar rescue the formation of erythroid progenitors in 61% (38/62) and 68% (50/74) of mon embryos, respectively. In-vivo metabolomics analyses identified nucleotide metabolism as the most significantly altered process in mon mutants, with elevated levels of uridine monophosphate and low levels of nicotinamide adenine dinucleotide. This increase is functionally linked to a reduced oxygen consumption rate. DHODH is the only enzyme of the pyrimidine de novo synthesis pathway located on the inner mitochondrial membrane and its activity is coupled to that of the electron transport chain (ETC) via coenzyme Q (CoQ). Rotenone, a potent ETC complex I inhibitor reverses the rescue of the erythroid progenitor defect by DHODH inhibition in mon embryos. Through parallel genome-wide transcriptome and chromatin immunoprecipitation analyses, we found that genes encoding CoQ metabolic enzymes are direct TIF1γ targets. Treatment with the CoQ analog decylubiquinone results in rescue of erythroid progenitors in 26% (33/126) of mon embryos. These results demonstrate a tight coordination of nucleotide and mitochondrial metabolism as a key function of tif1γ-dependent transcription and reveal that TIF1γ activity regulates a metabolic program that drives cell fate decisions in the early blood lineage. Our work highlights the importance of transcription regulatory processes such as transcription elongation for tuning metabolism during lineage differentiation and could have therapeutic implications for blood diseases.

Keywords: Erythroid progenitor, Mitochondria, Metabolism

12:05 – 12:13

EOMESODERMIN GOVERNS THE HEMOGENIC COMPETENCE OF MURINE YOLK SAC MESODERMAL PROGENITORS

Harland, Luke T.[1], Bikoff, Elizabeth[1], Costello, Ita[1], De Bruijn, Marella[2], Greder, Lucas[2], Porcher, Catherine[2], Robertson, Elizabeth[1], Senft, Anna[1] and Simon, Claire[1]

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The gene regulatory networks that coordinate hematopoietic commitment in the developing murine yolk-sac (YS) remain ill-defined. Here we report that the T-box transcription factor Eomesodermin (Eomes) is transiently expressed in mesodermal progenitors that generate virtually all YS hematopoietic and endothelial cells. Using an embryonic stem cell (ESC) differentiation system, we find that Eomes activity is essential for the production of primitive erythrocytes and definitive hematopoietic progenitors but dispensable for the development of endothelial cells. Bulk RNA-seq and single-cell-RNA-seq experiments demonstrate that in the absence of Eomes function Flk-1+/PdgfRa- hematovascular mesoderm is specified but upon further differentiation it is diverted towards an endothelial rather than hematopoietic fate. Utilizing ESC reporter lines, we show that Eomes is expressed prior to both SCL and Runx1 during mesoderm patterning. Interestingly, Eomes activity is dispensable for the expression of SCL but is essential for the normal development of Runx1+ hemogenic endothelium (HE). ATAC-Seq experiments reveal that Eomes governs the accessibility of numerous hematopoietic enhancers that SCL normally utilizes to specify primitive erythrocytes and HE. Finally, ChIP-seq experiments suggest that Eomes coordinates the development of hemogenic competent mesoderm in the context of Activin/Nodal and Tead-Yap signaling at mesodermal stages of development. Collectively, these experiments demonstrate that Eomes sits at the

top of the transcriptional hierarchy, functioning upstream of Runx1 expression and SCL functional activity, to promote hemogenic competence of the entire YS mesodermal lineage. These results ultimately suggest that hemogenic competence is endowed earlier during murine embryogenesis than previously appreciated and have widespread implications for the generation of HE from pluripotent stem cell sources.

Funding source: The work was supported by grants from the Wellcome Trust (214175/Z/18/Z E.J.R, 10281/Z/13/Z L.T.G.H).

Keywords: hemogenic endothelium, mesoderm, gastrulation

12:15 – 12:30
AN UNUSUAL CELLULAR STATE OCCUPIED BY THE FAST CYCLING HEMATOPOIETIC PROGENITORS

Guo, Shangqin[1], Eastman, Anna E[2], Chen, Xinyue[3], Hu, Xiao[3] and Hartman, Amaleah[4]

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The stochastic and rare cell fate transition driven by the Yamanaka factors to convert somatic cells into pluripotency becomes fast, synchronous and efficient in a subset of the granulocyte-monocyte progenitors (GMPs). An important feature of such GMPs is an ultrafast cell cycle, which on average lasts ~8 hours/cycle. Given the unusual behavior of this GMP subset, and the suspected commonality between reprogramming and malignant transformation, we investigated whether the ultrafast cycling GMPs also underlie leukemic transformation. Indeed, the fastest cycling 3% GMPs acquire malignancy with near certainty when MLL-AF9 is expressed. Since the most recognizable feature of this remarkable cellular state is an ultrafast cell cycle, we designed a fluorescence-based cell cycle speed reporter. This reporter is based on the color-changing Fluorescent Timer (FT) protein, which emits blue fluorescence when newly synthesized before maturing into a red fluorescent protein. We generated a mouse strain expressing an H2B-FT fusion reporter from a universally active locus, and demonstrate that faster-cycling cells can be distinguished from slower-cycling ones based on the intracellular fluorescence ratio between the FT's blue and red states. This reporter can resolve the native cell cycle speed distribution of primary hematopoietic stem and progenitors, and enable their prospective purification based on cell cycle speed. Ongoing investigation is aimed to decipher how cell cycle speed contributes to cell fate determination in hematopoietic progenitors.

Keywords: cell cycle speed, granulocyte-monocyte progenitors (GMPs), H2B-FT, reprogramming, leukemic transformation

FRIDAY, JUNE 26, 11:00 – 12:45

CONCURRENT - CLINICAL APPLICATIONS: CARDIAC AND MUSCLE

11:05 – 11:20
ADVANCES IN HEART-ON-A-CHIP ENGINEERING

Radisic, Milica

University of Toronto, ON, Canada

There is an urgent need to develop more predictive tissue models to determine drug efficacy and safety in advance of clinical testing. For many years, the cost of drug discovery has been steadily rising due to the limited predictability of the existing models that involve 2D cell culture and animal models. Through the convergence of microfabrication and tissue engineering, organ-on-a-chip technologies are poised to disrupt the drug discovery industry by offering an alternative to the conventional tests. In this presentation, organ-on-a-chip technologies will be broadly categorized according to three distinct aspects of human physiology they focus on emulating: barrier properties, parenchymal tissue function, and multi-organ interactions. The current challenges in the field will be highlighted and recent organ-on-a-chip startups will be discussed. The presentation will also highlight recent advances in the development of heart-on-a-chip for drug testing and disease modelling. An ideal heart-on-a-chip platform should be able to yield distinct atrial and ventricular tissues of high biological fidelity; yet cardiac tissue engineering starting from human pluripotent stem cells has focused on reproducing ventricular myocardium and assessing adverse ventricular events. Our goal is to develop a versatile resource for the community, a platform that enables creation of electrophysiologically distinct atrial and ventricular tissues, and that is capable of providing months long biophysical stimulation of 3D tissues to model a polygenic disease. This platform, termed Biowire II, enables growth of thin, cylindrical tissues, similar to human trabeculae, suspended between two parallel polymer wires whose deflection can be used to conveniently quantify passive and active forces simultaneously with Ca2+ transients. With appropriate choices of directed differentiation protocols and optimized electrical conditioning, atrial vs. ventricular specification is robustly achieved. We demonstrate chamber specific drug responses, specifically that a low concentrations of 4-AP (<50μM) prolonged AP duration in atrial but not ventricular tissues. Unique-

ly, electrical conditioning for up to 8 months enables modeling of polygenic left ventricular hypertrophy starting from cells of hypertensive patients.

Keywords: heart, myocardium, atria, ventricle, iPSC

11:25 – 11:33

GENERATION OF IPSC-DERIVED MYOGENIC PRECURSORS TO TREAT DUCHENNE MUSCULAR DYSTROPHY IN A MOUSE MODEL

Domenig, Seraina, Lenardic, Ajda and Bar-Nur, Ori

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Duchenne muscular dystrophy (DMD) is an X-linked heritable disease affecting approximately 1 in 3,500 boys worldwide. DMD is caused by a mutation in dystrophin, an essential protein for normal skeletal muscle function. Absence of dystrophin results in progressive muscle degeneration, leading to paralysis and premature death of patients. Intramuscular transplantation of muscle stem cells offer an attractive approach to treat DMD, as dystrophin is highly expressed in myofibers and satellite cells, however obtaining high quantity of muscle stem cells for transplantation is challenging. Here we report on generation of induced pluripotent stem cells (iPSCs) from mouse embryonic and adult fibroblasts of the DMD mouse model for Duchenne. DMD-iPSCs could propagate long term in vitro and express canonical pluripotency markers including alkaline phosphatase, Oct4, Sox2 and Nanog. Capitalizing on the clonogenicity of iPSCs, we utilized the CRISPR-Cas9 genome editing system to correct the dystrophin mutation in DMD-iPSCs using an exon-skipping based strategy. Corrected DMD-iPSCs were subjected to a step-wise directed differentiation protocol to generate myogenic precursors positive for the satellite cell and myoblast markers Pax7, Myf5, MyoD and Myog. The myogenic precursors could further differentiate into contractile multinucleated myotubes positive for dystrophin and myosin heavy chain. These findings thus report on a facile method to generate high numbers of autologous and corrected myogenic precursors for the treatment of DMD in a mouse model, paving way to devise similar therapeutic approaches with human cells.

Keywords: Duchenne Muscular Dystrophy, iPSC-based cell therapy, Genome editing

11:35 – 11:43

TRANSIENT REPROGRAMMING OF RAT AND MOUSE CARDIOMYOCYTES IN VITRO AND IN VIVO

De Lazaro, Irene[1], Kisby, Thomas[2], Cossu, Giulio[2] and Kostarelos, Kostas[2]

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Zebrafish and urodele amphibians regenerate their myocardium via de-differentiation, or reprogramming, of existing cardiomyocytes (CMs) followed by transient proliferation. Adult mammalians have lost such capacity. We have previously demonstrated that somatic cells in mouse liver and skeletal muscle can be transiently de-differentiated to a proliferative state by overexpression of Oct3/4, Klf4, Sox2, and c-Myc (OKSM) and contribute to tissue repair. Whether this strategy could induce CM reprogramming that contributes to myocardial regeneration remains unknown. Here, we first investigated if rat and mouse CMs could be reprogrammed in vitro. Neonatal CMs were isolated, cultured and transduced with OKSM-encoding adenovirus, which triggered upregulation of the pluripotency master regulators Nanog and Oct4 and downregulation of CM specific genes. De-differentiation was confirmed by sarcomere disassembly, cessation of beating and reduced expression of cardiac troponin-T. Signs of increased cell proliferation and mesenchymal to epithelial transitions were also found. Lineage tracing was used to confirm the cardiomyocyte origin of reprogrammed cells. We did not observe the generation of pluripotent stem cell-like colonies throughout the length of the study (20 days), even when standard embryonic stem cell culture conditions were used and, by day 15 after transduction, CM gene expression was restored and contractile activity re-established. This suggests that reprogramming was only partial and transient. Given the potential advantages of partial and transient reprogramming for regenerative medicine strategies, avoiding the pluripotent state and the risk of tumorigenesis, we tested if a similar cell fate conversion could be achieved in adult mice in vivo. OKSM-encoding adenoviral vectors were injected in the myocardial wall of healthy mice and mice after myocardial infarction (MI) and triggered upregulation of pluripotency markers Gdf3 and endogenous Oct4. However, no Nanog expression was detected, suggesting that a less de-differentiated state was achieved in vivo. No mice developed teratomas or tissue dysplasia for the course of the study (120 days). We continue to investigate the differentiation state of reprogrammed cells in vivo and their contribution to myocardial regeneration after MI.

Keywords: cardiomyocyte, reprogramming, OKSM

11:45 – 11:53

INHIBITION OF ROS-MEDIATED CAMKII ACTIVATION RESTORES ABNORMAL CALCIUM HANDLING AND IMPAIRED CONTRACTION IN BTHH SYNDROME

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Mutation of the gene Tafazzin (TAZ) causes Barth syndrome (BTHS), which is characterized by cardiac and skeletal muscle myopathy. TAZ catalyzes the remodeling of cardiolipin, the signature phospholipid of the mitochondrial inner membrane. Cardiomyopathy and risk of sudden cardiac death are prominent features of Barth syndrome (BTHS), but the mechanisms by which impaired cardiolipin biogenesis cause cardiac muscle weakness and arrhythmia are poorly understood. Here we used genetically engineered cardiomyocytes derived from human induced pluripotent stem cells (iPSC-CMs) as a model to gain insights into the mechanism connecting TAZ mutation to abnormal Ca2+ handling and impaired force generation. Compared to isogenic control cells, we found that BTHS iPSC-CMs have reduced systolic stress as well as abnormal Ca2+ handling characterized by increased diastolic Ca2+, reduced Ca2+ transient amplitude, and increased frequency of improper Ca2+ release. Our data uncovered a molecular pathway linking TAZ mutation to these Ca2+ abnormalities and contraction defects through ROS-mediated activation of CaMKII and subsequent RYR2 phosphorylation. Scavenging ROS, inhibiting CaMKII, or ablating the RYR2 site phosphorylated by CaMKII, we normalized BTHS iPSC-CM Ca2+ handling abnormalities and contraction force. In addition, we validated this molecular pathway and treatment strategy in isolated neonatal or adult ventricular cardiomyocytes with mutated TAZ. In summary, this study identifies a ROS-CaMKII pathway that likely contributes to reduced force generation as well as arrhythmia in BTHS and shows that targeting this pathway may represent a therapeutic avenue to treat this disease.

Keywords: Barth syndrome, iPSC-CMs, CaMKII activation

11:55 – 12:03

SUCCESSFUL ENGRAFTMENT OF HUMAN IPSC-DERIVED CARDIAC LINEAGE CELLS IN A MODEL OF RIGHT VENTRICLE HEART DISEASE USING IMMUNOSUPPRESSED MACAQUES

Secreto, Frank J.[1], Nelson, Timothy[1], Scholz, Jodi[2], Kurian, Joe[3], Mejia, Andres[4], Wobig, Joan[1], Cannon, Bryan[5], Padley, Doug[6], Reece, Chelsea[7] and Emborg, Marina[8]

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Our laboratory is currently engaged in developing a process for producing clinical-grade human iPSC-derived cardiac-lineage cells (iPSC-CL) for single right ventricle (RV) disease, with particular focus on Hypoplastic Left Heart Syndrome (HLHS). In preparation for an impending Investigational New Drug (IND) proposal, we designed a pilot study involving an established large animal model for the study of stem cell therapies (rhesus macaque) to investigate whether our hiPSC-CL cell product will engraft into the myocardium of a diseased RV. All surgeries took place at the Wisconsin National Primate Research Center utilizing iPSC- CL procured from Regen Theranostics Inc., Rochester, MN, with pre-determined release criteria. Surgical procedures and perioperative care were provided in compliance with the "Guide for the Care and Use of Laboratory Animals." Animals underwent a left surgical thoracotomy for placement of a pulmonary artery band (PAB) to induce right ventricular pressure overload (n=4). After 2-3 weeks post-PAB, right thoracotomy was performed, and iPSC-CL cells (10 X 106 cells/kg; 6-15 200µl injections) were injected into the myocardium of the RV using a 27 gauge single-needle device. Animals were immunosuppressed prior to cell injection using methylprednisolone, abatacept, and tacrolimus. Animals were followed for 4 and 8 weeks with periodic echocardiography and arrhythmia detection via an implantable loop recorder (ILR). After euthanasia of the first 2 animals, analysis of histological samples stained with an antibody specific for monkey cardiac-troponin I revealed engraftment of iPSC-CL at 4 (n=1) and 8 (n=1) weeks post-injection. Cellular engraftment was generally localized to the area of injection. No signifi-

cant safety issues were observed, and although sinus tachycardia was detected (240-270 bpm), heart rate variability was normal. Based upon these findings, we have initiated an expanded safety study with engraftment being verified at 4 and 12 weeks post-injection.

Funding source: The Todd and Karen Wanek Family Program for Hypoplastic Left Heart Syndrome

Keywords: iPSC, Engraftment, Cardiac

12:05 – 12:13
ENGINEERED HUMAN CARDIAC TISSUES WITH PERFUSABLE EMBEDDED VASCULATURE

Uzel, Sebastien[1], Lewis, Jennifer[2] and Skylar-Scott, Mark[1]

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Biomanufacturing vascularized functional human tissues derived from patient-specific cells, and ultimately full organs, is a grand challenge. Specifically, the cellular density and microarchitecture of the organ of interest must be replicated in order for the engineered construct to match the function of the native counterparts. Traditional bioprinting techniques offer promising approaches but fail to generate tissue constructs with the cellular and structural complexity necessary for proper function at a therapeutic scale. On the other hand, 3D cell culture systems such as induced pluripotent stem cells (iPSC)-derived organoids exhibit exquisite multicellular arrangements and elements of function, but in the absence of perfusable channels, those structures are limited to submillimeter diameters. By combining these two strategies, we developed a new assembly paradigm that allows the vascularization of organ-specific tissues with the cellular density and function needed for large-scale tissue engineering. With this technique called Sacrificial Writing into Functional Tissue (SWIFT), cellular aggregates or organ building blocks (OBBs) are assembled into a densely packed living matrix into which vascular channels can be free-form templated via embedded printing of a sacrificial material. We have demonstrated that this method allows for the rapid assembly and perfusion of more than 2 milliliters of tissues, composed of hundreds of thousands of embryoid bodies, making up half a billions cells at a density of approximately 200 million cells per milliliter. Applied to differentiated cardiac cells, we show that the perfused construct composed of individually and asynchronously beating OBBs evolve over time towards a continuous tissue exhibiting coordinated contraction and responsive to electrical pacing and drug treatment. This scalable approach, bridging iPS-derived organoids and additive manufacturing, provides a new avenue towards full-scale organ manufacturing.

Keywords: 3D bioprinting, Cardiac engineering, Tissue vascularization

12:15 – 12:30
CREATING A LANDSCAPE OF STEM CELL SIGNATURES

Horwitz, Rick

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The Allen Institute for Cell Science is bringing together a number of new technologies to develop a landscape of hiPS cell signatures, identifying cell types and states, study changes in their cellular organization during differentiation of hiPSCs to cardiomyocytes, and elucidate the principles of cellular organization. The focus is on high-quality genome edited stem cells in cultures and organoids, high replicate imaging, single cell genomics, and quantitative image analysis, machine learning, and computational models and visualization methods. To do this, we have developed a toolkit that includes methods for GFP-tagging proteins that localize to particular cellular structures and organelles, novel methods of generating and analyzing high replicate fluorescence and brightfield images, models for the integrative visualization of many structures in the same cell, novel visualization methods, and a simulation platform. With this toolkit, we are creating a collection of plasmids and genome-edited hiPSC lines, a large image database for analysis and exploration, 3D cellular models and simulations, and educational materials that are all publicly available (www.allencell.org). The Institute is presently using these tools to address scientific programs that: 1. Conjoin cellular images with gene expression profiles in the same cell using multiplexed fluorescent in situ hybridization (FISH) to ask whether gene expression profiles can be predicted from cellular organization and vice versa. 2. Model nuclear organization, creating a nuclear “state space” to identify and predict cell types and states. 3) Quantify cell variance, and its sources. 4) Investigate the relationship between genotype to phenotype in normal and pathological contexts with a library of isogenic disease mutations. The presentation will discuss the status of these programs, the tools used to address them, and the use cases that can parallel research activities by others.

Keywords: stem cells, imaging, gene editing

FRIDAY, JUNE 26, 11:00 – 12:45

CONCURRENT - MODELING DEVELOPMENT AND DISEASE: GENE EDITING IN EARLY EMBRYO AND PLURIPOTENT STEM CELLS

11:05 – 11:20
DEVELOPMENTAL DISEASE MODELS AT SINGLE-CELL RESOLUTION: SCIENTIFIC AND ETHICAL BENCHMARKING

Testa, Giuseppe

University of Milan, Italy

In vitro models of human development are transforming biomedicine by making the developmental potential of individual genomes experimentally tractable. For developmental disorders as well as for late-onset disorders with an early developmental seeding, this patient-specific re-enactment of development promises to bridge altered developmental trajectories to clinical phenotypes at unprecedented resolution, especially through the pairing of single cell multi-omics with digital phenotyping. With the increasing scalability of in vitro organoidogenesis, the prospect is thus within reach for a systematic exploration, including a mechanistic dissection, of how genetic variation shapes the human developmental space, an intellectual pursuit with far reaching implications that requires scientific as well as ethical benchmarking. Here I lay out the principles for such dual benchmarking, starting from our most recent modelling of a particularly informative set of neurodevelopmental disorders caused by point mutations or dosage imbalances in chromatin regulators that operate in inter-related pathways. I present our single-cell level deconvolution, in brain organoids, of dosage-dependent alterations in developmental pathways along with a transcriptome-based benchmarking that allows us to empirically assess how far different paradigms of brain organoidogenesis recapitulate human brain development and match specific experimental needs. This benchmarking pipeline is then applied to the quest for convergent dysregulation across neurodevelopmental disorders and to multiplexed designs, experimental and computational alike, in order to advance the field towards population-level scalability, with the attending analysis of its ethical and societal impact.

Keywords: organoids; disease modelling; ethics

11:25 – 11:33
ANALYSIS OF HAPLOINSUFFICIENCY DISORDERS IN HUMAN EMBRYONIC STEM CELLS

Zaken Gallily, Roni, Yilmaz, Atilgan, Sagi, Ido, Golan-Lev, Tamar and Benvenisty, Nissim

Department of Genetics, The Hebrew University of Jerusalem, Israel

Haploinsufficiency occurs when one functioning allele of a gene is insufficient for wild type phenotype. Although haploinsufficiency underlies many human diseases, its extent and molecular basis are not fully understood. To date, the effect of haploinsufficiency on human embryonic stem cell (hESC) growth and proliferation has not been broadly studied. We aimed to identify genes that require two functioning alleles for the normal growth of hESCs. To establish a genome-wide library for heterozygous mutations, we fused a loss-of-function mutant library of haploid hESCs with normal haploid hESCs. By screening for the heterozygous mutations that negatively affect the hESC growth, we could identify which of the haploinsufficiency-related disorders show growth retardation phenotype even in early embryonic cells. Furthermore, we examined the distribution of our list of putative haploinsufficient genes in the human genome and in different cellular compartments. Interestingly, a large fraction of the essential genes with heterozygous mutations was found to be localized to the extra-cellular matrix and the plasma membrane, this being in strike difference to essential genes with homozygous mutations, which were predominantly found in the nucleus and mitochondria. These findings suggest that essential homozygous and heterozygous genes work via different mechanisms/cellular compartments. Next, we searched for pathways and interactions common for several essential heterozygous gene, discovering three main signal transduction pathways critical for hESC growth. We suggest that hESCs require high levels of proteins in these pathways, thus showing growth retardation even when a single allele is mutated. Overall, we have constructed a novel model system for studying the effect of haploinsufficiency and identified important dosage-dependent pathways involved in hESC growth and survival.

Keywords: Haploinsufficiency, Human Embryonic Stem Cells, Genetic Screening

11:35 – 11:43
MECHANISMS OF ANEUPLOIDY IN HUMAN EMBRYONIC STEM CELLS

Godek, Kristina M., Deng, Chenhui and Compton, Duane

Department of Biochemistry and Cell Biology, Geisel School of Medicine at Dartmouth, Hanover, NH, USA

During cell division chromosomes must be accurately segregated to produce daughter cells with the correct numbers of chromosomes whereas segregation errors generate aneuploid cells with abnormal numbers of chromosomes. In normal human somatic cells, chromosome segregation errors and aneuploidy are rare. In contrast, in human totipotent and pluripotent embryonic cells meiotic and mitotic errors are common, resulting in aneuploidy being the leading cause of miscarriages and birth defects. Yet, we do not understand the mechanisms responsible for this, particularly for mitotic errors. Here, we directly compare cell division between pluripotent human embryonic stem cells (hESCs) and somatic cells to investigate both the mechanisms that cause chromosome mis-segregation in hESCs and that allow aneuploid hESCs to propagate. Using quantitative live-cell imaging and chemical approaches, we show that mitotic errors, including lagging chromosomes, are elevated in hESCs compared to somatic cells. Moreover, we show that increasing mitotic duration or decreasing chromosome microtubule attachment stability in hESCs decreases the frequency of mitotic errors. These results demonstrate that hESCs do not efficiently correct chromosome microtubule attachment errors and that multiple pathways contribute to the inefficient error correction. Furthermore, we show that, unlike somatic cells, hESCs fail to arrest in G1 following an aberrant mitosis suggesting that the G1 cell cycle structure of hESCs permits a tolerance to aneuploidy. Collectively, our data demonstrates that pluripotent embryonic cells are inherently different from somatic cells with respect to mechanisms that support chromosome segregation fidelity and in their response to aneuploidy. Importantly, these results offer new strategies to improve the genome stability of human embryonic cells grown in culture that is critical to the success of reproductive and regenerative medicine therapies.

Keywords: Aneuploidy, Mitosis, G1

11:45 – 11:53
DEFINING ALTERNATIVE HUMAN NAÏVE PLURIPOTENCY CONDITIONS DEVOID OF MEK/ERK INHIBITORS

Bayerl, Jonathan[1], Ayyash, Muneef[2], Shani, Tom[1], Novershtern, Noa[1], Viukov, Sergey[1] and Hanna, Jacob[1]

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In mouse and human naïve pluripotency conditions, the use of MEK/ERK inhibition is the major mediator for inducing global DNA hypomethylation which in turns leads to sporadic erosion of imprinting that becomes more severe with extended passaging. In mice, using alternative naïve conditions that do not employ MEK/ERK inhibitors allows isolating genetically and epigenetically stable murine PSCs with all features of naivety. The latter murine cells are fully naïve and are capable of generate all-iPS mice with contribution to the germline, and thus provide a safer route for exploiting defined mouse naïve PSCs. However, such alternative naïve conditions have not been described so far with human cells. Here we engineer reporter systems that allow functional screening for conditions that can endow both the molecular and functional features expected from human naive pluripotency in the absence of MEK/ERK inhibitors. We establish defined alternative naïve conditions in which MEK/ERK inhibition is substituted with inhibition for a novel signaling pathway that allow obtaining alternative human naïve PSCs with diminished risk for loss of imprinting and deleterious global DNA hypomethylation. Furthermore, following refinement of these growth conditions we are able to overcome limited propensity for in vitro developmental potential in human primordial germ cell commitment and enhance interspecies-chimaerism competence following microinjection into mouse morulas for in vivo differentiation tracing. Our findings set a new framework for the signaling foundations of human naïve pluripotency and may advance its utilization in future translational applications.

Keywords: Human naive pluripotency, MEK/ERK inhibition, Inter-species chimaera

11:55 – 12:03
MEASURING AND CONTROLLING THE SPATIOTEMPORAL DYNAMICS OF FGF SIGNALING IN A HUMAN GASTRULATION MODEL

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During mammalian gastrulation, embryonic cells simultaneously undergo differentiation to form the germ layers, and morphogenesis to establish the body axes. However, how these processes are coordinated, and how they affect each other is largely unknown. Among the signaling pathways involved in gastrulation, FGF plays a key role in driving both mesodermal differentiation as well as the proper migration of cells, placing it at the crossroads of patterning and morphogenesis. However, the complex intracellular signal transduction and dynamic nature of this pathway have made its characterization in the context of gastrulation difficult. To understand the dynamics and versatility of FGF signaling, we have generated a multicolor embryonic stem cell line that allows us the simultaneously image the dynamics of Erk, Akt, and PLC-γ – the 3 primary intracellular components of the FGF pathway. We have quantified the dynamics of these components in a novel in vitro differentiation system that captures the full

cellular diversity of human gastrulation in spatially defined domains. Finally, we have generated additional lines containing an optogenetic variant of the FGF receptor, allowing us to manipulate the spatial and temporal dynamics of this pathway in the context of gastrulation-like differentiation. We believe that these tools will allow us to dissect the role of FGF in coordinating cellular movements and patterning during early human development.

Keywords: Gastrulation, FGF, Optogenetics

12:05 – 12:13
EMBRYOIDS, SYNTHETIC EMBRYOS AND CELL-CULTURE MODELS OF EARLY HUMAN DEVELOPMENT: THE NEED FOR IMPROVED NAMING CONVENTIONS

Matthews, Kirstin R.[1], Wagner, Daniel[2] and Warmflash, Aryeh[2]

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In 2014, scientists found that they could manipulate and organize human embryonic stem cells (hESCs) to obtain cellular characteristics and germline layers similar to a developing embryo during gastrulation; an entity that some call embryoids but may be given a variety of names. Embryoids are an attractive alternative due to the difficulty in obtaining human embryos and strong ethical concerns regarding their use. Several different embryoid models have now been described and confusion regarding their creation, development, and potential has led to uncertainty regarding how they should be regulated. Much of the confusion regarding how to regulate and use embryoids is linked to contradictory, inaccurate and confounding names scientists and science journalists have used, which in turn leads regulators to make inaccurate assumptions about the entities potential to develop to a functioning human embryo. Names can hold power in public perception. In this presentation, we will review numerous names for embryoids and broadly classify them into three types: general, time-based, and cell/tissue-based. Analyzing the strengths and weaknesses of each, we conclude there is a need for a consensus on naming embryo-like entities. In addition, we will recommend a nomenclature structure to allow specific entities to be defined based on what they are and what they intend to model. This system will help scientists communicate more effectively with each other through explicit names as well as communicate with the public with broad names. Ultimately, if scientists do not want unnecessary regulations on embryoids, especially primitive versions that do not have the capability of growing to a fetus, then they must work to help communicate with the public what embryoids are and are not.

12:15 - 12:30
DNA REPAIR RESPONSE IN HUMAN EMBRYOS

Shoukhrat Mitalipov

Oregon Health & Science University, USA

Applications of genome editing ultimately depend on DNA repair triggered by targeted double-strand breaks (DSBs). We observed that DSBs selectively induced on a mutant allele in heterozygous human embryos are repaired by gene conversion using an intact wildtype homolog as a template in up to 40% of targeted embryos. Additionally, conversion tracks may expand bi-directionally well beyond the target region leading to an extensive loss of heterozygosity (LOH). Our study demonstrates that gene conversion and NHEJ are two major DNA DSB repair mechanisms in preimplantation human embryos.

Keywords: Human Embryo, Gene Conversion, Loss of Heterozygosity

FRIDAY, JUNE 26, 11:00 – 12:45

CONCURRENT - TISSUE STEM CELLS AND REGENERATION: CANCER AND STEM CELLS

11:05 – 11:20
MICRORNA REGULATION OF PLURIPOTENCY AND DEVELOPMENT

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MicroRNAs function through multiple targets and pathways to regulate cell fate decisions. The mir-290/miR-302 family promotes pluripotency in vitro and suppresses premature differentiation in vivo. It does so through a large network of targets that regulate multiple cellular process. Our lab has been dissecting these mechanisms. In embryonic stem cells, we have recently discovered that the miR-290/miR-302 family regulates ERK signaling by suppressing PFN2, a regulator of dynamin-regulated endocytosis. In the absence miRNA suppression, increased levels of PFN2 suppress endocytosis and ERK signaling. The resulting cells show reduced proliferation and a failure to differentiate. These findings link dynamin- regulated endocytosis to signaling and ultimately embryonic stem cell fate. Previously, we have shown that these microRNAs also suppress the cell cycle checkpoint and cell cycle driven phasic gene expression in embryonic stem cells. Recently, we have been studying these roles in vivo, focusing on the miR-302 cluster. The loss of miR-302 results in the premature differentiation of multiple

somatic tissues including neurons within the neural tube. We asked whether phasic expression could be linked to this defect. We find that phasic expression is established following gastrulation. The loss of miR-302 results in premature establishment of phasic expression, while loss of one of its targets, the checkpoint protein P27, results in a delay in phasic expression. Importantly, the changes in phasic expression precede any other evidence of premature differentiation, suggesting a mechanistic link between phasic expression and cellular differentiation, a link that we are currently testing. Together, our results underscore how miRNAs can integrate multiple cellular mechanisms to regulate cell fates.

Keywords: Pluripotency, microRNAs, embryonic development, embryonic stem cells, cell cycle, cell fate, developmental timing

11:25 – 11:33
CO-OCCURING PROLIFERATION AND DIFFERENTIATION BEHAVIORS DEFINE EPIDERMAL REGENERATION

Cockburn, Katie[1], Annusver, Karl[2], Kawaguchi, Kyogo[3], Kasper, Maria[2] and Greco, Valentina[1]

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Maintenance of adult tissues depends on the differentiation of stem cell populations to replace functional cell types that are continuously being shed, but how this process is orchestrated in vivo remains poorly understood. In the mammalian skin epidermis, highly proliferative stem cells reside in an underlying basal layer and differentiate upwards to replenish the outermost protective barrier of the skin. Although basal cells that have initiated differentiation are thought to have lost their proliferative capacity, how the commitment to differentiation and loss of stem cell characteristics are temporally coordinated in individual cells during this process is not known. Here we use 2-photon intravital imaging to elucidate the early steps of stem cell differentiation in the epidermis of living adult mice. Using a live reporter of differentiation status, together with single-cell sequencing to track global transcriptional changes, we find that cells commit to their differentiated fate several days before they begin to delaminate up and out of the basal layer. Surprisingly, we observe that a large proportion of these differentiation-committed cells can divide, producing daughter cells that later complete the differentiation trajectory and exit the stem cell compartment. These divisions occur in response to neighboring delamination events, suggesting that differentiating cells proliferate to preserve cell density within the stem cell compartment and not necessarily as part of an obligate transit amplifying program. To test this, we block divisions specifically within the differentiation-committed basal population and find that the tissue can adapt to maintain the size of the differentiating cell pool in their absence. Together, these results demonstrate that cell fate commitment and loss of proliferative capacity are temporally uncoupled during epidermal regeneration, with differentiating cells able to divide if needed to preserve cellularity in the stem cell compartment.

Keywords: Epidermis, Differentiation, Live imaging

11:35 – 11:43
ONCOGENIC MELANOCYTE STEM CELLS, DRIVEN BY REGENERATIVE NICHE SIGNALS, GIVE RISE TO HETEROGENEOUS MELANOMA RESEMBLING HUMAN MELANOMA

Sun, Qi[1], Katehis, Ioanna[1,2], Lee, Wendy[1,2], Mohri, Yasuaki[3], Takeo, Makoto[1,2], Lim, Chae Ho[1,2], Xu, Xiaowei[4], Myung, Peggy[5], Atit, Radhika[6], Taketo, M. Mark[7], Moubarak, Rana[8], Schober, Markus[2], Osman, Iman[1], Gay, Denise[2], Saur, Dieter[9], Nishimura, Emi[3] and Ito, Mayumi[2]

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Melanoma, the most lethal form of skin cancer, is rarely curable at its advanced stages. The early events of this disease, during which treatment would be beneficial, remain poorly elucidated. Melanocyte stem cells (McSCs) residing in the hair follicle niche have been proposed to be cells-of-origin for melanoma. To understand the cellular and molecular mechanisms regulating the initiation and progression of McSC derived melanoma, we have established a novel c-Kit-CreER-driven melanoma mouse model that enabled us to specifically target McSCs and trace their oncogenic behaviors. Coupling this model with an advanced imaging technology, we now demonstrate that oncogenic McSCs first expand in the niche and then migrate to the epidermis to form epidermal melanoma that later invade into the underlying dermis and undergo metastasis. Furthermore, normal Wnt and Endothelin signals, secreted by epithelial niche cells during hair anagen onset, can be hijacked to promote the malignant transformation of McSCs to

melanoma. Finally, transcriptional profiling revealed a strong resemblance between murine McSC-derived melanoma and human melanoma in heterogeneity and gene signatures. These results suggest that follicular McSCs can be an ultimate origin of melanoma and that follicular niche can control McSC oncogenic transformation. The similarities of McSC derived melanoma with human melanoma in epidermal to dermal progression, heterogeneity and gene expression suggest the potential utilization of this mouse model as a pre-clinical model for human melanoma.

Funding source: NIH NIAMS R01AR059768 and R01AR066022 NIH NIEHS R21ES023034 NYSTEM institutional training grant C026880 Melanoma Research Alliance 654765

Keywords: Melanocyte stem cell, Melanoma mouse model, Skin cancer

11:45 – 11:53

AN ENCOMPASSING PRECURSOR LESION ATTRACTOR STATE PRECEDES NEURAL CREST REACTIVATION IN MELANOMA

McConnell, Alicia M, Mito, Jeffrey, Weiskopf, Erika, Gosselink, Irene and Zon, Leonard

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Melanoma arises due to a malignant transformation of melanocytes, which are embryonically derived from the neural crest. We previously found that single melanocytes in BRAFV600E;p53-/- zebrafish reactivate an embryonic neural crest progenitor state and go on to form a tumor. By injecting a reporter for the melanocyte master regulator mitf, we found that neural crest re-activation occurs within a large area of adjacent adult melanocytes expressing high levels of mitf, which we term "precursor" lesions. These precursor lesions consist of hundreds of melanocytes that have dysplastic morphology with retracted dendritic processes and altered gene expression, including an increase in RHO/RAC signaling and a decrease in cell adhesion. Despite the presence of constitutively active BRAF, pERK is inactive prior to precursor lesion formation, with pERK activation first occurring in mitfhigh melanocytes. To characterize the transcriptional and chromatin states of precursor lesion and crestin+ cells, we performed scRNA-seq, bulk RNA-seq, and ATAC-seq on melanocytes sorted from different stages of initiation. We identified distinct sets of transcription factors that drive either precursor lesion formation or neural crest re-activation and validated their presence in human melanoma samples. Precursor lesions had high expression of BMP pathway members and targets, whereas WNT, NOTCH, and IRF3 signaling was high in crestin+ melanocytes. Overexpression of the BMP target ID1 resulted in an increase in the number of precursor lesions without increasing the number of neural crest reprogramming events per patch (n= 35, $p \leq 0.0001$), whereas IRF3 overexpression increased the number of crestin patches, but not precursor lesions (n=37, $p \leq 0.01$). Overexpression of the neural crest master regulator SOX10 increased both the number of precursor lesions and crestin patches (n=116, $p \leq 0.0001$). This demonstrates that there are independent regulatory programs that reprogram melanocytes to an mitfhigh state, and subsequently to a neural crest progenitor-like state. Together, our data show that melanoma initiates through transcriptionally-driven cell attractor states that define a distinct field of cells with the potential to initiate melanoma.

Funding source: Alicia McConnell, PhD was supported by a Postdoctoral Fellowship, PF-18-150-01-DDC, from the American Cancer Society.

Keywords: neural crest, melanoma, in vivo reprogramming

11:55 – 12:03

THE CIRCADIAN CLOCK GENE, BMAL1, SUPPRESSES TUMORIGENESIS BY REGULATING INTESTINAL STEM CELL SIGNALING

Karpowicz, Phillip[1], Stokes, Kyle[1], Nunes, Malika[1], Trombley, Chantelle[1], Flores, Danilo[2], Wu, Gang[2], Taleb, Zainab[1], Curran, Colin[1], Alkhateeb, Abed[3], Rueda, Luis[3], Harris, Chris[4], Love, Oliver[4] and Hogenesch, John[2]

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Circadian rhythms are daily changes in physiology driven by the circadian clock: a self-sustaining 24-hour transcriptional feedback mechanism comprising the E-Box transcription factors Clock/Bmal1 and their repressors Per/Cry. Rhythmic expression of circadian E-Box target genes causes daily rhythms in hormones, inflammation, and metabolism. A particular area of interest has been the role of circadian rhythms in cancer, a disease that is elevated in shift-workers subjected to circadian disruption. However, in animal models, circadian rhythms have been shown to both promote or inhibit cancer, and certain studies have been questioned due to genetic background effects. Intestinal stem cells reside in the intestinal epithelium where they are responsible for tissue maintenance throughout adulthood. Intestinal stem cells are intimately connected to colorectal cancer; the deregulation of the Wnt signaling pathway required in intestinal stem cells also drives tumorigenesis. It is not known whether the loss of circadian rhythms has any consequences for intestinal stem cells. We tested the role of Bmal1 in stem cells and tumorigenesis using the APCmin model of colorectal can-

cer, with mice bred on the same genetic background. Adenomas have heterogenous and weak clock function that is not self-sustaining, suggesting that loss of circadian rhythms is a feature of early tumours. Bmal1 mutants and intestine-conditional Bmal1 mutants both exhibit increased tumorigenesis in an inflammation-dependent manner. Intestinal organoids derived from wildtype and Bmal1 mutants, in normal versus APCmin backgrounds, show that Bmal1 inhibits intestinal stem cell self-renewal and growth. A genome-wide analysis of self-sustaining clock target genes reveals that Bmal1 regulates regeneration through the regulation of intestinal stem cell signaling pathways. The Hippo component, Tead4, is rhythmic, and APCmin Bmal1 mutant adenomas exhibit higher Hippo activity but lower Wnt activity. Our data identify the circadian clock as a regulator of intestinal stem cell regenerative pathways that impact colorectal tumorigenesis, and we provide a resource of circadian target genes for organoids in regenerative medicine.

Funding source: Canadian Institutes of Health Research; Ontario Institute for Regenerative Medicine; Crohn's and Colitis Foundation of Canada

Keywords: intestinal stem cells, circadian, intestinal organoid

12:05 – 12:13
AQP5 ENRICHES FOR STEM CELLS AND CANCER ORIGINS IN THE DISTAL STOMACH

Barker, Nick

Department of Epithelial Stem Cells, Institute of Medical Biology, Singapore

Lgr5 marks resident adult epithelial stem cells at the gland base in the mouse pyloric stomach, but the identity of the equivalent human stem cell population remains elusive due to a lack of surface markers facilitating its prospective isolation and validation. Lgr5+ intestinal stem cells are major sources of cancer following Wnt pathway hyperactivation in mice. However, the contribution of pyloric Lgr5+ stem cells to gastric cancer following Wnt pathway dysregulation, a frequent event in human gastric cancer, is unknown. Here, we employed comparative profiling of Lgr5+ stem cell populations along the mouse gastrointestinal tract to identify, then functionally validate the membrane protein AQP5 as a marker that enriches for mouse and human adult pyloric stem cells. We show that stem cells within the Aqp5+ compartment are a source of Wnt-driven, invasive gastric cancer in vivo using new Aqp5-CreERT2 mouse models. Additionally, tumour-resident Aqp5+ cells can selectively initiate organoid growth in vitro, indicating that this population harbours potential cancer stem cells. In human gastric cancer, AQP5 is robustly expressed in primary and metastases of intestinal and diffuse subtypes, often displaying altered cellular localization compared to healthy tissue. These new markers and mouse models will be an invaluable resource for deciphering early gastric cancer formation and for isolating and characterizing human stomach stem cells as a prerequisite to potentially harnessing their regenerative medicine potential in the clinic.

Funding source: A-STAR

Keywords: AQP5, Gastric stem cell, Gastric cancer

12:15 – 12:30
LUNG EPITHELIAL CELL ORGANOIDS MODEL CELL-CELL INTERACTIONS IN LUNG DISEASE AND LUNG CANCER

Kim, Carla

Boston Children's Hospital, Boston, MA, USA

A major focus of our group has been to develop tools to characterize progenitor cells in the adult lung and in lung cancer. We created three-dimensional co-culture organoid systems that have begun to define cell-cell crosstalk between epithelial progenitors and other supporting cell types in the lung. We can now model the formation of airway- and alveolar-like structures from lung progenitor cells, and we have a platform to understand differentiation control at the molecular level. Our ongoing research program seeks to further develop lung organoids to interrogate the molecular underpinnings of cell-cell interactions between epithelial progenitor cells and their environment in homeostasis and in diseased lung. We are determining the signals through which epithelial progenitors are regulated by mesenchymal cells during lung injury response and repair. We have also developed a new organoid tool to rapidly recapitulate lung cancer progression in vitro and a window into the transcriptional changes that immediately follow oncogenic KRAS expression in epithelial cells, revealing candidate targets for early intervention of lung cancer and other KRAS-driven cancers. Finally, we are also developing a lung progenitor cell transplantation assay, a critical need in the lung community for the study of progenitors and for regenerative medicine approaches.

Keywords: lung cancer, organoids, progenitor cells

FRIDAY, JUNE 26, 11:00 – 12:45

CONCURRENT - TISSUE STEM CELLS AND REGENERATION: NEURAL

11:05 – 11:20
MECHANISMS OF NEUROGENESIS-INDUCED REMODELING IN THE ADULT AND AGING HIPPOCAMPUS

Schinder, Alejandro

Leloir Institute, Buenos Aires, Argentina

Neural stem cells in the dentate gyrus of the mammalian hippocampus generate neurons during the entire

life. This ongoing process begins in the embryo and decreases with time but it never shuts down. The pre-existing working circuits maintain their function while simultaneously integrate newly generated neurons that grow, connect and, ultimately, transform the manner in which signals are processed. There are a myriad of developmental and homeostatic mechanisms acting in concert to coordinate such a complex process in a manner that is adaptive for behavior, while minimizing the damage that might arise from wrong connectivity. New neurons navigate through distinctive functional properties as they develop, undergoing periods of enhanced excitability and activity-dependent plasticity that confer unique properties to the local network. This dynamic functionality is determined by both the intrinsic properties of new neurons and by the nature of their input and output synaptic connections. Synaptogenesis follows different timing and rules for excitatory and inhibitory networks; the manner in which this map is assembled is heavily sensitive to network activity and it is shaped by behavior and aging. The preexisting network is also required to deploy mechanisms to support the incorporation of new neurons. We are deconstructing the rules guiding these processes to reveal their logic and their molecular players. A thorough understanding of these fundamental mechanisms will contribute to develop novel strategies for regeneration and repair in non neurogenic areas of the brain.

Keywords: neural stem cells, brain repair, regeneration, dentate gyrus

11:25 – 11:33
FUNCTIONAL CHARACTERIZATION OF A NOVEL GENE IN THE ANTIGEN PRESENTATION PATHWAY ALLOWS SPECIFIC IMMUNOTHERAPEUTIC TARGETING OF GLIOBLASTOMA STEM CELLS

Kidwell, Reilly L.[1], Li, Xiqing[2] and Rich, Jeremy[1]

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Glioblastoma is the most prevalent and aggressive primary brain tumor in adults, with a median survival of approximately one year. Glioma stem cells (GSCs) drive poor clinical outcomes by contributing to tumor initiation, growth, maintenance, and immune evasion. Immune checkpoints (e.g. PD-1/PD-L1) modulate anti-tumor response to promote self-tolerance. GSCs and other cancer cells co-opt these mechanisms to evade immune destruction. Immune checkpoint inhibition has been successful in treating several solid tumors, but has largely failed to improve outcomes in glioblastoma. Here, we interrogated functional variants in immune response modulators specific to GSCs. RNA-seq data from GSCs and matched differentiated glioma cells (DGCs) identified a novel gene in the antigen presentation pathway, which is specifically upregulated in GSCs. Further characterization revealed that this upregulation was associated with decreased immune cell infiltration into tumor tissue, and reduced CD8+ T cell-mediated GSC lysis. shRNA targeting increased sensitivity to T cell killing, with increased cell lysis compared to both controls and anti-PD-L1 treatment. We next investigated peptide binding targeted to MHC class I. Control GSCs exhibited minimal peptide binding (decreased antigen presentation), whereas gene knockdown reversed this interaction, resulting in increased binding (improved antigen recognition). In addition, GSCs increased induction of the B7 ligand CD276 (a known inhibitor of T cell activation) compared to DGCs. CRISPR-Cas9 mediated gene knockout diminished CD276 in GSCs at the protein level. Finally, mice bearing GSC xenografts transduced with targeting shRNA displayed decreased tumor burden, demonstrating facilitation of T cell-mediated tumor destruction. shRNA knockdown also prolonged overall survival compared to both controls and mice given anti-PD-L1 therapy. Stratification of glioblastoma patient data revealed a correlation between high gene expression and poor clinical prognosis/decreased overall survival, supporting the clinical relevance of this discovery. This work suggests one explanation for the observed immunotherapeutic resistance of glioblastoma, and illuminates a therapeutic vulnerability unique to glioblastoma cancer stem cells.

Funding source: This work was partially supported by the National Institutes of Health, Grant TL1TR001443 of CTSA funding. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Keywords: Immune Checkpoint Inhibition, Antigen Presentation Pathway, Glioblastoma

11:35 – 11:43
REGENERATION OF FUNCTIONAL RETINAL GANGLION CELLS BY NEURONAL IDENTITY REPROGRAMMING

Liu, Hongjun, Wei, Xiaohu, Zhang, Zhenhao and Qiao, Na

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

Degeneration of retinal ganglion cells (RGCs) and their axons underlies vision loss in glaucoma and various optic neuropathies. There are currently no treatments available to restore lost vision in patients affected by these diseases. Regenerating RGCs and reconnecting the retina to the brain, using endogenous cellular sources, represent an ideal therapeutic strategy, and hold great promise for vision restoration in these patients. However, unlike lower vertebrates, mammals including humans do not have a reservoir of retinal stem/progenitor cells poised to produce new neurons in adulthood. Here, we regenerated RGCs in adult mice by direct lineage reprogramming of other retinal neurons. By combinatorial expression of transcription

factors essential for RGC specification, we successfully converted retinal amacrine and displaced amacrine interneurons into RGCs. Regenerated RGCs project axons into the optic nerve, and extend axonal terminals into discrete retinorecipient areas in the brain. They can convey visual information to the brain in response to visual stimulation, and are able to transmit electrical signals to postsynaptic brain neurons, in both normal animals and in an animal model of glaucoma where original RGCs have been damaged by elevated intraocular pressure. The generation of functional RGCs in adult mammals points to a new therapeutic strategy for vision restoration in patients.

Keywords: Retina, Retinal ganglion cell, Regeneration

11:45 – 11:53
HUMANIZED MODELS FOR ASSESSING IN VIVO NEURAL CONVERSION

Hoban, Deirdre B.[1], Nolbrant, Sara[2], Giacomoni, Jessica[2], Bruzelius, Andreas[3], Birtele, Marcella[2], Rylander Ottosson, Daniella[3], Goldman, Steven[4] and Parmar, Malin[2]

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Direct in vivo conversion is emerging as a novel therapeutic strategy for brain repair. Glial progenitor cells (GPCs) are an exciting target for direct neuronal conversion since these cells are found in abundance throughout the adult brain and can renew. To date, in vivo conversion studies have been conducted using resident rodent glia and the question whether human glia can be converted into neurons within the adult brain remains to be resolved. In this study, we have therefore established a novel rat-human glial chimeric animal model whereby human glia are transplanted into the rat brain, uniquely allowing us to study in vivo conversion of human glia into clinically relevant neurons in the rodent brain. The model is established using human glial progenitor cells (hGPCs) generated from human embryonic stem cells (hESCs). hGPCs are transplanted in the rodent brain, both to adult animals maintained under daily immunosuppression and nude athymic animals. Transplanted hGPCs survive, proliferate and migrate throughout the host brain and express markers of astrocytes and oligodendrocyte progenitors, but no neuronal markers. In in vitro studies using hGPCs, we have identified conversion factor combinations that efficiently convert hGPCs into midbrain dopaminergic (mDA) neurons, the cell type that selectively degenerates in Parkinson's disease (PD). The induced neurons express markers characteristic of DA neurons and become functionally mature. Studies assessing if these conversion factor combinations can also convert hGPCs in vivo are ongoing. The results of these studies will enhance our understanding of how cell fate specification and reprogramming operates during in vivo neural conversion and define the key factors that control this process, allowing for future studies for conversion of human glia to neurons in a model of PD.

Funding source: The New York Stem Cell Foundation (NYSCF), Swedish Research Council (2016-00873), Swedish Parkinson Foundation (Parkinsonfonden), and Knut and Alice Wallenberg Stiftelse (KAW 2018-0040). M.P is a NYSCF Robertson Investigator.

Keywords: neuroregeneration, in vivo conversion, human glial progenitor cells

11:55 – 12:03
ELEVATED HERV-K(HML-2) EXPRESSION NEGATIVELY IMPACTS HUMAN CORTICAL DEVELOPMENT

Vincendeau, Michelle[1], Padmanabhan Nair, Vidya[1], Liu, Hengyuan[2], Ciceri, Gabriele[3], Jungverdorben, Johannes[3], Frishman, Goar[4], Tchieu, Jason[3], Cederquist, Gustav[3], Rothenaigner, Ina[5], Schorpp, Kenji[5], Ruepp, Andreas[4], Mayer, Jens[6], Hadian, Kamyar[5], Frishman, Dmitrij[2] and Studer, Lorenz[3]

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Currently, one of the major challenges towards deciphering the blueprint of life is to understand how functional elements of the genome influence key biological processes. However, the functions of large parts of the human genome including human endogenous retroviral (HERV) elements remain elusive. Recent studies have started to investigate the regulation of retrotransposons including HERVs, by manipulating their cellular regulators such as KAP1. However, as KAP1 regulates the dynamic organization of chromatin structure and hence almost all transposable elements, it is not possible to dissect whether the identified changes in gene regulatory networks are caused by HERVs or other transposable elements. Interestingly, results from our work demonstrate that HERV groups are differentially expressed during the course of neuronal differentiation into distinct neuronal subtypes. Therefore, to unravel the functional contribution of HERV-K(HML-2)

within neuronal development, we developed a novel approach to directly manipulate expression of specific HERV groups in human embryonic stem cells and subsequently differentiate these into neurons. Here, we applied the CRISPRactivation system to target multiple LTRs of HERV-K(HML-2) and efficiently elevate transcription of this HERV group. Excitingly, HERV-K(HML-2) overexpressing cells showed a clear reduction in the neuronal marker MAP2 after differentiation into cortical neurons and almost completely lost their neuronal functionality. In contrast, transcriptional activation of HERV-K(HML-2) LTRs during differentiation into dopaminergic neurons had no effects on MAP2 levels. HERV-K(HML-2) transcriptional activation also affected the expression of specific cellular genes implicated in neurodegeneration supporting the discovery that HERV-K(HML-2) transcriptional activation negatively impacts cortical development. Our novel findings shed light on how differences in HERV transcriptional activity can influence differentiation of pluripotent stem cells into distinct neuronal cell types. In the future, our work will help to elucidate how de-regulation of these elements may drive neurodegenerative disorders.

Keywords: Human Cortical Development, Human Endogenous Retroviruses, Neurodegeneration

12:05 – 12:13

TISSUE WIDE COORDINATION OF CORNEAL HOMEOSTASIS REVEALED AT THE SINGLE STEM CELL LEVEL BY 2-PHOTON LIVE IMAGING

Rompolas, Panteleimon and Farrelly, Olivia

Dermatology, University of Pennsylvania, USA

The cornea consists of a stratified epithelium that lines the outermost surface of the eye. Even though the cornea serves a similar barrier function to the skin epidermis, it is characterized by unique epithelial cell behavior and hierarchical stem cell organization. A significant challenge in elucidating the cellular mechanism of corneal homeostasis and regeneration is accessibility to the live limbal niche where the bone-fide stem cells are believed to reside. Here, we visualize the stem cell niche in the live mouse eye and track the activity of single stem cells in their native environment by 2-photon microscopy. We identify previously unknown niche compartments and show that long implicated slow-cycling cells form separate lineages in the outer limbus, with only local clonal dynamics. Instead, we find distinct stem cells in the pericorneal limbus to be required for corneal regeneration. Unbiased photo-labeling captures the stem cell progeny exiting the niche, then moving centripetally in unison before undergoing terminal differentiation. This study demonstrates how a compartmentalized stem cell organization coordinates tissue wide homeostatic maintenance of the eye surface epithelium.

12:15 – 12:30

INITIATING A PHASE I /IIA CLINICAL TRIAL FOR AN AUTOLOGOUS IPS CELL THERAPY FOR MACULAR DEGENERATION: FROM BENCH-TO-BEDSIDE

Bharti, Kapil

National Eye Institute, NIH, Bethesda, MD, USA

Induced pluripotent stem cells (iPSCs) provide immune-compatible autologous replacement tissue for the treatment of potentially all degenerative diseases. Age-related macular degeneration (AMD) is caused by the progressive degeneration of retinal pigment epithelium (RPE), a monolayer tissue that maintains photoreceptor function and survival. Combining developmental biology with tissue engineering we developed clinical-grade iPSC-derived RPE-patch on a biodegradable scaffold. This patch performs key RPE functions like photoreceptor phagocytosis, water transport, and polarized cytokine secretion. We confirmed the safety of this patch in immune-compromised rat models and confirmed its efficacy in a laser model of RPE injury. A phase I/IIa Investigational New Drug (IND)-application for iPSC-derived ocular tissue to treat AMD was recently cleared by the FDA. This Phase I/IIa clinical trial will test safety, feasibility, and integration of an autologous iPSC-derived RPE-patch in twelve advanced AMD patients. This work is helping leverage other similar autologous cell therapies in various other degenerative diseases.

Keywords: Cell therapy, retinal degeneration

FRIDAY, JUNE 26, 16:00 – 18:00

PLENARY IV: DISSECTING ORGANOGENESIS

16:05 – 16:20

NEW DIRECTIONS FOR HUMAN BRAIN ORGANOIDS

Lancaster, Madeline

Medical Research Council Laboratory of Molecular Biology, Cambridge, UK

The human brain exhibits a number of unique characteristics, such as dramatic size expansion and variation in relative abundance of specific neuron populations. In an effort to better understand human brain development, we developed an in vitro human model system, called cerebral organoids. These 3D tissues are generated from human pluripotent stem cells through directed differentiation and a supportive 3D microenvironment to generate organoids with the stereotypic organization of the early human fetal brain. We have now extended these methods to model later events in neurodevelopment, including neuronal migration and positioning, axon guidance, and neuronal maturation.

These newer approaches can model CNS tract formation with the generation of neural circuits, and even functional output through a motor circuit to modulate and activate muscle contractions. Finally, we are continuing to advance these methods and have devised choroid plexus organoids as a model of the human blood-CSF barrier for testing CNS penetrance. These methods are now allowing us to tackle questions previously impossible with more traditional approaches.

Keywords: Organoids, brain development, blood-brain-barrier

16:25 – 16:40
HUMAN TIME VS. MOUSE TIME WITH RECAPITULATED SYSTEMS

Ebisuya, Miki

EMBL Barcelona, Spain

Different species have different tempos of embryonic development: larger animals tend to grow more slowly than smaller animals. My group has been trying to understand the molecular basis of interspecies differences in developmental time by using in vitro segmentation clock as a model system. The segmentation clock is the oscillatory gene expressions that regulate the timing of body segment formation from presomitic mesoderm (PSM) during embryogenesis. We have recently succeeded in inducing PSM from both human iPS cells and mouse ES cells, recapitulating the oscillation and traveling wave of segmentation clock in vitro. Interestingly, the oscillation period of human segmentation clock was 5-6 hours while that of mouse was 2-3 hours. Taking advantage of our in vitro system, we measured several biochemical reaction parameters of the core gene of the oscillation mechanism, Hes7, finding out that the degradation and production processes of Hes7 are 2-3 times slower in human PSM cells compared to mouse cells. Our mathematical model quantitatively explained how the slower biochemical reactions in human cells give rise to the longer oscillation period in the human segmentation clock.

Keywords: segmentation clock, interspecies difference, presomitic mesoderm

16:45 – 17:00
LUNG ORGANOIDS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

Snoeck, Hans

Columbia University Medical Center, New York, NY, USA

Organoids are in vitro three-dimensional (3D) organ-like cultures derived from pluripotent or adult stem cells that at least partially recapitulate anatomical and functional features of in vivo tissues. Organoid-based models have proven valuable for studying the dynamics of organ development and to model disease. We have developed a 3D organoid model of human lung development derived from human pluripotent stem cells (hPSCs) that allowed the modeling of pulmonary fibrosis associated with Hermansky-Pudlak syndrome as well as infection with human respiratory viruses. Using this model, we identified interleukin-11 (IL-11) as a factor necessary and sufficient for the induction of fibrosis. As these organoids were staged at the second trimester of human gestation, a stage where multipotential progenitors are present at the tips of the branching airway tree, we attempted to detect, isolate and expand such cells from the organoids. These studies led to a strategy isolate and expand putative human distal lung progenitors (pDTP). pDTPs could engraft and repopulate airway and distal lung of immunodeficient NSG mice after bleomycininjury. The availability of an expandable and engraftable lung progenitor population might open avenues for the treatment of patients afflicted with lung diseases that would theoretically be amenable to stem cell therapies, including a fraction of patients with cystic fibrosis, infants with respiratory failure caused by surfactant deficiencies and victims of severe inhalation injury. Efficiency of engraftment and identification of an appropriate conditioning regimen of the recipient remain major challenges however.

Keywords: lung, pluripotent stem cells, regeneration, fibrosis, organoids

17:05 – 17:13
KNEE IN A DISH: SPECIFICATION OF ESC-DERIVED JOINT PROGENITORS WITH DIFFERENTIAL JOINT TISSUE POTENTIAL

Craft, April M.[1], Raj, Suyash[1], Cutia, Tom[1], Menghini, Stefano[1], Young, Mariel[2], Ramos-Mucci, Lorenzo[3], Poulet, Blandine[3] and Capellini, Terence[2]

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Joint tissues such as the articular cartilage and ligaments fail to heal properly following injury, accelerating joint degeneration, and contributing to the high prevalence of osteoarthritis in the general population. Strategies for regenerating injured joint tissues would greatly benefit from a more complete understanding of the hierarchy of cells that give rise to these tissues during embryogenesis. We thus sought to develop an ESC-based model by which we could investigate the signaling pathways responsible for these processes, effectively decoupling limb bud morphogenesis and cell fate determination. We generated several mouse and human ESC fluorescent reporter lines for joint progenitor cells (Gdf5), joint tissue surface cells (Prg4), and cartilage (Col2a1), which broadly represent the majority of joint tissues. By modifying our existing differentiation approach for cartilage, we identified important signaling pathways (e.g., TGFb, Wnt and MAPK) that enrich for joint progenitors and skeletal lineages that are

transcriptionally similar to those found in mouse embryonic limb buds and adult joint tissues using single cell RNA seq. We found TGFb to be both necessary and sufficient to induce Gdf5+ cells, consistent with previous reports of a key role for this pathway in joint development. Despite the presence of Wnt ligands in the early joint space, we found that inhibition of Wnt greatly increased the percentage of Gdf5+ cells in a dose-dependent manner. Inhibition of MAPK signaling likewise resulted in an increase in Gdf5+ cells, which emerged several days earlier than TGFb alone. Interestingly, Wnt-inhibited cells differentiated into articular cartilage, while cells with transcriptional similarities to ligaments and synovial tissue were enriched upon MAPK inhibition. This temporal specification of Gdf5+ cells linked to differential joint tissue fates is consistent with in vivo studies that suggest early Gdf5-lineage cells contribute preferentially to ligaments, while cells recruited later are incorporated in the articular cartilage. Our work represents the first report of in vitro-derived joint progenitor cells, and the establishment of a model system by which joint tissue specification can be illuminated towards the goal of generating specialized joint tissues for therapeutic applications.

Keywords: directed differentiation, joint cartilage, pluripotent stem cells

17:15 – 17:23
AXIAL ELONGATION OF CAUDALIZED HUMAN PLURIPOTENT STEM CELL ORGANOIDS MIMICS NEURAL TUBE DEVELOPMENT

McDevitt, Todd, Libby, Ashley, Joy, David, Elder, Nicholas, Bulger, Emily and Krakora, Martina

Gladstone Institutes, University of California San Francisco, CA, USA

During vertebrate development, the embryo elongates along its anterior-posterior axis as the neural tube is patterned to form the spinal cord. However, neural tube development in mammals occurs post-implantation, making it technically challenging to study and thus, restricting research to model organisms. Here we report a human iPSC organoid model of axial extension that recapitulates many of the morphogenic features of early neural tube development. A modified protocol for spinal interneuron differentiation performed in 3D suspension culture resulted in aggregates that spontaneously developed elongating protrusions and displayed internal epithelial sheets that resembled tube like structures. Immunohistochemical analysis revealed that the extending aggregates contained cells that co-expressed brachyury(T) and SOX2, suggesting the emergence of neuromesodermal progenitors (NMPs), which contribute to neural tube and paraxial mesoderm formation in vivo. Increasing the concentration of the Wnt small molecule agonist (CHIR) from 2mM to 6mM during the first 5 days of differentiation increased the number of SOX2(+)/T(+) cells and resulted in more pronounced axial extension. Single cell RNA sequencing during elongation revealed increased neural progenitor populations in extending aggregates in addition to a MEOX1(+) population, a marker of paraxial mesoderm differentiation. Extending aggregates displayed regionalized HOX genes, with hindbrain (HOXB1) expressed at the base of extensions, and brachial (HOXC6) and thoracic (HOXB9) gene expression in the extended regions. In order to further modulate dorsoventral cell fate patterning, the BMP inhibitors Noggin and Chordin were transiently silenced in human iPSCs using inducible CRISPR interference prior to elongation. Knockdown of BMP inhibitors resulted in increased expression of gene markers for dorsal neuronal fates (i.e. V0 andV1 interneurons) and altered the kinetics of axial extension. By recapitulating many of the morphologic and cell fate decision events associated with axial elongation, this novel organoid model of neural tube development can permit interrogation of the multicellular interactions that enable the development of the human nervous system.

Funding source: CIRM

Keywords: neural tube, axial elongation, neuromesoderm progenitors

17:30 – 17:50
ISSCR MOMENTUM AWARD LECTURE: MECHANISM AND IN VITRO RECONSTITUTION OF MAMMALIAN GERM-CELL DEVELOPMENT

Saitou, Mitinori

Institute for the Advanced Study of Human Biology, Kyoto University, Kyoto, Japan

The germ-cell lineage ensures the creation of new individuals, perpetuating/diversifying the genetic and epigenetic information across the generations. We have been investigating the mechanism for germ-cell development, and have shown that mouse embryonic stem cells (mESCs)/induced pluripotent stem cells (miPSCs) are induced into primordial germ cell-like cells (mPGCLCs) with a robust capacity both for spermatogenesis and oogenesis and for contributing to offspring. These works have served as a basis for exploring the mechanism of key events during germ-cell development such as epigenetic reprogramming, sex determination, and meiotic entry. By investigating the development of cynomolgus monkeys, we have defined a developmental coordinate of the spectrum of pluripotency among mice, monkeys, and humans, and have identified the origin of the germ-cell lineage in cynomolgus monkeys in the amnion. Accordingly, we have succeeded in inducing human iPSCs (hiPSCs) with a primed pluripotency into human PGCLCs (hPGCLCs) with properties of human early PGCs. Furthermore, we have shown that hPGCLCs can be induced into oogonia/early oocytes with appropriate epigenetic reprogramming and a precursory state for meiotic entry in xenogenetic reconsti-

tuted ovaries. Here, I would like to discuss a brief history of our work and our latest findings regarding the mechanism of and in vitro reconstitution of mammalian germ-cell development.

Keywords: Germ cell development, Primordial germ cells (PGCs), Primordial germ cell-like cells (PGCLCs)

FRIDAY, JUNE 26, 20:00 – 21:45

CONCURRENT - CELLULAR IDENTITY: EARLY DEVELOPMENT AND PLURIPOTENCY

20:05 – 20:20
NUCLEAR CONDENSATES IN GENE CONTROL AND CELL IDENTITY

Young, Richard

Whitehead Institute for Biomedical Research, Cambridge, MA, USA

Recent studies have shown that the diverse functions of the nucleus - DNA synthesis, DNA damage repair, transcription, splicing and nuclear transport - all occur within bimolecular condensates, which are membrane-less bodies that exhibit dynamic liquid-like behavior. Condensates are believed to be phase-separated bodies that compartmentalize and concentrate protein and RNA molecules that participate in shared functions. I will discuss evidence that key regulators of euchromatin and heterochromatin operate under the principles of phase separation, explain how these contribute to the control of cell identity and development, and note where condensates become dysfunctional in disease. I will also discuss how this new understanding of biological regulation is revolutionizing the study of biomedical problems and the development of new therapeutics.

Keywords: nucleus, transcription, cell identity, condensates, phase separation, therapeutics

20:25 – 20:33
ESSENTIAL GENES FOR DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS INTO ALL THREE GERM LAYERS

Yilmaz, Atilgan, Braverman-Gross, Carmel, Bialer-Tsypin, Anna and Benvenisty, Nissim

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

Pluripotent stem cells can differentiate into all three embryonic germ layers, a process that is orchestrated by the dissolution of pluripotency network and activation of pro-differentiation pathways. Yet, the genes essential for these drastic cell fate transitions in human remain elusive. Recently, we have derived haploid human pluripotent stem cells (hPSCs) and generated a loss-of-function library in these cells targeting 18,000 coding genes utilizing CRISPR/Cas9 technology. This library enabled us to define the genes essential for the normal growth and survival of undifferentiated hPSCs. Here, we set out to map the essential genes for the differentiation of hPSCs into all three embryonic germ layers, by using the loss-of-function library in haploid hPSCs. Through the analysis of essential and inhibitory genes for the differentiation of hPSCs into ectoderm, mesoderm and endoderm we defined the essentialome of each germ layer separately. Furthermore, we could also identify the genes that are common for the transition from pluripotency stage into all three embryonic germ layer fates and hence potentially regulate the exit from pluripotency in human. Although we have identified unique sets of transcription factors that have an essential role in the differentiation of hPSCs into each specific germ layer, the group of common essential genes for differentiation into all germ layers was devoid of transcription factors. Interestingly, this group was enriched by genes that are localized to the Golgi apparatus and the endoplasmic reticulum and regulate membrane and secreted molecules, highlighting the key role of signaling events during these dynamic cell state transitions. In addition to the essential genes for hPSC differentiation, we have also analyzed common inhibitory genes for this process across germ layers and identified members of a specific signaling pathway. Overall, our work sheds light on the gene networks regulating early gastrulation events in human by defining essential drivers of specific embryonic germ layer fates and essential and inhibitory genes for the exit from pluripotency.

Keywords: Germ layer differentiation, Human embryonic stem cells, Genome-wide CRISPR screens

20:35 – 20:43
TWO 129-STRAIN LOCI ARE SUFFICIENT TO CONFER SUSCEPTIBILITY TO SPONTANEOUS TERATOMAS IN MICE

Nicholls, Peter K., Bellott, Daniel, Skaletsky, Helen, Cho, Ting-Jan and Page, David

Whitehead Institute, Cambridge, MA, USA

The 129-strain of mice is uniquely capable of producing spontaneous gonadal teratomas, a tumor arising from primordial germ cells (PGCs). This unique susceptibility laid the foundation for several important advances in biology, including the first derivation of pluripotent stem cells (both embryonal carcinoma [EC], and embryonic stem [ES] cells), and later, for the generation of mutant mice by targeted mutagenesis. Despite the tremendous legacy of the 129-strain to modern biology and medicine, the genetic basis for spontaneous teratomas arising from the germline – which facilitated each of these technical accomplishments – remains unknown. To resolve this long-standing question, we conducted a sensitized gene mapping study in crosses

of C57BL/6N and 129S4/SvJae mice. Through analyses of 575 mice, we find that teratoma susceptibility is a recessive trait, which can be transferred between mouse strains to enable the development of spontaneous teratomas. Through a genome-wide approach, we identify two unlinked loci derived from the 129-strain of mice on Chromosomes 13 and 18 that, together, are sufficient to confer teratoma susceptibility to the B6 strain. In males homozygous for 129-strain alleles at both loci, PGCs migrate to the gonad, but bypass otherwise lethal checkpoints. Our genetic studies resolve the long-standing question of the 129-strain's unique capacity for spontaneous teratoma production, and implicate an escape from apoptosis in the improved derivation and germline contribution of pluripotent cell lines from the 129-strain. We present a model whereby spontaneous gonadal teratomas arise from PGCs that colonize the embryonic gonad but fail to undertake germ cell commitment, escape from an apoptotic fate, and subsequently initiate tumorigenesis.

Funding source: This work is supported by the HHMI, and by a Frontier Program award from the Koch Institute at MIT. PKN is a recipient of a Hope Funds for Cancer Research Fellowship, and an Early Career Fellowship (NH&MRC, Australia).

Keywords: Teratoma, Gene mapping, Germline

20:45 – 20:53

ANTAGONISM BETWEEN YOUNG AND OLD TRANSPOSABLE ELEMENTS MEDIATES INTRA-ORGANISM SELECTION IN THE EARLY HUMAN EMBRYO

Izsvak, Zsuzsanna[1], Cortes, Jose L.[2], Widmann, Thomas[2], Kondrashkina, Aleksandra[1], Römer, Christine[1], Wang, Jichang[1], Garcia-Perez, Jose[2], Hurst, Laurence[3] and Singh, Manvendra[1]

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While much of our genome is relic of inactive transposable elements (TEs), some Young TEs remain transpositionally active. Given Young TE activity in the early human embryo, we ask whether it can filter TE-damaged cells. We uncover a cell-type that segregates shortly after embryonic gene activation that expresses Young TEs, DNA-damage response genes, apoptotic factors and no lineage-specific markers. Their ontogenetic sisters, defining the human inner cell mass (ICM), by contrast express lineage-specific genes, have a developmental future and don't express mutagenic TEs. ICM and its epiblast (EPI) derivative do, however, express phylogenetically old non-transposing TEs, one of which, HERVH, not only enables pluripotency-specification but suppresses the younger TEs. We conclude that there is a race to inactivate Young TEs and that Old TEs have been recruited to aid this suppression, failure of which can lead to cell death. This has implications for possible causes of infertility. The active HERVH-based transcription is a requirement to safeguard genome stability of human pluripotent stem cells (hPSCs). Consequently, PSCs with HERVH knocked-down have transpositional activity, DNA-damage response and apoptosis. Intriguingly, using HERVHhi(gh) PSCs allows an unprecedently high improvement of generating viable human-animal interspecies chimeras. The human HERVHhi cells are tracked and could be detected both in the placenta and in various organs, but not in the gonads of the chimeric embryo.

Keywords: Early embryogenesis, Transposable elements, Human specificity

20:55 – 21:03

PRIMATE EMBRYO PROFILING FROM ZYGOTE TO GASTRULATION DEMARCATES PLURIPOTENT STATES IN VITRO

Slatery, Erin M.[1], Bergmann, Sophie[1], Penfold, Chris[1], Siriwardena, Dylan[1], Clark, Stephen[2], Drummer, Charis[3], Reik, Wolf[4], Behr, Rudiger[3] and Boroviak, Thorsten[1]

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Pluripotent cells of the early embryo have the remarkable ability to form all somatic tissues and the germ line. Most of our knowledge about mammalian development is based on studies in mouse. However, human and non-human primate embryogenesis radically diverges from the canonical mouse model. Primates form a flat embryonic disc, segregate amnion directly after implantation, give rise to a secondary yolk sac and specify extraembryonic mesoderm prior to gastrulation. Despite the tremendous opportunities for biomedical research, little is known about the molecular mechanisms of primate embryogenesis in vivo. Here, we illuminate early marmoset postimplantation development by spatial embryo profiling. Laser capture microdissection combined with Smart-Seq2 allowed us to track the physical location of each sample. We virtually reconstructed the primate embryo to render genome-wide expression patterns before and after primitive streak formation. Moreover, we identify transcriptional signatures and examine developmental trajectories for embryonic and extraembryonic lineages. Integration of single-cell RNA-seq datasets of marmoset preimplantation embryos enabled us to generate a blueprint from zygote to late gastrulation in a non-human primate. We employed this transcriptional reference map to interrogate the developmental state of marmoset embryonic stem cells (ESCs) by single-cell RNA-seq. Conventional (primed) marmoset

ESCs interclustered with the postimplantation embryonic disc, while somatic tissues as well as neural cells in vitro clustered away from both embryonic and extraembryonic lineages. To further probe the system, we reset marmoset ESCs to an earlier (naïve) developmental state. Remarkably, the transcriptome of naïve marmoset ESCs corresponded to the preimplantation epiblast. Our embryo profiling approach provides unprecedented insights into the molecular features of primate embryogenesis and a robust platform to assess developmental states in vitro.

Funding source: The research presented here is supported by the Wellcome Trust Sir Henry Dale Fellowship, the Centre for Trophoblast Research, and the Newton Trust.

Keywords: Spatial transcriptomics, Pluripotency, Nonhuman primate

21:05 – 21:13

APEX2-BASED SPATIAL PROTEOMIC ANALYSIS OF A HUMAN PLURIPOTENT STEM CELL-DERIVED EPIBLAST-LIKE MODEL

Wang, Sicong[1], Cortez, Chari[2], Lin, Chien-Wei[3], Johnson, Craig[2], Townshend, Ryan[2], Basrur, Venkatesha[4], Nesvizhskii, Alexey[5], Zou, Peng[6], Duncan, Mara[2], Fu, Jianping[7], Gumucio, Deborah[2] and Taniguchi, Kenichiro[1]

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The pro-amniotic cavity of the implanting human embryo forms from an unpolarized mass of epithelial pluripotent cells. This process requires apico-basal polarization and radial organization, followed by central lumen expansion. To gain global mechanistic insights into these critical but poorly understood early steps in human embryological development, we developed a spatially targeted approach to compare the proteomes of apical vs. basolateral membrane territories in a human pluripotent stem cell (hPSC)-derived epiblast-like model (hPSC-cyst). We employed APEX2-based proximity biotinylation, coupled with quantitative mass spectrometry and a downstream bioinformatics pipeline tailored for spatial proteomics datasets. This analysis confirmed the location of several established apical (e.g., NHERF1, MOESIN, RADIXIN) and basolateral (e.g., CDH1, ITGB1, CTNNB1) proteins, and revealed several proteins that were not previously known to be localized to specific membrane sub-domains. Interestingly, the bioinformatic analysis revealed dozens of candidates for critical cell trafficking regulators that have not been directly implicated in regulating cell polarization and epithelial morphogenesis. Further experiments demonstrated the requirement of selected apically enriched proteins (EZR, SNX27, RAB35, BASP1, AP1G1) in hPSC-cyst morphogenesis by gain- and loss-of-function assays. These findings highlight the power of spatially resolved cell polarity proteome profiling in discovering regulators of epithelial remodeling during early human embryogenesis, as well as the general utility of the APEX2-based proximity labeling approach to identify novel regulators of morphogenesis in stem cell-based systems.

Keywords: hPSC-derived early human embryo model, APEX2-based spatial proteomics, cell polarity and epithelial remodeling

21:15 – 21:30

A HILL ON WADDINGTON'S EPIGENETIC LANDSCAPE: HOW CAN CELLS EXPAND THEIR DIFFERENTIATION POTENTIAL DURING DEVELOPMENT?

Wysocka, Joanna, Zalc, Antoine, Sinha, Rahul, Swigut, Tomek and Weissman, Irv L

Stanford University, Stanford, CA, USA

Cell differentiation progresses via a continuous lineage restriction process where cell potential is progressively reduced as the embryo develops. In the early embryo, pluripotent embryonic cells can beget all somatic cell types, but this capacity is rapidly restricted during the formation of the three germ layers: endoderm, mesoderm, and ectoderm—each giving rise to specific and distinct cell types and organs. Uniquely among vertebrates, a stem cell-like population called neural crest challenges this paradigm. Within the ectodermal lineage, neural crest cells are induced as an epithelial cell type, and they subsequently undergo an epithelial-to-mesenchymal transition (EMT), and migrate through the embryo to populate ventral locations where they differentiate into diverse cell types. Remarkably, neural crest cells arising from the most rostral part of the embryo, called cranial neural crest cells (CNCC), generate not only derivatives typical of ectoderm, such as neurons and glia, but also give rise to cell types canonically associated with the mesoderm lineage such as bone, cartilage and smooth muscle. The unique ability of CNCC to expand their differentiation potential beyond their germ layer of origin raises the question of whether this pluripotency is induced de novo in the ectoderm or, alternatively, retained from the early pluripotent embryo in a specific subset of neuroepithelial cells. Using single cell transcriptome analyses we probed cellular heterogeneity at the onset of murine neural crest formation and identified a novel epithelial precursor population, which gives rise

to CNCC. I will discuss how our subsequent analysis of this precursor population sheds light into the mechanisms by which cells can gain developmental plasticity and expand their differentiation potential during embryogenesis.

Keywords: pluripotency, chromatin, neural crest

FRIDAY, JUNE 26, 20:00 – 21:45

CONCURRENT - CELLULAR IDENTITY: NEURAL

20:05 – 20:20
MITIGATING ANTAGONISM BETWEEN TRANSCRIPTION AND PROLIFERATION ALLOWS NEAR-DETERMINISTIC CELLULAR REPROGRAMMING

Ichida, Justin[1], Galloway, Kate E[2], Kisler, Kassandra, Babos, Kimberley, Shi, Yingxiao, Li, Yichen and Zlokovic, Berislav

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Although cellular reprogramming enables the generation of new cell types for disease modeling and regenerative therapies, reprogramming remains a rare cellular event. By examining reprogramming of fibroblasts into motor neurons and multiple other somatic lineages, we find that epigenetic barriers to conversion can be overcome by endowing cells with the ability to mitigate an inherent antagonism between transcription and DNA replication. We show that transcription factor overexpression induces unusually high rates of transcription and that sustaining hypertranscription and transgene expression in hyperproliferative cells early in reprogramming is critical for successful lineage conversion. However, hypertranscription impedes DNA replication and cell proliferation, processes that facilitate reprogramming. We identify a chemical and genetic cocktail that dramatically increases the number of cells capable of simultaneous hypertranscription and hyperproliferation by activating topoisomerases. Further, we show that hypertranscribing, hyperproliferating cells reprogram at 100-fold higher, near-deterministic rates. Therefore, relaxing biophysical constraints overcomes molecular barriers to cellular reprogramming.

Keywords: reprogramming, DNA torsion, neurons

20:25 – 20:33
FORWARD PROGRAMMING OF PHOTORECEPTORS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

Zuzic, Marta[1], Kempe, Anka[1], Karl, Mike[2] and Busskamp, Volker[3]

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Transplantation of cells derived by in-vitro differentiation of hiPSCs is one of the main goals of regenerative medicine. Differentiation protocols that follow the developmental cues driven by soluble factors and specialized culturing techniques are often slow and laborious. Overexpression of transcription factors (TFs) to activate differentiation is, on the other hand, often faster and more robust. The TF "recipes" for engineering desired cell types, including photoreceptors, are mostly unknown. The eye is an immune-privileged organ and well-suited for cell therapies. Therefore, it would be of utmost importance to produce vision-restoring photoreceptors in high quantity and quality. To this end, we aimed to find TFs that drive efficiently hiPSCs to photoreceptors. We performed a TF screen based on a photoreceptor-reporter hiPSCs line and systematically screened combinations of 1748 human TFs. The reporter line, coding for a fluorescent marker driven by a photoreceptor-specific promoter, allowed for single-cell sorting. Of the sorted cells, 87% expressed photoreceptor-specific genes. By sequencing we identified a combination of three TFs that, when overexpressed for 10 days without adding any additional factor, trigger hiPSC differentiation into cone photoreceptor-like cells. The differentiation efficiency was 25% and when using the cell cycle inhibitor AraC, it increased to 50%. Two of the identified TFs are known players in photoreceptor development. The third one however, was never associated with photoreceptors before. Our developed TF screening method was very precise: by using other reporter cassettes, it can easily be adapted for other cell types of interest. Furthermore, we showed that a TF that was previously never connected to photoreceptor development is essential, indicating that in-vitro processes might be uncoupled from the ones we know from in-vivo studies. Our in-vitro engineered photoreceptor-like cells will serve as biomedical testbeds and donor material for cell transplantation to treat neurodegenerative pathologies of the retina.

Funding source: Dresden International Graduate School for Biomedicine and Bioengineering (DIGS-BB) European Research Council (ERC-StG-678071 - ProNeurons)

Keywords: human induced pluripotent stem cells, cell type engineering, retina

20:35 – 20:43

CELL TRAJECTORY MAPPING OF MOUSE IN VITRO NEUROGENESIS BY SINGLE-CELL MASS CYTOMETRY IDENTIFIES DISTINCT NEURAL SUBTYPES AND PROVIDES A COMPARATIVE ANALYSIS OF IN VITRO AND IN VIVO NEURAL DEVELOPMENT

Fread, Kristen[1], VanDeusen, Amy[2], Williams, Corey[1], Goggin, Sarah[2], Puleo, Emily[1] and Zunder, Eli[1]

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Defects in neurodevelopmental processes have been implicated in a range of neurological disorders including autism, Alzheimer's disease, and epilepsy. Identifying the molecular mechanisms that control the progression of neurogenesis can provide critical insight into the underlying processes of neural cell specification. Recent advances in high dimensional single-cell-omic technologies have allowed us to survey the unique protein and transcriptomic profiles of cellular development. However, we have yet to fully apply protein-based high dimensional analysis techniques to understanding neurogenesis within mouse in vitro models. Doing so would provide a platform to interrogate the precise hierarchy of cell diversification as well as the signaling that drives cell lineage choices. Toward this end, we applied single-cell mass cytometry to molecularly profile neural populations over the course of a 14-day neural differentiation of mouse embryonic stem cells, using a specific neural targeted antibody panel for protein level readout of 40 surface markers, transcription factors, neural filament proteins, and cell signaling molecules. After mass cytometry measurement, cell lineage relationships and differentiation trajectories were inferred by force-directed graph layout analysis (FLOWMAP), revealing heterogeneous cell populations including both neuron like (MAP2+, NeuN+, Tuj1+) and glial like cells (Sox2+, Nestin+, BFBP+). Overall, we identified cellular markers that define the early, intermediate, and late differentiated populations of in vitro neurogenesis. Additionally, we compare in vitro populations identified to in vivo mouse embryonic neurogenesis, finding neuronal progenitors and glial cell types in vitro that match in vivo phenotypes, as well as populations that define in vitro specific cell types. This approach to analyzing neural differentiation creates a cell trajectory map of in vitro differentiation, providing high dimensional protein-level insight into neural cell development and relevance to in vivo systems. This scalable approach for rapid cell phenotyping can be used in future experiments to probe the effect of inhibitors, cytokines, and genetic manipulation on neural differentiation and, once applied in disease models, to neurodevelopmental disorders.

Funding source: NIGMS training grant 5T32GM008715

Keywords: mass cytometry, neural differentiation, high dimensional analysis

20:45 – 20:53

THE ROLE OF AUTISM-RELATED CHROMATIN REMODELER CHD8 AND ITS INTERACTORS IN HUMAN NEURAL STEM CELL IDENTITY

Meert, Lize, Dekker, Mike, Pelicano de Almeida, Mariana and Poot, Raymond

Department of Cell Biology, Erasmus Medical Center, Rotterdam, Netherlands

Autism spectrum disorders (ASDs) are a very heterogeneous group of neurodevelopmental disorders that for 90% are determined by genetic factors. The main characteristics of ASDs are deficits in communicative and social interaction skills. Patients also display restricted interests and repetitive behavior, which often coincides with impaired cognitive functions. Previous studies have identified de novo mutations in chromatin regulators by whole exome sequencing of ASD-patients and their unaffected parents or siblings. The most frequently mutated gene in autism patients is CHD8, an ATP-dependent chromatin remodeling factor. Interestingly, CHD8 mutations give rise to a specific subset of autism that often coincides with macrocephaly. Macrocephaly is present in 30% of autism patients and is likely caused by neural stem cell over-proliferation. In order to better understand the role of CHD8 and how it is linked to autism with macrocephaly, we determined the protein interaction network of CHD8 in human neural stem cells (hNSCs). To do so, we endogenously tagged both CHD8 alleles in hNSCs with a FLAG-V5 tag using CRISPR-Cas9. Subsequently, we purified the CHD8 protein and its interaction partners using the introduced FLAG-tag. Mass spectrometry analysis of multiple CHD8 purifications revealed around 50 consistent interaction partners, mostly involved in transcription elongation, transcription pause-release and the regulation of alternative splicing. We identified TRRAP and most subunits of the transcription co-activator complex TRRAP/TIP60 as top novel interaction partners of CHD8. In addition, we are characterizing the phenotype of CHD8 KO hNSCs. CRISPR-Cas9 induced CHD8 KO hNSCs were viable but could no longer differentiate to neurons. This suggests that the lack of CHD8 results in difficulties in adapting the chromatin structure to change cell identity. We are currently investigating the role of the TRAPP/TIP60 complex in CHD8 mediated gene regulation in hNSCs and further analyzing the CHD8 KO phenotype in hNSCs.

Keywords: Autism spectrum disorders, CHD8, Human neural stem cells

20:55 – 21:03
SINGLE-CELL ANALYSIS ON DIRECT REPROGRAMMING TO NEURAL PROGENITORS BY PLURIPOTENCY FACTORS

Kim, Janghwan[1], Im, Ilkyun[1], Kim, Beomseok[2], Choi, Yoon Ha[2], Ha, Jeongmin[1], Lee, Mi-Ok[1], Kim, Jongpil[3], Son, Mi-Young[1], Ding, Sheng[4] and Kim, Jong Kyoung[2]

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Direct reprogramming by the four factors Oct4, Sox2, Klf4, and c-Myc (OSKM) holds immense potential for creation of somatic cell populations for research and therapeutic purposes, but concerns about their safety continue to be raised. Here, we provide a time-course single-cell RNA sequencing data set comprising transcriptional profiles of 33,966 single cells in which we compared OSKM-mediated reprogramming under conditions that generate either iPSCs or iNSCs. We found the cells on the iNSC reprogramming that express Pou5f1 without co-expression of other representative pluripotency markers, such as Nanog, implying that reprogramming to iNSCs bypasses the transient iPSC-like state. Notably, trajectory analysis revealed that three reprogramming paths to iNSCs classified by different level of Pou5f1 expression according to the exogenous OSKM expression. Our study provides support for the safety of OSKM-mediated direct reprogramming approaches and also provide mechanistic insights of early and intermediate stage of reprogramming driven by OSKM.

Funding source: Samsung Research Funding Center of Samsung Electronic under Project Number SRFC-MA1601-06, KRIBB research initiative program, and NRF-2015M3A9C7030128, and NRF-2018R1A5A1025511 funded by Korean Ministry of Science and ICT.

Keywords: direct reprogramming by pluripotency factors, neural progenitors, single-cell analysis

21:05 – 21:13
HARNESSING P53 TO STABILIZE ACCELERATED, DUAL-PHASE REPROGRAMMING

Galloway, Kate E.

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The tumor-suppressor protein p53 regulates proliferation and transcription, binds nucleosome-dense regions, triggers apoptosis, and maintains genomic integrity, serving as a guardian of cellular identity. In reprogramming, inhibition and knockdown of p53 enhances reprogramming suggesting that p53 functions as an inhibitor of cell fate transitions. However, we find that maintaining p53 levels enhances reprogramming to motor neurons. This paradox may be resolved by our observation that cells proceed through two distinct phases of reprogramming: a proliferation-dominant phase and a transcription-dominant phase. We define these phases as separated by an inflection point observed in non-monotonic changes in proliferation, transcription, and chromatin accessibility. Our previous work indicates that transcription and proliferation represent dual competing demands. Cells that rapidly proliferate and establish high transcription rates at early timepoints reprogram near-deterministically across diverse protocols. Interference with wildtype p53 via a p53 mutant increases proliferation and the p53 presence in the nucleus. Loss of either proliferation or nuclear p53 reduces the population of hyperproliferating, hypertranscribing cells (HHCs) and reprogramming. In vitro and cell lines, p53 activity controls genome stability via Topoisomerase I (Top1). We find that loss of Top1 phenocopies loss of p53 in reducing transcription, HHCs, and reprogramming while increasing genomic stress. Together our data suggest a model in which p53 serves dual roles in inhibiting and promoting reprogramming through regulation of cell cycle and transcription, respectively. This more nuanced perspective of p53 highlights temporally distinct molecular mechanisms by which p53 restricts or confers cellular plasticity.

Funding source: NA

Keywords: Cellular reprogramming, p53, Topoisomerase

21:15 - 21:30

IDENTITY THEFT: CONVERSION OF ASTROCYTES INTO NEURONS REVERSES PARKINSON'S-LIKE DISEASE

Cleveland, Don W.[1,2], Hao Qian[1], Roy Maimon1[-2], Carlos Marinas[2], Xinjiang Kang[3,4], Jing Hu[1], Dongyang Zhang[5], Zhengyu Liang[1], Fan Meng[1], Xuan Zhang[1], Yuanchao Xue[1,6], Steven F. Dowdy[1], Neal K. Devaraj[5], Zhuan Zhou[3], William C. Mobley[7], and Xiang-Dong Fu[1,8]

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The initial development of methods to transdifferentiate non-neurons into a wide range of neural cells opened a new era for treating neurodegenerative diseases. We now report an efficient single-step cell "identity theft" that converts astrocytes into functional neurons in human brain organoids and in the adult mouse brain following transient depletion of the RNA binding protein PTB. Suppression of PTB using a therapeutically viable approach with injection of an antisense oligonucleotide (ASO) into cerebral spinal fluid of healthy adult mice is shown to convert astrocytes into new neurons, especially in the hippocampus. Focal suppression of PTB in mice with chemically induced Parkinson's disease potently reverses disease through converting astrocytes into new substantia nigral neurons, which act to restore striatal dopamine levels and partially reestablish the nigrostriatal dopamine pathway. These findings identify a generalizable, therapeutically feasible strategy for treating neurodegenerative disorders through replacing lost neurons.

FRIDAY, JUNE 26, 20:00 – 21:45

CONCURRENT - CLINICAL APPLICATIONS: PANCREAS

20:05 – 20:20

DEVELOPMENT OF STEM-CELL DERIVED, ISLET REPLACEMENT FOR TYPE 1 DIABETES

D'Amour, Kevin

ViaCyte, San Diego, CA, USA

ViaCyte Inc. is a clinical stage company developing a stem cell-based islet replacement therapy for treatment of patients with diabetes. The therapy is a combination product comprised of pancreatic endoderm cells encapsulated within a retrievable delivery device. After implantation, progenitor cells differentiate into glucose-responsive, insulin-secreting cells. The renewable starting material for cell product manufacturing is human embryonic stem cells that are directed to differentiate to the pancreatic endoderm cell product using scalable processes. The bio-stable delivery device is designed to contain cells and facilitate formulation and delivery of the product to subcutaneous tissue sites.

Keywords: Cell, Device, Diabetes

20:25 – 20:33

DEVELOPMENT OF FUNCTIONAL HUMAN ISLETS FOR CELL REPLACEMENT THERAPY TO TREAT DIABETES

Millman, Jeffrey R.

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Differentiation of human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) into insulin-producing islet organoids would enable development of a novel cell replacement therapy for patients with diabetes. While many approaches have been published in recent years detailing generation of stem cell-derived islets (SC-islets), these cells are immature both transcriptionally and functionally compared to primary islets from adult donors. Here we present a multi-faceted strategy for generating and studying SC-islets with enhanced maturation. We optimized and innovated our prior reports on this technology by combinatorial testing of (1) soluble factors targeting glucose-stimulated insulin secretion and expression of the maturation gene SIX2 and (2) evaluating the molecular effects of key microenvironment factors, including islet-associated extracellular matrix proteins, direct modulation of the actin cytoskeleton, and non-endocrine cell co-cultures. This resulted in development of a new differentiation approach that is reproducible across a large number of cell lines, including hESCs and control- and patient-derived hiPSCs (Type 1 diabetes, Type 2 diabetes, Wolfram Syndrome,

and MODY). Differentiated SC-islets in vitro achieved both first and second phase dynamic insulin secretion in addition to other islet characteristics. We transplanted both hESC-derived and patient hiPSC-derived SC-islets into mice with severe pre-existing diabetes (>500 mg/dl blood sugar) and rapidly restored normoglycemia within 1-2 weeks, which could be maintained for at least 6 months. Single-cell RNA sequencing of transplanted cells revealed even further transcriptional maturation and commitment to β cells and other islet endocrine cell fates. Using a combination of soluble and insoluble cues, we have advanced SC-islet technology towards better enabling next generation cell therapeutic strategies for patients with diabetes to replace the insulin-producing tissue that is dead or dysfunctional with a renewable, stem cell-derived source.

Funding source: NIH (5R01DK114233), JDRF (5-CDA-2017-391-A-N), Washington University-Centene Personalized Medicine Initiative

Keywords: diabetes, organoids, single-cell RNA sequencing

20:35 – 20:43
BETA AND ALPHA CELL ENRICHMENT IMPROVES STEM CELL GRAFT FUNCTION IN MICE

Collins, Samantha, Hyoje-Ryu Kenty, Jennifer and Melton, Douglas

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Our ability to differentiate human pluripotent stem cells into pancreatic islets provides a promising opportunity for the treatment of type 1 diabetes, a disease characterized by the autoimmune destruction of beta cells and subsequent dysfunction of the pancreatic islets they reside in. Cell replacement therapy aims to reverse the lifelong dependence on exogenous insulin and close glucose monitoring resulting from this destruction and dysfunction, although the ideal stem cell-derived graft composition remains unknown. Current in vitro differentiation protocols yield stem cell-derived islets (SC-islets) that are heterogenous, composed not only of beta- and alpha-like cells, but also enterochromaffin and pancreatic exocrine cells that are not found in endogenous human islets. Despite their ability to be glucose responsive, SC-islets' ability to respond to changes in glycemia both in vitro and in mouse transplant models pales in comparison to human islets. We recently identified beta and alpha cell surface markers, CD49a and CD26 respectively. These markers give us the opportunity to generate stem cell-derived "designer islets" (SC-dIs) by dissociating SC-islets and reaggregating isolated CD49a+ beta cells and CD26+ alpha cells into islet-like clusters. This new ability enables us to test the functional value of various ratios of beta and alpha cells, without the potential interference of foreign cell types. Here we show all transplanted SC-dIs maintain their purified in vitro cellular composition over time. In assessing glucose stimulated C-peptide secretion, we show SC-dIs composed of 100% SC-betas demonstrate appropriate glucose responsiveness as early as 7 days post-transplantation while SC-islets take 4 weeks. We also demonstrate transplanted SC-dIs are more efficient than SC-islets by 30 weeks, as all SC-beta majority grafts secrete more human C-peptide than SC-islets despite smaller total cellular volumes transplanted. Finally, we show SC-dIs composed of 100% SC-betas have the ability to return diabetic mice to euglycemia as early as 17 days post-transplant, 62 days prior to SC-islets, and display robust glycemic control in glucose tolerance tests. These results will guide us in determining the ideal composition of a stem cell-derived graft for therapeutic management of type 1 diabetes.

Keywords: Type 1 Diabetes, Stem Cell Enrichment, in vivo Graft Function

20:45 – 20:53
A NANOFIBER-SKIN, HYDROGEL-CORE ENCAPSULATION DEVICE FOR SAFE DELIVERY OF INSULIN-PRODUCING CELLS

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With rapid advancements in stem cell technologies in recent years, there are great hopes that stem cell-derived beta (SC-beta) cells may be used one day to replace the missing ones in patients and cure T1D. However, in a foreseeable future, a device that is both functional and safe may be needed to deliver the cells. Such a device should at minimum 1) prohibit the invasion of immune cells, 2) prevent the escape of the stem cell-derived cells, 3) allow the exchange of oxygen and nutrients and 4) be mechanically robust for long-term use without rupture or any potential to break. In this presentation, I describe such a device - a Nanofiber Integrated Cell Encapsulation (NICE) device - to facilitate safe and functional cell transplantation for T1D. Transplantation of human SC-beta cells reversed hyperglycemia in diabetic NSG mice immediately and last for as long as 120 days. SC-beta cells are glucose responsive after transplantation for 2 weeks or 8 weeks by measuring human C-peptide in blood after stimulation of high glucose solution. Immunofluorescence staining showed that the SC-beta cells were positive for markers such as insulin, glucagon, C-peptide and Nkx6.1. Quantitatively, percentage of insulin positive cells, glucagon positive cells and polyhormonal cells per islet were about 38%, 34% and 1% respectively. Percentage of C-peptide positive, Nkx6.1 positive cells and double positive cells per islet were 43%, 37% and 19% respectively. No cell penetration or cell escape was observed. After implantation of NICE device in healthy dogs for 2 weeks, devices with minimal adhesion could be retrieved from i.p. space using minimally invasive

laparoscopic surgeries. H&E and immunofluorescence staining showed that the SC-beta cells were viable and positive for insulin. The NICE device composed of nanofiber-skin and hydrogel-core prevented cell penetration or escape. SC-beta cells were viable, glucose responsive and positive for hormone markers in the device. Devices with SC-beta cells can be scaled up and retrieved from healthy dogs following laparoscopic procedures. The device is safe, biocompatible and functional, which holds promise to deliver insulin- secreting beta cells to treat Type I diabetes.

Funding source: Funding for this work was partially provided by Juvenile Diabetes Research Foundation (JDRF) and the Novo Nordisk Company.

Keywords: stem cell derived beta cells, stem cell therapy, encapsulation device

20:55 – 21:03

MUILTIOMIC SINGLE CELL ANALYSIS TO IDENTIFY MECHANISMS OF PANCREATIC BETA CELL MATURATION DURING HUMAN PLURIPOTENT STEM CELL DIFFERENTIATION

Zhu, Han[1], Zhu, Chenxu[2], Kovsky, Jenna[3], Nguyen-Ngoc, Kim-Vy[3], Jun, Yesl[3], Wang, Jinzhao[3], Ren, Bing[4] and Sander, Maike[3]

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Human pluripotent stem cell (hPSC)-derived islet cells provide a promising resource for diabetes research and cell therapies due to their unlimited supply. Recent studies have demonstrated feasibility of in vitro generation of glucose-responsive insulin-secreting pseudo-islets from hPSCs (SC-islet). These islet-like clusters consist of heterogenous populations of cells, including beta-like cells (SC-beta cells) which exhibit glucose-responsive insulin secretion and express genes characteristic of mature functional beta cells. An open question is whether the SC-beta cell lineage arises de novo at late stages of in vitro differentiation or whether SC-beta cells are specified early and gradually mature over time. To address this question, we conducted single cell transcriptome (scRNA-seq) analysis and glucose-stimulated insulin secretion (GSIS) assays at multiple time points of hPSC differentiation from the emergence of the first insulin+ cells to the acquisition of a robust GSIS response. scRNA-seq profiling revealed that a population of cells with molecular features resembling late stage SC-beta cells precedes the gain of a GSIS response, suggesting that SC-beta cells are specified early and gradually mature in vitro. Comparison of gene expression profiles of this population before and after maturation showed that immature SC-beta cells express higher levels of genes associated with fatty acid metabolism, while mature SC-beta cells are enriched for transcripts involved in endoplasmic reticulum lumen and vesicle transportation. To gain insight into the signals and transcription factors that regulate this maturation process, we generated single cell chromatin accessibility maps (snATAC-seq) from immature and mature SC-beta cells. We also employed Paired-seq for parallel analysis of transcriptome and accessible chromatin in the same cell, to integrate the deeply sequenced scRNA-seq and snATAC-seq datasets. Analysis of these data will allow us to correlate chromatin dynamics with gene expression changes during human beta cell maturation, as well as identify transcription factors and signaling events that regulate this process.

Keywords: hPSC-derived pancreatic islet, multiomic single cell analysis, human beta cell maturation

21:05 - 21:13

ENGINEERING VASCULARIZED ISLET ORGANOIDS FROM HUMAN INDUCED PLURIPOTENT STEM CELL

Connor Wiegand and Banerjee, Ipsita

University of Pittsburgh, USA

Induced pluripotent stem cell (iPSC) derived islet organoids have great promise in regenerative medicine and drug discovery application. Our goal is to engineer an such an islet mimetic, by reproducing the structural and functional components of a pancreatic islet. We achieve this by controlled integration of relevant biophysical cues in parallel to chemical cues, in each stage of islet derivation. We propagate and differentiate the hPSCs in a 3D hydrogel platform, which results in improved phenotype of the derived cells, as compared to differentiation in suspension culture. Pancreatic islet development is initiated as an epithelial sheet, and adopts its spheroidal configuration in parallel with endothelialization and subsequently attains functional maturation. Accordingly, we have designed a novel substrate for controlled aggregation of the derived cells into size controlled spheroids as well as co-aggregation with stromal and endothelial population into organoids. Importantly, the hPSC-islet spheroids thus derived demonstrate insulin responsiveness to glucose, with insulin secretion doubling to 0.32 micro-insulin units (uIU) per aggregate with increased exposure to glucose. Further, the heterotypic islet organoids generated by co-aggregation with adipose derived microvessel fragments and human Mesenchymal Stem Cells demonstrate a more distinctive insulin responsiveness with over triple insulin secretion to 0.58uIU per aggregate with increased glucose. Interestingly, exposure to islet microenvironment induced phenotyp-

ic change which start to express markers specific to islet endothelial cells. In the final stage of maturation, the hPSC-islet spheroids and organoids thus engineered are incorporated in a microfluidic device and exposed to dynamic perfusion culture, to parallel with in-vivo condition. The derived islets thus maintained under simulated interstitial flow, retained high viability over multiple weeks. The flow conditions also enhanced endothelial growth and induced network formation within the organoids, and enhanced islet function over parallel static culture. Overall our results demonstrate the advantages of integration of biophysical cues, mimetic of physiologic conditions, in parallel with chemical induction of differentiation.

Keywords: vascularized Islet organoid, dynamic perfusion culture,hydrogel for controlled aggregation

21:15 – 21:30

GENERATING FUNCTIONAL PANCREATIC ENDOCRINE CELLS FROM EXTENDED PLURIPOTENT STEM CELL

Deng, Hongkui

Peking University, Beijing, China

Using stem cells to generate fully functional cells is promising, yet challenging. Our lab has been studying the stepwise differentiation of human pluripotent stem cells into functional cells, while at the same time seeking new sources of pluripotent stem cells. In 2005, we established the first protocol for the generation of pancreatic β cells from embryonic cells. Over the years, we fine-tuned the differentiation process by deepening our knowledge of β cell development. Notably, by knock-in studies of genes involving the pancreatic development axis, we revealed a series of transition events in real time during cell fate patterning, paving a path towards the generation of functionally mature β cells in vitro. In 2017, we derived extended pluripotent stem (EPS) cells, a new type of pluripotent cell bearing both embryonic and extra embryonic developmental potential, while possessing also naïve pluripotent features of pre-implantation epiblast. Given their superior developmental potential, EPS cells exhibit characteristics of a promising cell source for differentiation. Very recently, we demonstrated the derivation of functional endodermal lineage through stepwise differentiation from EPS cells (Wang et al., Cell Research 2020). This work also revealed a simple pre-treatment method that enabled EPS cells to acquire the capacity for multi-lineage differentiation, which enables EPS cells to be easily adapted to current differentiation protocols for conventional hESC/iPSCs. I will be presenting our recent progress in the derivation of functional β cells from EPS cells, which integrates the advantages of EPS cells and our established differentiation methods. I will also discuss main challenges and exciting advances in clinical translations using stem cell-derived cells.

Keywords: pluripotent stem cells, pancreatic β cells, differentiation

FRIDAY, JUNE 26, 20:00 – 21:45

CONCURRENT - MODELING DEVELOPMENT AND DISEASE: HEMATOPOIETIC AND ENDOTHELIAL CELLS

20:05 – 20:20

MONOGENIC AND POLYGENIC INHERITANCE BECOME INSTRUMENTS FOR CLONAL SELECTION IN HEMATOPOIETIC STEM CELLS

McCarroll, Steve[1,2,3], Loh, Pu-Ru[1,4] and Genovese, Giulio[1,2,3]

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Clonally expanded blood cells with somatic mutations (clonal hematopoiesis, CH) are commonly acquired with age and increase risk of blood cancer. The blood clones identified to date contain diverse large-scale mosaic chromosomal alterations (mCAs: deletions, duplications, and copy-neutral loss of heterozygosity [CN-LOH]) on all chromosomes, but the sources of selective advantage that drive expansion of most clones remain unknown. To identify genes, mutations and biological processes that give selective advantage to mutant clones, we analyzed genotyping data from the blood-derived DNA of 482,789 UK Biobank participants, identifying 19,632 autosomal mCAs which we analyzed for relationships to inherited genetic variation. Fifty-two inherited, rare, large-effect coding or splice variants in seven genes associated with greatly increased (odds ratios 11 to 758) vulnerability to CH with specific acquired CN-LOH mutations. Acquired mutations systematically replaced the inherited risk alleles (at MPL) or duplicated them to the homologous chromosome (at FH, NBN, MRE11, ATM, SH2B3, and TM2D3). Three of the seven genes (MRE11, NBN, and ATM) encode components of the MRN-ATM pathway, which limits cell division after DNA damage and telomere attrition; another two (MPL, SH2B3) encode proteins that regulate stem cell self-renewal. In addition to these monogenic inherited forms of CH, we found a common and surprisingly polygenic form: CN-LOH

mutations across the genome tended to cause chromosomal segments with alleles that promote hematopoietic cell expansions to replace their homologous (allelic) counterparts, increasing polygenic drive for blood-cell proliferation traits. Readily-acquired mutations that replace chromosomal segments with their homologous counterparts appear to interact with pervasive inherited variation to create a challenge for lifelong cytopoiesis.

Keywords: clonal hematopoiesis, mutations, human genetics

20:25 – 20:33

ENGINEERING ADULT RED BLOOD CELLS FROM HUMAN IPSCS THAT SICKLE IN VITRO: A NOVEL THERAPEUTIC PLATFORM FOR SICKLE CELL ANEMIA

Conway, Ashlee J.[1], Rosanwo, Tolulope[2], Williamson, Thomas[1], Landry, Samuel[1], Kinney, Melissa[1], Vo, Linda[3], Rowe, Grant[4], North, Trista[1] and Daley, George[1,4]

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Human induced pluripotent stem cells (iPSC) are an invaluable resource in tissue and blood cell engineering due to their multi-lineage potential in culture systems. iPSC-derived hematopoietic progenitors that undergo successful erythropoiesis would allow for the in vitro modeling of inheritable red blood cell (RBC) diseases, such as Sickle Cell Anemia (SCA), for novel therapeutic design and pre-clinical testing. Here, we describe an optimized method of generating induced RBCs (iRBCs) in vitro from healthy and sickle cell homozygous iPSCs, created from patient leukocytes. Using a human plasma-supplemented erythroid differentiation media (EDM), our iRBCs mature in vitro into GlyA+CD71- cells which successfully enucleate at a high rate (>80%). mRNA profiles demonstrate iRBCs undergo three distinct waves of globin activation and suppression throughout erythroid development, which closely mimics the in vivo globin-switching profile modeled from humans. Furthermore, these iRBCs become robust adult -globin expressing cells, which is confirmed at the protein level. Using hypoxic culture incubation, -globin expression in WT iRBCs is increased 4-fold, however, cells harboring the (HBB)E6V mutation respond poorly to hypoxic stress. Sickling is readily observable in mutant iRBCs, regardless of oxygen conditions, along with other morphological abnormalities characteristic of SCA (microcytosis, hypochromasia, cell-cell adhesion, fragmentation etc). RNA-seq analysis was performed on iRBCs to compare WT and SCA transcriptional profiles, in order to identify novel disease phenotypes; a practice that could help drive patient-specific therapies. Importantly, this erythroid differentiation protocol has been optimized to be xeno-free with the successful replacement of BSA with an inorganic alternative, PVA. Together, this study demonstrates that enucleated, hemoglobinized iRBCs can be engineered from other somatic cell sources by passing through the iPSC state. Obtaining terminal maturity in these iRBCs using this optimized protocol allows for modeling of human blood diseases with visually recognizable phenotypes. Future cellular products generated using a xeno-free EDM therefore have the capacity for live human testing as an autologous source of transfusable blood products.

Funding source: 1R01DK098241, 4T32HL007893 (North) NIH-NHLBI (UO1-HL134812) (Daley)

Keywords: iPS, remodeling, hematopoiesis

20:35 – 20:43

GENERATION OF RETINOIC ACID-DEPENDENT DEFINITIVE HEMATOPOIETIC PROGENITORS FROM HUMAN PLURIPOTENT STEM CELLS

Luff, Stephanie[1], Dege, Carissa[1], Creamer, John[1], Scarfo, Rebecca[2], Morris, Samantha[3], Ditadi, Andrea[2] and Sturgeon, Christopher[1]

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The generation of the hematopoietic stem cells (HSCs) from human pluripotent stem cells (hPSCs) is a major goal for regenerative medicine. In the embryo, HSCs derive from a HOXA+ population known as hemogenic endothelium (HE) in a retinoic acid (RA)-dependent manner. We have previously identified a WNT-dependent (WNTd) CDX4+ mesodermal population that gives rise to a clonally multipotent, HOXA+ definitive HE. However, this HE lacks HSC-like capacity in the absence of exogenous transgenes, and is RA-independent, with exogenous RA treatment failing to confer HSC potential. Thus, identification of RA-dependent HE has remained elusive. We therefore asked if the developmental stage of RA-dependence is developmentally earlier than currently appreciated. Through single cell RNAseq analyses, we identified that hPSC-derived hematopoietic mesoderm, prior to HE specification, is comprised of three distinct KDR+ populations that are distinguishable by CD235a and CXCR4 expression. Transcriptome analyses revealed that these populations recapitulate those found in the early murine embryo, with similar populations being identified between E8-8.75. Interestingly, KDR+CD235a-CXCR4- mesoderm expressed CYP26A1, an RA degrading enzyme,

and was the only population capable of giving rise to multipotent definitive HOXA+ CD34+ HE. In contrast, KDR+CD235a-CXCR4+ mesoderm expressed ALDH1A2, a key enzyme in RA synthesis, suggesting this population may be responsive to RA signaling. Critically, we found that stage-specific application of retinol or all-trans retinoic acid to this CXCR4+ population resulted in the robust specification of CD34+ HE with multi-lineage definitive erythroid, myeloid, and lymphoid hematopoietic potential. Further, these CD34+ cells have >3 fold higher proliferative potential, a fetal-like HOXA expression pattern, and uniquely display transcriptional similarity to primary human fetal HSC-competent HE. Collectively, this represents the first ever characterization of stage-specific RA-dependent hPSC-derived definitive hematopoiesis and its mesodermal progenitor, recapitulating a key developmental process during HSC development. This novel insight into human hematopoietic development will ultimately provide the basis for the transgene-free generation of HSCs from hPSCs.

Keywords: hematopoietic stem cell, hemogenic endothelium, mesoderm

20:45 – 20:53
HUMAN TYPE-1 INNATE LYMPHOID CELLS SECRETE TGFB1, DRIVING EPITHELIAL AND MATRIX REMODELLING IN HIPSC-DERIVED INTESTINAL ORGANOIDS

Jowett, Geraldine M.[1], Norman, Michael[2], Rosell, Patricia[2], Yu, Tracy[2], Read, Emily[3], Neves, Joana[3] and Gentleman, Eileen[2]

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Type-1 innate lymphoid cells (ILC1) are enriched in patient mucosa with active inflammatory bowel disease (IBD), but the impact of this accumulation remains elusive, and -IFNγ therapeutics against their signature cytokine lack clinical efficacy. To shed light on their role in IBD, we established co-cultures of murine small intestine organoids (SIO) with ILC1, and human iPSC-derived intestinal organoids (HIO) with patient ILC1. We used pico-SMARTSeq2 transcriptomics to demonstrate that IFNγ sensitises co-cultured SIO to Fas-mediated apoptosis. However, this also revealed that ILC1 drive expansion of the epithelial stem cell crypt through p38γ phosphorylation and aberrant Cd44v6 expression, which is unexpectedly regulated by ILC1-derived TGFβ1, not IFNγ. We next establish that human ILC1 also secrete TGFβ1, and drive CD44v6 expression in both HIO epithelium and mesenchyme. Notably, this phenotype is only recapitulated by ILC1 from patient biopsies with active inflammation. As TGFβ1 is a master regulator of fibrosis, the leading indicator for surgery in IBD, we next characterised the ability of ILC1 to regulate matrix remodelling. We developed a functionalized, synthetic hydrogel system, which we harnessed to perform microrheology and atomic force microscopy on encapsulated HIO. We show that ILC1 drive both stiffening and degradation of this system, which we posit occurs through a balance of MMP9 degradation and TGFβ1-induced FN1 deposition by HIO fibroblasts. Thus, our synthetic organoid co-culture system enabled us to tease apart an important role for intestinal ILC1 in epithelial and matrix remodelling, which may contribute to wound healing or pathology in IBD. Moreover, our system provides a broader platform for dissecting interactions between complex 3D hiPSC-derived tissues and rare cell subtypes in development and disease.

Keywords: Murine and hiPSC intestinal organoids, Innate lymphoid cells, Functionalised PEG-hydrogels

20:55 – 21:03
THE ALS/FTD ASSOCIATED GENE PRODUCT C9ORF72 FUNCTIONS IN MURINE HEMATOPOIETIC STEM AND PROGENITOR CELLS

Burberry, Aaron[1], Eggan, Kevin[1,2], Scadden, David[1] and Van Gastel, Nick[1]

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder involving loss of motor neurons in the motor cortex, brainstem and ventral horn of the spinal cord resulting in progressive paralysis and death. A GGGGCC hexanucleotide repeat expansion within the first non-coding intron of Chromosome 9 Open Reading Frame 72 (C9ORF72) is the most common genetic contributor to ALS, accounting for approximately 40% of familial cases and 6% of sporadic cases. The expansion is hypothesized to promote disease through a collaboration of toxic gain of function and loss of function (LOF) mechanisms. The intron containing the microsatellite expansion is transcribed, potentially leading to disruption of RNA metabolism. Repeat containing transcripts are also translated in each reading frame producing toxic aggregate-prone repetitive peptides. Furthermore, the mutated allele is epigenetically silenced in a manner similar to the FMR1 gene in Fragile X Syndrome, resulting in reduced abundance of C9ORF72 coding mRNA. The murine ortholog (C9orf72) regulates endo-lysosomal trafficking and functions in mice to prevent systemic and neural inflammation. Here we find that hematopoietic stem cells (HSC) from C9orf72 LOF mice accumulate mitochondria and are more likely to exit quiescence, resulting in an expanded pool of hematopoietic stem and progenitor cells (HSPC). In serial competitive transplantations mutant HSC outcompete their wildtype counterparts, especially in lymphoid lineages, demonstrating a cell intrinsic function of C9orf72 within cells at the zenith of the hematopoi-

etic hierarchy. These studies warrant further investigation into mechanisms by which C9orf72 loss of function sensitizes the immune system to fatal inflammatory disease and suggest that hematopoietic replacement in C9ORF72 carriers might be considered as a prophylactic therapy.

Funding source: Support was provided by 5R01NS089742. A.B. was supported by 5K99AG057808-02

Keywords: Hematopoietic stem cell, Amyotrophic lateral sclerosis, Frontotemporal dementia

21:05 – 21:13

MOUSE EMBRYONIC PRE-HSCS LYMPHOCYTES CONTRIBUTE TO AUTOIMMUNITY

Ditadi, Andrea[1], Cascione, Sara[2], Rigoni, Rosita[1], Squadrito, Mario[1] and Anna, Villa[1]

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Autoimmune disorders (ADs) arise from deregulated self-reactive immune response. Currently, little is known about AD molecular and cellular triggers, thus hampering the design of targeted treatments. We sought to study the cell of origin of ADs exploiting the context of Omenn Syndrome (OS), a genetic disease that offers an unparalleled platform to study autoimmunity in the absence of the adaptive immune system. In OS, hypomorphic mutations in RAG and IL7R genes lead to the paradoxical coexistence of severe combined immunodeficiency (SCID), due to V(D)J recombination impairment in lymphocytes, and autoimmunity. In OS patients, autoreactive T-cells, showing highly restricted TCR usage with common specificity across patients, trigger the typical autoimmune inflammation in the skin and intestine. The peculiar features of OS T-cells closely resemble those of the first lymphocytes emerging before, and independently from, hematopoietic stem cells (HSCs). Therefore, we speculated that OS autoreactive T-cells are mostly generated in the embryo independently from HSCs. To test this hypothesis, we isolated E9.5 HSC-independent hematopoietic progenitors of the yolk sac and para-aortic splanchnopleura as well as adult bone marrow HSCs from both wild-type (wt) and the RAG2R229Q OS mouse model and analyzed their T-lymphoid potential. While both E9.5 cells and adult HSCs from wt mice can differentiate in CD4+CD8+ (DP) CD3+ T-cells in vitro, in the OS setting only embryonic progenitors can generate mature CD3+ T-cells. Conversely, OS adult HSC-derived T-lymphopoiesis shows the characteristic RAG-SCID differentiation blockage prior the DP stage. Of note, the fetal reprogramming of OS adult HSCs by Lin28 ectopic expression rescued their lymphoid potential, confirming that embryonic T-lymphopoiesis is unaffected by OS mutations. Finally, the thymus of adult OS mice is devoid of the DP population normally observed in wt mice, further supporting our hypothesis that mature T-cells in this model do not originate from adult HSCs. Taken together, our studies describe the previously unappreciated contribution of embryonic HSC-independent progenitors to the pool of autoimmune T-cells, underscoring the importance of determining the ontological source in ADs for the design of new and more specific therapeutic treatments.

Keywords: autoimmunity, embryonic hematopoiesis, Omenn Syndrome

21:15 – 21:30

MODELING HUMAN PRE-LEUKEMIC HEMATOPOIETIC STEM CELLS USING CRISPR ENGINEERING

Ravindra Majeti

Stanford University, Stanford, CA, USA

AML develops from the sequential acquisition of multiple mutations in a single lineage of cells. These mutations initially occur in HSCs, termed pre-leukemic HSCs, and are enriched in genes involved in regulation of the epigenome. Similar mutations are found in individuals with no history of hematologic malignancy with increasing frequency with age, a condition termed clonal hematopoiesis. The pre-leukemic cells acquire additional mutations, often in genes involved in proliferation, resulting in development of AML. Stratification of a cohort of AML patients into high or low pre-leukemic HSC groups demonstrated that the high group had much worse overall and relapse-free survival, indicating that the presence of pre-leukemic HSC may be critical for clinical outcomes. The methods by which these pre-leukemic HSCs outcompete normal cells and progress to myeloid malignancy and/or AML is unknown, yet likely involves both cell autonomous and cell non-autonomous mechanisms. In order to model this pre-leukemic state, we used CRISPR/Cas9 methods coupled with homology directed repair to engineer these mutations into normal cord blood-derived hematopoietic stem and progenitor cells. We assessed the effects of these mutations in vitro and in vivo on HSC self-renewal, differentiation, and engraftment. Moreover, we observed that similar to human individuals, in some cases these engineered cells would spontaneously progress to myeloid disease in vivo. Thus, we have established a reliable model for human pre-leukemic hematopoietic stem cells and their progression.

Keywords: Hematopoiesis, Stem Cells, CRISPR

FRIDAY, JUNE 26, 20:00 – 21:45

CONCURRENT - TISSUE STEM CELLS AND REGENERATION: CARDIAC AND MUSCLE

20:05 – 20:20
USING DEVELOPMENTAL BIOLOGY TO INFORM TISSUE REGENERATION

Red-Horse, Kristy

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Developing organisms create tissues de novo, and the underlying instructions could be used to regenerate diseased organs. With this mindset, we study coronary arteries, which bring blood flow to heart muscle, in hopes of eventually addressing coronary artery disease. To date, we have discovered how mouse coronary arteries are built during embryogenesis, and then reinstated developmental pathways in adults to aid regrowth and injury recovery.

Keywords: regeneration, coronary, heart

20:25 – 20:33
MOUSE AND HUMAN ARTICULAR CARTILAGE REGENERATION BY ACTIVATION OF SKELETAL STEM CELLS

Koepke, Lauren S.[1], Ambrosi, Thomas[1], Chan, Charles[1], Hoover, Malachia[2], Longaker, Michael[1], Murphy, Matthew[3], Steininger, Holly[1], Wang, Yuting[1] and Weissman, Irv[2]

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Osteoarthritis (OA) is a degenerative disease resulting in irreversible, progressive destruction of hyaline cartilage lining articular joints. The etiology of OA is complex and involves a variety of factors, including genetic predisposition, acute injury, and chronic inflammation. Here we investigate the ability of resident skeletal stem cell (SSC) populations to regenerate cartilage in relation to age, a possible contributor to the development of osteoarthritis. We demonstrate that with greater maturity, the frequency of SSC in joints progressively decreased in both mice and humans corresponding to diminished chondrogenesis in mature adult tissue. However, a local expansion of SSC could still be triggered in the chondral surface of adult limb joints by stimulating a regenerative response following microfracture (MF) surgery. While MF-activated SSC tend to form fibrous tissues, we found that localized co-delivery of BMP2 and soluble VEGFR1 (sVEGFR1), a VEGF receptor antagonist, in a biochemical hydrogel can drive the differentiation of MF-activated SSC towards generation of articular cartilage. These data suggest that following MF, there is a therapeutic window to skew MF-activated SSC differentiation fate towards robust formation of de novo cartilage for treating localized chondral disease in OA. Our findings provide a new stem cell paradigm for regenerating cartilage that is validated in both mouse and human tissues. To our knowledge this is the first time that stable articular cartilage has been successfully regenerated in mice by activation and fate control of autologous tissue-resident SSC populations.

Keywords: osteoarthritis, cartilage, regeneration

20:35 – 20:43
SINGLE CELL DECONSTRUCTION OF MUSCLE STEM CELL SENSITIVITY TO THE NEURO-MUSCULAR SYNAPSE DURING AGING

Aguilar, Carlos A.[1], Ulintz, Peter[2], Larouche, Jacqueline[1], Mohiuddin, Mahir[3], Castor Macias, Jesus[1], Choi, Jeongmoon[4], Brown, Lemuel[5], Markworth, James[5], Chakkalakal, Joe[6], Jang, Young[7] and Brooks, Susan[8]

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During aging and neuromuscular diseases, there is a progressive loss of volume and function in skeletal muscle that impacts mobility and quality of life. Muscle loss is often associated with muscle fiber denervation, but neither the impact of a loss of innervation on resident muscle stem cells (satellite cells or MuSCs) nor the role of MuSCs in the initiation or progression of denervation has been resolved. Herein, using a combination of single-cell transcriptomic analysis, high-resolution immunofluorescence imaging and transgenic young and aged mice as well as from mice with neuromuscular degenerative disease (Sod1-/-), a compensatory neuro-regenerative function for a subset of MuSCs was identified. Genetic rescue of motor neurons in Sod1-/- mice reduced this subset of MuSCs and restored integrity of the neuro-muscular junction (NMJ) in a manner akin to young muscle. Administration of severe neuro-

muscular trauma induced MuSCs to specifically engraft in a position proximal to the NMJ and contrasting the expression programs of young and aged MuSCs after muscle injury at the single cell level, we observed distinctive gene expression programs between responses to neural trauma and muscle trauma. Collectively, these data reveal MuSCs sense synaptic perturbations and display exert support for the NMJ, which is attenuated during aging and neuro-muscular disease.

Funding source: NIAMS-NIH P30 AR069620 (CAA, SVB), 3M Foundation (CAA), AFAR (CAA), NIA P30 AG024824 (CAA, SVB), DoD-CDMRP W81XWH18SBAA1-12579992 (CAA, YCJ), NIA P01 AG051442 (SVB), and NIA R01 AG051456 (JVC).

Keywords: Aging, Neuromuscular Junction, Sod1

20:45 – 20:53

ZEBRAFISH CHEMICAL COMPOUND SCREEN UNCOVERS INDUCERS OF SKELETAL MUSCLE ENGRAFTMENT ACROSS SPECIES

Tavakoli, Sahar[1], Adatto, Isaac[1], Ashrafi Kakhki, Sara[1], Chan, Victoria[1], Fotowat, Haleh[2], Gähwiler, Eric[3], Manning, Margot[1], Messemer, Kathleen[1], Rangan, Apoorva[1], Wagers, Amy[1], Yang, Song[4] and Zon, Leonard[4]

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Genetic muscle disorders compromise muscle function through degeneration of muscle fibers, increased inflammation and impaired muscle regeneration. Transplantation of genetically normal muscle progenitors could be an approach to rescue muscle wasting and boost repair; however, this approach has shown limited utility thus far due to the typically poor engraftment efficiency of cultured progenitors. To define regulators of muscle engraftment, we developed a novel cross-species screening platform, employing zebrafish and mouse, to discover chemical compounds that promote muscle progenitor engraftment in vivo. Muscle cells derived from zebrafish blastomeres were treated for 4 hours with biomolecules and transplanted into the flanks of adult zebrafish (n=30/ biomolecule and 188 biomolecule). Using limit-dilution assays, "hits" from our primary screen were identified and re-evaluated in replicate transplantation experiments. We discovered two lipids that promote zebrafish muscle progenitor cell engraftment in vivo: lysophosphatidic acid (LPA) and niflumic acid (NFA). Using bioluminescence imaging, we further ascertained that both NFA and LPA enhance muscle stem cell engraftment in mouse as well (mean BLI radiance ± SEM- NFA: 27.2E+6 ± 6.8 E+6 p/s; LPA: 25.6 E+6 ± 4.4 E+6 p/s; vehicle-treated cells: 8.2 E+6 ± 1.4 E+6 p/s; n=15, 1-way ANOVA, $p \leq 0.05$), indicating conservation of the pro-myogenic activities of these compounds across vertebrate species. Studies in sapje-like (dystrophin mutant) fish transplanted with NFA-treated or LPA-treated cells showed higher engraftment efficiency, significantly better swimming performance and greater ability to swim against a water current. Mechanistically, the pro-myogenic activities of LPA and NFA appear to be associated with increased cytoplasmic Ca2+ and down-regulation of muscle development genes. RNA sequencing analysis also revealed upregulation of myoblast fusion regulating genes, including myomaker (Tmem8c) and Ccl8, in LPA-treated satellite cells. In summary, successful application of this cross-species approach has uncovered evolutionarily conserved pathways regulating muscle regeneration, suggesting new potential opportunities for treating muscle disease by enhancing myogenic contributions of transplanted muscle progenitors.

Keywords: cell therapy, cross-species muscular dystrophy, niflumic acid lysophosphatidic acid

20:55 – 21:03

TRANSLATIONAL CONTROL OF MOUSE MUSCLE STEM CELLS QUIESCENCE EXIT BY CPEB1

Zeng, Wenshu, Cheung, Tom, Lam, Sheung Wai, So, Wai Kin, Yue, Lu and Zhang, Wenxin

Division of Life Science, Hong Kong University of Science and Technology, Hong Kong

Somatic stem cells are required for tissue homeostasis and repair. In low turn-over tissues, somatic stem cells are maintained in quiescence for a prolonged period. Quiescent stem cells (QSCs) are capable to exit quiescence and respond rapidly to stimuli. Dysregulation of quiescence exit results in stem cell pool depletion and tissue regeneration impairment. However, regulations of quiescence exit are not well understood. Translational control is essential for cell fate determination and cellular stage transition. Using skeletal muscle stem cells, or satellite cells (SCs), we revealed a translational regulation of SC quiescence exit mediated by Cytoplasmic Polyadenylation Element Binding protein 1 (CPEB1). CPEB1 loss function study reveals its requirement for SC activation. By next-generation sequencing technique, we revealed that transcripts targeted by CPEB1 are critical for ribosome biogenesis, RNA transportation, and RNA polymerase function, suggesting that CPEB1 regulates SC quiescence exit by enhancing transcription and translation capacity. Further analysis suggested that CPEB1 regulates translation of the transcripts that are highly upregulated during SC quiescence exit. Specifically, we identified a translocation of Myod1 transcripts from nuclei to cytoplasm prepared for rapid translation during SC quiescence exit and subsequently demonstrated that CPEB1 regulates

Myod1 protein rapid expression via cytoplasmic polyadenylation elements (CPEs) within Myod1 3'UTR. CPEB1 phosphorylation is required for translation regulation. We observed a rapid switch of CPEB1 phosphorylation during SC quiescence exit, which is essential for the rapid Myod1 protein expression during SC quiescence exit. Our study collectively revealed that CPEB1 regulates SC quiescence exit by translational control. Moreover, manipulation of CPEB1 phosphorylation in vivo influences muscle regeneration and SC self-renewal, thus suggesting a potential therapeutic strategy for skeletal muscle diseases.

Funding source: Hong Kong Research Grant Council (GRF660313, C6015-14G, C6003-14G, C6009-15G, AoE/M-604/16, T13-607/12R), Lee Hysan Foundation (LHF17SC01) and the Croucher Innovation Award from Croucher Foundation

Keywords: Stem Cell Quiescence Exit, Translational Control, CPEB1

21:05 – 21:13
TRANSCRIPTOMIC ENTROPY ENABLES CROSS-STUDY AND CROSS-SPECIES QUANTIFICATION OF CARDIOMYOCYTE MATURATION AT SINGLE CELL LEVEL

Kannan, Suraj[1], Farid, Michael[1], Lin, Brian[2], Miyamoto, Matthew[2], Hawthorne, Robert[1], Tung, Leslie[1], Kass, David[2] and Kwon, Chulan[2]

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Pluripotent stem cell-derived cardiomyocytes (PSC-CMs) offer tremendous potential for a range of applications in regenerative medicine, drug screening, and disease modeling. However, clinical application of PSC-CMs has been severely constrained by the failure to mature these cells to a fully adult-like phenotype. Moreover, comprehensive metrics to benchmark the maturation status of PSC-CM have not yet been established. Comparison against an in vitro control is often used to show that certain interventions can improve PSC-CM maturation. However, without directly comparing to in vivo CM maturation, the true efficacy of these treatments remain unclear. Therefore, we established a novel approach to quantify CM maturation by directly comparing their transcriptomic entropy to that of in vivo CMs. Transcriptomic entropy is a measure of the breadth of gene expression of individual cells, and is calculated from single cell RNA-seq (scRNA-seq) data. This approach is based on the observation that less mature cells have a more promiscuous (hence, high entropy) gene expression distribution, while more mature cells hone in to a more narrow (thus, low entropy) distribution. We found that transcriptomic entropy is robust across datasets regardless of differences in isolation protocols, library preparation methods, and other potential batch effects. We analyzed over 30 scRNA-seq datasets and over 45,000 CMs to establish a cross-study, cross-species reference of CM maturation based on transcriptomic entropy. We subsequently computed the maturation status of PSC-CMs by direct comparison to in vivo development. We studied PSC-CMs generated by common differentiation protocols as well as following treatment with thyroid hormone, glucocorticoids, and fatty acids. Lastly, we demonstrated that transcriptomic entropy can be used to reconstruct maturation trajectories for in vivo and PSC-CMs. We used this approach to identify factors dysregulated over the course of PSC-CM maturation, highlighting potential targets for future interventions. Our study presents a robust, interpretable, and easy-to-use metric for quantifying CM maturation. Moreover, our study is the first large-scale meta-analysis of cardiac scRNA-seq data, offering a new approach for leveraging publicly available data to study cardiac biology.

Funding source: The lead author was funded by an AHA Predoctoral Fellowship, 20PRE35200028.

Keywords: cardiomyocyte, pluripotent stem cells, single cell RNA-seq

21:15 – 21:30
DIRECT REPROGRAMMING INTO CARDIOVASCULAR LINEAGES

Ieda, Masaki

University of Tsukuba, Tsukuba City, Japan

Heart failure increases as a cause of cardiac death, and regenerative therapy is highly demanded. We hypothesized that direct reprogramming of resident cardiac fibroblasts into cardiomyocytes may become a new strategy for the treatment of heart failure. We previously demonstrated that a combination of cardiac-enriched transcription factors, Gata4, Mef2c, and Tbx5 (GMT), directly reprogrammed mouse fibroblasts into functional cardiomyocytes (Ieda et al., Cell, 2010). Gene transfer of Sendai virus vectors expressing GMT in the mouse infarct hearts converted resident cardiac fibroblasts into cardiomyocytes in vivo and improved cardiac function after MI (Miyamoto et al, Cell Stem Cell, 2018). We also identified that a mesoderm-enriched transcription factor, Tbx6, induced a nascent mesoderm program in fibroblasts by screening of 58 candidate genes. Tbx6 induced nascent mesoderm from mouse and human PSCs and determined cardiovascular and somite lineage specification via its temporal expression (Sadahiro et al. Cell Stem Cell, 2018). However, barriers to cardiac reprogramming associated with aging remain undetermined. More recently, we screened 8400 chemical compounds and found that diclofenac, a non-steroidal anti-inflammatory drug, greatly enhanced cardiac reprogramming in combination with defined factors in postnatal and adult fibroblasts (Muraoka et al., Nat Commun, 2019). Direct re-

programming approach may be useful for regenerative medicine and cardiovascular research.

Keywords: Fibrosis, mesoderm, cardiomyocyte

FRIDAY, JUNE 26, 23:00 – 23:59

PLENARY V: STEM CELLS AND AGING

23:05 – 23:20
MAPPING MICROGLIA STATE AND FUNCTION IN ALZHEIMER'S DISEASE

Stevens, Beth

Boston Children's Hospital, Boston, MA, USA

Microglia are essential for proper brain development and response to injury or diseases. As the brain's resident immune cells, microglia are highly dynamic and reactive to environment and genetic challenges. Single cell transcriptomic studies reveal diverse microglial states in human and mouse brains, however we currently lack specific approaches to track and manipulate specific populations of microglia in Alzheimer's and other diseases. While it is clear that microglia adopt disease- associated transcriptional states, it is not known whether or how specific microglia populations are beneficial or detrimental to disease progression. To understand how microglia- specific pathways contribute to circuit dysfunction we developed and optimized iPS-induced microglia models to dissect how AD genetic risk and brain environment alter microglia transcriptional and functional states and the consequences of specific microglia populations on synapse loss and neuroinflammation.

Keywords: microglia, Alzheimer's disease, cell state

23:25 – 23:40
PROGRAMMING AND REPROGRAMING OF AGING

Liu, Guanghui

Institute of Zoology, Chinese Academy of Sciences, Beijing, China

Age is the major risk factor for most chronic human diseases. As a consequence, "anti-aging" strategies are being pursued as a way to prevent and treat age-related disorders. As we age, our stem cells undergo functional decay and exhaustion. This decline leads to compromised tissue regeneration, which in turn promotes organismal aging. Therapeutic approaches that promote tissue regeneration and repair could therefore potentially mitigate aging and its deleterious effects. Currently, there are three main methods to promote tissue regeneration: (1) Supplement tissues with exogenous stem/progenitor cells; (2) Chemically stimulate in-situ stem cell expansion, differentiation, and/or somatic cell transdifferentiation; and (3) Rejuvenate endogenous stem cell pools with specific biological factors. In animal models, these methods can alleviate diverse aging syndromes, including neurodegeneration, vascular degeneration, myocardial infarction and osteoarthritis. Technological advances allow us to further improve these strategies to repair degenerating organs. For instance, genetically enhanced stem cells and vascular cells with improved efficacy and safety were recently generated by editing longevity genes and tumor suppressors. Approaches to activate tissue regeneration could also be optimized by targeting cellular senescence and regeneration pathways. Overall, engineering tissues and organs that resist aging would transform regenerative medicine, providing a potential "silver bullet" against chronic disease.

Keywords: aging, stem cells

23:45 – 23:59
STEM CELL COMPETITION FOR SKIN HOMEOSTASIS AND AGING

Nishimura, Emi

Tokyo Medical and Dental University, Tokyo, Japan

Stem cells underlies tissue homeostasis, yet the actual stem cell dynamics during aging and the relevance to organ aging are still unknown. In mammalian hair follicles, dynamic elimination of aged stem cells underlies hair ageing phenotypes. However, the exact cellular identity and dynamics of the stressed or damaged cells in large vital organs such as the skin, and their contribution to organ ageing, are still largely unknown. In renewing tissues, stem cells undergo 'neutral stem cell competition' by which some somatic stem cells expand clonally while others are lost. It has been largely unknown whether this process represents neutral drift, biased competition or non-cell autonomous cell competition. Here we report that the expression of the hemidesmosome component collagen XVII (COL17A1) by epidermal stem cells fluctuates physiologically through genomic/oxidative stress-induced proteolysis, and that the resulting differential expression of COL17A1 in individual stem cells generates a driving force for cell competition to eliminate unfit cells. In vivo clonal analysis in mice and in vitro 3D modelling show that clones that express high levels of COL17A1, which divide symmetrically, outcompete and eliminate adjacent stressed clones that express low levels of COL17A1, which divide asymmetrically. Stem cells with higher potential or quality are thus selected for homeostasis, but their eventual loss of COL17A1 limits their competition, thereby causing ageing. The resultant hemidesmosome fragility and stem cell delamination deplete adjacent melanocytes and fibroblasts to promote skin ageing. Conversely, the forced maintenance of COL17A1 rescues skin organ ageing, thereby indicating potential new angles for anti-ageing therapeutic intervention.

Keywords: epidermal stem cells, aging, cell competition, epithelial tissues, epidermis

SATURDAY, JUNE 27, 00:05 – 00:20

00:05 - 00:20

MUSCLE STEM CELL SELF RENEWAL IS REGULATED BY ACETYLATION OF PAX7

Rudnicki, Michael

Ottawa Hospital Research Institute, Ottawa, ON, Canada

Acetyl-CoA is decreased and NAD+ levels increased under conditions of caloric restriction, which also have been suggested to have anti-aging effects. Interestingly, caloric restriction stimulates an increase in muscle stem cell number and engraftment capability in skeletal muscle. However, the precise mechanisms through which acetyl-CoA and NAD+ act to control muscle stem cell self-renewal remain obscure. The transcription factor PAX7 is a critical regulator of multiple muscle stem cell functions including self-renewal. We have determined that PAX7 is acetylated on two conserved lysine residues, and that acetylation is required for the efficient binding of PAX7 to chromatin and for full transcriptional activity. More specifically, acetylation of PAX7 is required for the binding of PAX7 to homeobox-containing target genes, and not to paired box or paired box-homeobox-containing target genes. Moreover, lack of acetylation of PAX7 results in down-regulation of target genes regulated by homeobox-only binding domains. We identified the acetyltransferase MYST1 and the deacetylase SIRT2 as responsible for the deposition and removal of the acetylation mark on PAX7 protein, respectively. We demonstrated that MYST1 and SIRT2 regulate the balance between symmetric versus asymmetric satellite cell divisions, thus controlling satellite stem cell fate. Correspondingly, loss of PAX7 acetylation disrupts the self-renewal balance of satellite cells, by favoring symmetric over asymmetric stem cell divisions. Consequently, disrupting PAX7 acetylation leads to expansion of the muscle stem cell pool and impairs muscle regeneration. Notably, SIRT2 is a NAD-dependent lysine deacetylase, and MYST1 lysine acetyltransferase activity is dependent on Acetyl-CoA levels. Accordingly, the level of PAX7 acetylation is closely linked to the metabolic state of muscle stem cells. These new findings demonstrate that Acetyl-CoA and NAD+ availability not only influences the global status of histone acetylation, but also regulate the transcriptional networks downstream of PAX7, a master regulator of muscle stem cell fate. Thus, our findings support a PAX7-dependent mechanism for fed-state and aging effects on muscle stem cell self-renewal.

Keywords: Muscle stem cell; PAX7; Ageing

00:25 – 00:33

PROFILING OF CHROMATIN ACCESSIBILITY IN HUMAN INDUCED NEURONS IN ALZHEIMER'S DISEASE

Lucciola, Raffaella[1], Mertens, Jerome[1,2], Linker, Sara[1], Schafer, Simon[1], Schlachetzki, Johannes[3], Herdy, Joseph[1], Bhnke, Lena[1,2], Traxler, Larissa[1,2], Reid, Dylan[1], Lee, Hyungjun[1], Zangwill, Dina[1], Fernandes, Diana[1], Yuan, Shauna[4], Glass, Christopher[3], Paquola, Apua[1,5], Goldstein, Lawrence[4], Galasko, Douglas[4], Gage, Fred[1]

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Alzheimer's disease (AD) is a neurodegenerative disorder that ultimately results in impaired cognitive functions. Characterizing chromatin organization and dynamic epigenetic code governing cell identity will accelerate our understanding of the mechanistic basis of the pathology and ultimately contributes to the development of targeted pharmacological approaches to treat the disease. In contrast to iPSC-derived neurons, directly fibroblast induced neurons (iNs) retain age signature and therefore represent an attractive model to study patient-specific AD features. Here we report on results from Assay for Transposase Accessible Chromatin with high-throughput sequencing (ATAC-seq), which is a robust and scalable chromatin profiling technique, that was used to map the genomic landscape of iNs directly derived from fibroblasts obtained from healthy (n = 10) and AD subjects (n = 10). Overall, we identified a consistent trend where AD iNs had more open chromatin compared to controls with 10,237 regions of accessible chromatin uniquely mapped in AD iNs compared to control. Interestingly, this signature of open-chromatin was largely independent of the position along the gene, indicating a genome-wide shift in chromatin structure in the context of AD. GO analysis of genes associated to these differential opening peaks showed enrichment for GO terms including 'nervous system development'. We clustered all the differential ATAC-seq peaks based on their genomic locations and further examined their degree of openness. Data confirmed that AD iNs are more open than controls and interestingly revealed that opening peaks located in intergenic and other non-coding regions are more accessible than regions located close to the TSS, that may reflect access to enhancer sites and may facilitate or prevent interactions between different and related genes. Next, we assessed enriched motifs within AD-associated ATAC-peaks. This extended annotation

revealed an enrichment for 85 binding sites for various transcription factors (TFs), including KLF9, CHOP and CEBP, matching the induction of KFL9, CHOP and CEBPB. Taken together, our study has provided new insights into the cis-regulatory chromatin landscape of neuronal cells during AD-related neurodegeneration.

Keywords: Alzheimer, neurons, ATACseq

00:35 – 00:43
A DIET-INDUCED PPAR/FAO AXIS ENHANCES STEMNESS AND ESTABLISHES A METABOLIC LIABILITY IN ADULT INTESTINAL STEM CELLS

Mana, Miyeko, Bahceci, Dorukhan, Hussey, Amanda and Yilmaz, Omer

Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology (MIT), Cambridge, MA, USA

Diet is an established risk factor for multiple diseases and comorbidities. How diet impacts stem cell function and alters tissue homeostasis that precipitates disease is now gaining more attention for prevention and therapeutic potential. In the mammalian intestine, a pro-obesity diet (high fat diet, HFD) promotes stem cell self-renewal and tumorigenesis by increasing intestinal stem cell (ISC) number and niche-independent growth. Enhanced activity of peroxisome proliferator-activated receptor delta (PPAR-d) is sufficient to recapitulate the effects that a HFD has on these ISCs. In the current study, we genetically ablate PPAR-d in the intestine to find that its loss is compensated by invoking PPAR-a activity. The combined genetic loss of PPAR-d and PPAR-a reverts the HFD ISC phenotype. In the HFD ISCs, the PPARs instruct a fatty acid oxidation (FAO) program necessary to increase intestinal stemness, whereby loss of mitochondrial fatty acid import into the mitochondria through Cpt1a reduces stem cell number and clonogenic capacity. Abrogated FAO by absence of functional Cpt1a diminishes tumor induction after removal of the tumor suppressor Apc, and pharmacologic inhibition of Cpt1a in established adenomas inhibits tumor progression in the HFD state. These findings highlight that a PPAR/FAO axis is employed in ISCs under a pro-obesity diet and suggests that adaptation to FAO renders stem cells vulnerable to FAO inhibition resulting in a targetable or exploitable aim for therapeutic benefit.

Funding source: American Cancer Society

Keywords: metabolism, diet, cancer

00:50 – 01:10
ISSCR TOBIAS AWARD LECTURE: IMMORTAL HEMATOPOIETIC STEM CELLS IN AGING AND CANCER

Goodell, Margaret A.

Baylor College of Medicine, Houston, TX, USA

The peripheral blood is composed of many different cell types which are constantly being replenished. When young, thousands of hematopoietic stem cells residing in the bone marrow are simultaneously working to regenerate the blood. Over the past few years, high throughput sequencing of peripheral blood cells from a large number of individuals has revealed that as we age, one or a few stem cells start dominating blood production, resulting in a condition termed “clonal hematopoiesis”, or “CH”. CH is increasingly common above the age of 70, and correlates with a higher chance of developing hematologic malignancies, as well as a higher rate of mortality due to other age-related diseases. While the drivers, other than age, of CH are poorly understood, somatically acquired mutations in around 20 genes are repeatedly observed. This has led to a concept of constant Darwinian competition between stem cells in the bone marrow, with selection over time for those that are most adapted to thrive in the aging environment. The stem cells thus acquire characteristics of “immortality”, even as the host is aging. The gene encoding DNA methyltransferase 3A (DNMT3A) is the most commonly mutated gene in CH, indicating that loss of its function confers longevity on the stem cell, even as it puts the host at risk for age-associated diseases. Dr. Goodell will discuss some of the cellular and molecular mechanisms that drive CH in the context of aging and DNMT3A mutations, and why individuals with CH are predisposed to cancer. Finally, Dr. Goodell will discuss how aging, more broadly, may be influenced by CH.

Keywords: Hematopoiesis, epigenetics, aging

SATURDAY, JUNE 27, 08:00 – 10:05

PLENARY VI: REPROGRAMMING AND REGENERATION

08:05 – 08:20
CELL TYPES OF THE HUMAN RETINA AND ITS ORGANOIDS AT SINGLE-CELL RESOLUTION

Roska, Botond

Institute of Molecular and Clinical Ophthalmology Basel, Switzerland

How closely human organoids recapitulate cell-type diversity and cell-type maturation of their target organs is not well understood. We developed human retinal organoids with multiple nuclear and synaptic layers. We sequenced the RNA of over 200’000 single cells from these organoids at six developmental time points and from the periphery, fovea, pigment epithelium and choroid of light-responsive adult human retinas, and performed histochemistry and functional imaging. Cell types in organoids matured in vitro to a stable ‘developed’ state at a rate similar to human retina de-

velopment in vivo and the transcriptomes of organoid cell types converged towards the transcriptomes of adult peripheral retinal cell types. The expression of disease-associated genes was significantly cell-type specific in adult retina and cell-type specificity was retained in organoids. Activation of cone photoreceptors evoked increases or decreases of neuronal activity in organoid cells in the inner nuclear and ganglion cell layers suggesting that cell types in retinal organoids are synaptically connected. We identify cellular targets for studying disease mechanisms in organoids and for targeted repair in adult human retinas.

Keywords: Human, Retina, Organoid

08:25 – 08:40
CARDIAC REPROGRAMMING: LEVERAGING BASIC SCIENCE FOR TRANSLATIONAL APPLICATION

Qian, Li

McAllister Heart Institute, University of North Carolina, Chapel Hill, NC, USA

The incidence of myocardial infarction is the leading cause of morbidity and mortality around the world. The underlying pathology is typically loss of cardiomyocytes that leads to heart failure. Over the years, we have worked on understanding the molecular mechanisms underlying direct cardiac reprogramming where endogenous cardiac fibroblasts were converted into cardiomyocyte-like cells (iCMs) to replenish the lost cardiomyocytes in infarcted hearts. By leveraging the knowledge that faithful cell fate conversion requires a precise dosage and temporal expression of transcription factors, we identified the optimal ratio of cardiac reprogramming factors for more complete and efficient iCM generation. Hypothesizing that reprogramming involves significant chromatin reorganization, we profiled the epigenetic repatterning events during early iCM induction and identified epigenetic barriers to iCM conversion. More recently, we applied single-cell omics to overcome the difficulties of studying reprogramming due to the inherent nature of its heterogeneity and asynchrony. Our single cell transcriptomics and epigenomics approaches allowed us to reconstruct the route of iCM formation and uncover intermediate cell populations, regulatory pathways and genes potentially involved in iCM induction. Through these efforts, we have obtained novel insights into the transcriptional, posttranscriptional and epigenetic regulation of iCM reprogramming, and concomitantly improved the quality and yield of iCMs for future clinical application. We also anticipate that the experimental and analytical methods presented here, when applied in additional cell programming or reprogramming contexts, will yield crucial insights about cell fate determination and the nature of cell type identity.

Keywords: Cardiac reprogramming, fibroblast, cell fate

08:45 – 08:53
METABOLIC CONTROL OF WNT SIGNALING AND STEMNESS IN COLORECTAL CANCER

Tran, Thai Q.[1] and Kong, Mei[2]

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Colorectal Cancer (CRC) remains a leading cause of cancer death worldwide, likely due to a lack of fundamental understanding about the disease progression as well as targeted therapy. Constitutive activation of Wnt signaling, most frequently caused by APC mutations, is the driver of cancer stemness and CRC initiation. However, APC mutations alone only cause benign polyps and the environmental factors that drive the transition from benign polyps to adenocarcinomas remain to be a central puzzle in the field. Colon cancer cells are subjected to diverse metabolic fluctuations in the gut microenvironment, yet little is known about the role of metabolism in regulating intestinal stem cells and CRC tumourigenesis. Here, we show that environmental glutamine restriction further augments Wnt signaling in APC mutant intestinal organoids to promote stemness and leads to adenocarcinoma formation in vivo. Mechanistically, low glutamine conditions deplete intracellular alpha-ketoglutarate (aKG) levels leading to drastic epigenetic reprogramming in intestinal stem cells, especially on the Wnt target genes. In contrast to the role of low glutamine in driving intestinal stemness, the treatment of aKG, a glutamine-derived metabolite, can suppress Wnt signaling and restore cellular differentiation in genetically-engineered tumor organoids and patient-derived organoids. Importantly, we demonstrate that dietary supplementation of aKG in drinking water drives terminal differentiation in colon cancer cells, thereby inhibiting tumour growth in patient-derived orthotopic tumour models and extending survival in APC Min mice. With the use of “multi-omics” approaches including metabolomics, genome-wide epigenomics, and transcriptomics in organoids models, our study reveals how metabolic microenvironment impacts intestinal stemness and identify aKG as a potent antineoplastic metabolite for CRC.

Keywords: Wnt signaling, epigenetic, metabolism

08:55 – 09:10
RECENT PROGRESS IN iPS CELL RESEARCH AND APPLICATION

Yamanaka, Shinya

Gladstone Institutes and Center for IPS Cell Research & Application, Kyoto University, Kyoto, Japan

Induced pluripotent stem cells (iPSCs) can proliferate almost indefinitely and differentiate into multiple lineages, giving them wide medical application. As a result, they are being used for new cell-based thera-

pies, disease models and drug development around the world. In the basic research of iPSCs, our goal is to understand the mechanisms underlying pluripotency and reprogramming. We have revealed that not only transcription factors, but also post-transcriptional regulation is critical for cell fate determination. For example, we have demonstrated that NAT1 (eIF4G2), a translation initiation factor, plays multiple roles in human ESCs and iPSCs depending on the differentiation status. Our research will reveal the complex interaction between transcriptional control and post-transcriptional regulation in cells and contribute to innovative therapeutic options including gene and cell therapies. At CiRA, we are proceeding with an iPSC stock project in which clinical-grade iPSC clones are being established from healthy donors with homologous HLA haplotypes. HLA homo donors are associated with a decreased immune response to the transplant and therefore lower the risk of transplant rejection. In 2015, we started distributing an iPSC stock clone to organizations in Japan, and clinical study using the iPSC stock began for the people with AMD in 2017, and 1-year follow-up study of 5 cases turned out the safety of allogenic transplantation using iPSC stock. Additionally, clinical trial for Parkinson's disease started using the iPSC stock-originated neurons in 2018 and the first surgery to transplant dopaminergic progenitors into the brain was conducted at Kyoto University Hospital. The clinical application of iPSCs has already began. However, donors with HLA homozygous are rare. Therefore, as an alternative to HLA homo donors, our institute reported HLA gene-edited iPSCs, which could widen the range of patients who benefit from iPSC therapies. Over the past decade iPSC research has made great progress, moving forward toward innovative therapeutic options for the people with intractable diseases by application of new findings from basic science and back translation from clinics. However, there are still various hurdles to overcome before all patients benefit.

Keywords: Stem Cell Biology, Cellular Reprogramming, Regenerative Medicine

09:15 – 09:30
ANNE MCLAREN MEMORIAL LECTURE: WHAT'S NEXT FOR HUMAN GENOME EDITING POLICY?

Charo, R. Alta

University of Wisconsin, Madison, WI, USA

Since the late 2018 announcement that twin girls had been born with edited genomes, policy activity has accelerated at both the national and international levels regarding heritable germline editing. The UK Royal Society and the the US National Academies of Science and of Medicine have collaborated on outlining the preclinical work needed to determine if this could ever be safe and effective enough to be used. The World Health Organization stood up a committee to make recommendations for global governance of heritable editing, and of non-heritable somatic editing. Until now, less attention has been paid to the rapidly increasingly number of non-heritable applications already in clinical trial, which offer their own set of policy challenges. These include whether and how to make somatic applications logistical and financially feasible to a wide population; anticipation of off-label uses; prevention of medical tourism to under-regulated jurisdictions offering unproven interventions; and, for editing of a fetus in utero, a host of questions about the ethics of research and care involving pregnant women. This presentation will survey the existing landscape and offer some observations about what may come next.

Keywords: policy, law, genome

09:40 – 10:00
ISSCR ACHIEVEMENT AWARD LECTURE: DNA DAMAGE AND REPAIR IN THE NEURONAL LINEAGE

Gage, Fred H., Wang, Meiyan and Reid, Dylan

The Salk Institute for Biological Studies, La Jolla, CA, USA

Using cellular reprogramming to generate human cells of the neuronal lineage from healthy and diseased individuals, I will explore where in the genome DNA damage occurs as well as how extensively these events are distributed throughout the genome. Surprisingly, these damaging events are not randomly distributed, but rather are restricted to specific sites in the genome. There are different mechanisms for DNA damage to occur and some of the mechanisms are a result of the vulnerability of the regions of the genome where they occur. While DNA damage is robust, on a daily basis the genome has evolved a remarkable repair system that relentlessly surveys the genome for damaged events. This repair molecular machinery is fueled by energy that is subject to challenges from internal genetic mutations as well as external environmental perturbations. I will discuss how, in light of decreases in energy availability, the DNA repair machinery makes non-random choices as to which DNA damaged events to repair. These findings are related to cell fate and cell survival.

SATURDAY, JUNE 27, 16:00 – 18:30

PLENARY VII: CLINICAL INNOVATION AND GENE EDITING

16:25 – 16:40
JOHN MCNEISH MEMORIAL LECTURE: HUMAN STEM CELL MODELING TO CLINICAL TRIAL: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED PHASE II TRIAL OF EZOGABINE ON CORTICAL AND SPINAL MOTOR NEURON EXCITABILITY IN AMYOTROPHIC LATERAL SCLEROSIS

Wainger, Brian

Harvard Medical School and Massachusetts General Hospital, Boston, MA, USA

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive degenerative disease of the motor nervous system. Clinical neurophysiological studies of cortical and spinal motor neurons using threshold tracking nerve conduction studies (TTNCS) and transcranial magnetic stimulation (TMS), respectively, have consistently demonstrated motor neuron hyperexcitability, the largest effect being reduced short-interval intracortical inhibition (SICI) measured by TMS. Using induced pluripotent stem cells (iPSCs) derived from ALS patients cultured for 2-4 weeks in vitro, we previously observed motor neuron hyperexcitability and reduced survival in ALS compared to control motor neurons. Treatment with ezogabine (also known as retigabine), a potassium channel activator, mitigated both of these phenotypes. Given that ezogabine was already approved for epilepsy, with well-defined CNS penetration, established side effect and PK profile, we initiated a multi-center, randomized, double blind, placebo-controlled study to assess the effect of ezogabine on neurophysiological excitability measurements in ALS patients. 65 ALS participants were randomized to placebo, low (600 mg/d) or high (900 mg/d) target dose ezogabine treatment for 10 weeks. The primary efficacy outcome was the change in SICI. Secondary outcomes included safety, tolerability, and changes in additional TMS and TTNCS measures of motor neuron excitability, ALSFRS-R, vital capacity, and strength. The results showed reduction of both upper and lower motor neuron excitability with ezogabine treatment. Thus, the study validates the paradigm of rapid translation from iPSC modeling to clinical trial without the intermediate use of mouse models.

Keywords: Amyotrophic lateral sclerosis, clinical trial, neuronal hyperexcitability

16:45 – 16:53
PHASE I/IIA CLINICAL TRIAL OF HUMAN EMBRYONIC STEM CELL (HESC)-DERIVED RETINAL PIGMENTED EPITHELIUM (RPE) TRANSPLANTATION IN ADVANCED DRY FORM AGE-RELATED MACULAR DEGENERATION (AMD): INTERIM RESULTS

Reubinoff, Benjamin E.[1], Banin, Eyal[2], Reimann, Christopher[3], Barak, Adiel[4], Boyer, Daviv[5], Do, Diana[6], Ehrlich, Rita[7], Jaouni, Tareq[2], McDonald, Richard[8], Telander, David[9], Ben Shabat, Avi[10], Mones, Jordi[11], Angelini, Diana[12], Hogge, Gary[12] and Reubinoff, Benjamin[13]

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Transplantation of RPE cells may be of therapeutic benefit in AMD. We developed RPE cells from hESCs using cGMP directed differentiation. Safety and tolerability of these cells is being evaluated in a Phase I/IIa clinical study in patients with dry AMD and geographic atrophy (GA) (NCT02286089). We report accumulated safety and imaging data from subjects in the fully enrolled first 3 cohorts (n=12) and ongoing 4th cohort (n=4). RPE cells in suspension (OpRegen; 50-200k) were subretinally transplanted under local anesthesia to the worse vision eye using either pars plana vitrectomy (PPV) and retinotomy or via an alternative surgical approach utilizing a suprachoroidal route of access. RPE cells ready for onsite thawing and immediate transplantation have also been evaluated. Short course perioperatively systemic immunosuppression is used. Systemic and ocular safety is closely observed, and retinal function and structure are monitored using various imaging modalities. Cohorts 1-3 are in long-term follow-up. Dosing of cohort 4 is ongoing. Treatment has been well tolerated and there have been no unexpected adverse events (AEs) or treatment-related systemic serious AEs. Using PPV, the most common ocular AEs were the formation of predominately mild epiretinal membranes (ERM), though 2 severe ERM were surgically peeled. One

PPV-treated patient experienced a retinal detachment of unknown origin. All 3 events were successfully treated. Visual acuity improvement has been noted in all four patients of cohort 4 to date (10-25 letters), which has been maintained for over 15 months in some. In several subjects, within the area of RPE cell transplant, improvements of the ellipsoid zone and RPE layers at the border of GA, as well as directional growth changes in the area of GA, has been seen. Persistent changes observed following treatment include, alterations in drusen appearance, subretinal pigmentation and hyper-reflective areas, suggestive of the presence of transplanted RPE cells. In conclusion, subretinal transplantation of hESC-derived RPE cells in patients with dry AMD and GA appears well tolerated. Imaging findings suggest presence of transplanted cells in the subretinal space. Encouraging structural and clinical changes observed in some patients will require additional follow-up.

Funding source: Lineage Cell Therapeutics, Inc.

Keywords: Cell Therapy, Retinal Pigment Epithelium, Age - Related Macular Degeneration

16:55 – 17:03

SUPRAPHYSIOLOGIC ENZYME RECONSTITUTION BY TRANSPLANTATION OF GENETICALLY-ENGINEERED AUTOLOGOUS HEMATOPOIETIC STEM AND PROGENITOR CELLS SHOWS PRELIMINARY BENEFITS IN CHILDREN WITH HURLER DISEASE

Gentner, Bernhard[1], Bernardo, Maria Ester[2], Tucci, Francesca[2], Zonari, Erika[1], Fumagalli, Francesca[3], Pontesilli, Silvia[4], Silvani, Paolo[5], Volpin, Monica[1], Miglietta, Simona[1], La Marca, Giancarlo[6], Parini, Rossella[7], Montini, Eugenio[1], Naldini, Luigi[1] and Aiuti, Alessandro[2]

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Autologous transplant of ex vivo genetically-engineered hematopoietic stem and progenitor cells (HSPC) may allow widespread delivery of therapeutic proteins throughout the body, including the central nervous system. We are conducting a phase I/II clinical study in children affected by severe type-1 mucopolysaccharidosis (Hurler syndrome) caused by biallelic germline mutations in the IDUA gene. Following myeloablative conditioning, 8 patients were infused with autologous HSPC transduced with an IDUA-coding lentiviral vector (IDUA-LV) using a short, 36h transduction protocol with prostaglandin E2 as a transduction enhancer, yielding a median transduction efficiency above 80% and a median vector copy number of 2.2 [1.0-5.2]. In preclinical studies, xenotransplantation of the drug product showed equivalent engraftment potential as patient-matched, non-manipulated CD34+ cells and a highly polyclonal multi-lineage hematopoiesis (>1000 and 100 unique LV integrations per mouse) in primary and secondary recipients, respectively. This suggested that our novel transduction protocol overcame the negative impact of longer ex vivo manipulations on HSPC function. Indeed, hematopoietic recovery in the patients was fast (median engraftment of platelets on day+15 [12-16], and neutrophils on day +21 [16-27]), accompanied by the appearance of gene-marked cells and instauration of IDUA activity in the blood above upper level of normal, which was stable (median follow up: 8 months). IDUA activity was detectable in the cerebrospinal fluid, resulting from local engraftment of microglia-like cells derived from transduced HSPCs. Pathologic glycosaminoglycan storage progressively decreased, with a logfold reduction in CSF by 3-6 months post gene therapy and reduction to normal urinary excretion levels in most patients. These results suggest that gene therapy accomplishes extensive metabolic correction of peripheral and central compartments. Patient 1, who reached 18 months follow-up by January 2020, has shown a stable cognitive score, improved findings on brain and spine MRI, resumed growth velocity and an amelioration of his skeletal phenotype. In summary, the preliminary safety and efficacy results from our phase I/II study suggest therapeutic potential for the treatment of Hurler syndrome.

Keywords: Hematopoietic stem cell (HSC) gene therapy, Metabolic storage disorder MPS1 Hurler, Microglia osteoclasts bone disease

17:05 – 17:20

CAR T CELLS: THE EMERGENCE OF SYNTHETIC IMMUNITY

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Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Natural immune responses fall short of eradicating tumors in most cancer patients. T cell engineering offers a means to repurpose immune cells to perform enhanced therapeutic functions. Chimeric antigen receptors (CARs) are synthetic receptors that redirect and reprogram T cells to mediate tumor rejection. CARs that target CD19 offer the prospect of complete remissions in patients with chemorefractory, relapsed B cell malignancies, especially acute lymphoblastic leukemia (ALL). The US Food and Drug Administration approved the first CD19 CAR therapies in 2017. Despite high CR rates (>80%), a number of patients will eventually re-

lapse, pointing to the need to further improve CAR design and T cell engineering strategies to increase the functional persistence of CAR T cells and reduce their toxicities. Using CRISPR/Cas9, we found that directing a CAR to the T cell receptor alpha chain (TRAC) locus not only results in uniform CAR expression in human peripheral blood T cells, but enhances T cell potency by attenuating CAR tonic signaling and T cell exhaustion, enabling TRAC-CAR T cells to outperform conventionally engineered CAR T cells. Further analyses of the signaling properties of the 19-28z CAR have identified signaling components that more effectively control T cell persistence and acquisition of effector functions, yielding new CAR designs that are better suited to balance T cell memory and anti-tumor activity. These advances in genome engineering and CAR design are integral to creating safe and effective CAR T cell therapies to tackle solid tumors and extend the use of CAR T cells beyond oncology, eg for the treatment of senescence-associated pathologies. They also enhance our efforts to generate CAR T cells from induced pluripotent stem cells.

Keywords: CAR, T cell, immunotherapies

17:25 – 17:40
GENE THERAPY FOR GENETIC DISEASE: LESSONS LEARNED FROM CLINICAL DEVELOPMENT PROGRAMS

High, Katherine

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The field of gene therapy now has three decades of experience with clinical investigation, and at least 5 products have been approved as gene therapies for genetic disease by either the FDA, the EMA or both. What lessons have been learned from clinical experience in gene therapies with AAV and lentiviral vectors that may have applicability to gene editing technologies? This presentation will review issues that emerged during clinical development programs for gene therapy, including: that animal models do not always accurately predict findings in clinical studies, a challenge that is not limited to novel classes of therapeutics; that both gene therapy and gene editing are approaching diseases that previously lacked any treatments, and therefore may not have well-established clinical endpoints; and that if clinical efficacy is compelling, it may be possible for an investigational agent to be approved on a relatively small clinical dataset. The role of regulatory guidance documents will also be discussed.

Keywords: Gene therapy, clinical trials, regulatory approval

17:45 – 18:15
KEYNOTE ADDRESS: GENOME EDITING MEDICINES TO MIMIC MUTATIONS PROTECTIVE AGAINST HEART ATTACK

Kathiresan, Sekar

Verve Therapeutics, Cambridge, MA, USA

Over the past 20 years, we and others have deciphered the genetic basis of risk for or resistance to heart attack, the leading cause of death in the world. Inherited factors that confer risk include a mutation in a single gene conferring a large effect (monogenic model) as well as many common polymorphisms acting in aggregate (polygenic model). However, in some, inherited factors confer resistance to disease. Mutations that disrupt any of eight genes lead to lifelong low levels of plasma LDL cholesterol and/or triglycerides and dramatic protection against heart attack. These eight liver-expressed genes all play a role in the metabolism of lipoproteins. These observations raise the possibility of identifying patients whose heart attack has a genetic basis and modifying their risk through in vivo therapeutic genome editing to mimic the naturally occurring protective mutations. Verve Therapeutics is developing a once-and-done gene editing therapy that permanently lowers plasma cholesterol and/or triglycerides, thereby conferring enduring protection against heart attack.

Keywords: genome editing, coronary disease, cholesterol

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