THE FUTURE STARTS HERE

ISSCR2021.ORG

#ISSCR2021

21–26 JUNE 2021

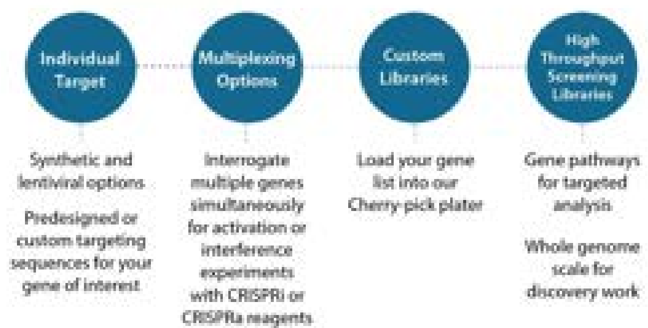
THE GLOBAL STEM CELL EVENT

PROGRAM GUIDE

Regardless of your genomic manipulation method, experimental scale, or where the work takes place, Horizon is your partner of choice

Manipulation Method

Experimental Scale



Capabilities

Gene Editing and Modulation Reagents

All the reagents you need to perform your gene editing and modulation experiments in your lab

Various formats of RNAi, CRISPRko/i/a, Orthogonal validation methods

Protocols and application notes specific to iPS cells

Proven designs with guarantees for editing and knockdown

Cell Line Engineering Services

Turnkey cell line engineering services to interrogate your gene of interest

The simplest to the most complex edits - knockout, SNP changes, knock-in of various reporters and tags

Access to over 200+ in-house cell lines

Onboard your cell line of interest free of charge

Screening Libraries

Investigate entire gene families or biological pathways

The reagents you need in high throughput format

CRISPR and RNAi

Gene families up to whole genome

Flexible vector formats

Horizon Discovery Innovation Showcase ISCCR Wednesday, June 23rd, 12-1pm EDT

- **Kevin Hemphill** CRISPRi and iPS cells
- **Amanda Haupt** Gene editing and modulation reagents
- **Yasmin Paterson** Deep dive into cell line engineering services and iPSCs
- **Max Blanck** Single Cell Screening services

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Dear Stem Cell Colleagues,

Welcome to ISSCR 2021! We are thrilled to introduce the new theme-based annual meeting, which has been completely redesigned to maximize the number of talks you can attend. The meeting is organized around five themes, representing key areas of stem cell research. Each theme program has been built by two co-chairs selected from the most prominent stem cell researchers in the world. Your program is their choice of "must knows" for 2021! You will experience seven signature Plenary sessions featuring outstanding leaders in the field, exciting emerging science across the five theme tracks, provocative Plenary Roundtable panels, and, for the first time, we are hosting an equity panel for an important conversation in the stem cell community.

The ISSCR has applied our rapid immersion in digital programming over the last 12 months to a new platform to deliver the 2021 Annual Meeting. This advanced digital meeting technology will help you discover meeting content through your preferences, take advantage of poster presentation options using live talks or recorded video, and participate in networking and career events in real-time with the chance to connect in groups in the Conversation Corner. There is so much this Annual Meeting has packed into six days, that we are rebroadcasting key sessions with live chat after their U.S. east coast debut time. This will make it easier for those of you in Asia, Australia, and the Pacific Coast of North America to participate while connecting live with colleagues in your regions. If you miss a session or two, you can take in the entire program on-demand for an entire month after the meeting concludes.

Yes, it's a fabulous new meeting organization! We will have five main themes: Tissue Stem Cells and Regeneration, Cellular Identity, Modeling Development and Disease, New Technologies, and Clinical Applications. Theme Sessions (formerly known as Concurrents) and Plenaries will identify with one of these core themes and each day of the meeting will focus on one or two themes. This makes it easier to find and network with scientists with your related interests. Just review the schedule to see the themes marked by icons - themes help make our large, comprehensive meeting smaller and easier to navigate, and provide the content you most care about in concentrated sessions.

Also new this year, we are hosting three Plenary Roundtables: The Fascination with Gastrulation: The Applications and Ethics of Modeling Early Development; Stem Cell Organoid Models as Empirical Testbeds for Personal Medicine Development – focusing on Cystic Fibrosis; and Engineered Tissues: Challenges to Bring to Clinic. These thought-provoking Plenary Roundtables are designed to welcome your discussion and input – come and talk to the experts!

ISSCR's 2021 scientific award honorees are each delivering a special lecture on different days throughout the program. We will hear from Valentina Greco, Madeline Lancaster, Stuart Orkin, and Janet Rossant about their amazing science and what's coming next.

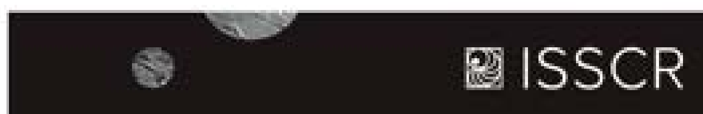
A full program of Focus Sessions and Innovation Showcases will introduce the latest tools, technologies, and techniques that are driving the field forward. We are particularly grateful to our sponsors and exhibitors who have taken this journey with us again in 2021 in a virtual format, and to our colleagues in Germany, we will be so thrilled to meet in person in Hamburg for the 2024 Annual Meeting. We appreciate the commitment and confidence all our members and partners have in the ISSCR.

There are countless opportunities to immerse yourself in stem cell science this week – and for the next month on-demand. Every year, the ISSCR annual meeting offers us the chance to learn, to reconnect with colleagues, and to uncover the potential of new collaborations. ISSCR 2021 remains The Global Stem Cell Event, inspiring the stem cell and regenerative medicine community worldwide. So, get ready for an extraordinary week of science!

Warmest wishes,

Christine L. Mummery
ISSCR President

Sally Temple
Program Chair



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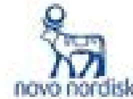

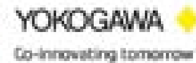

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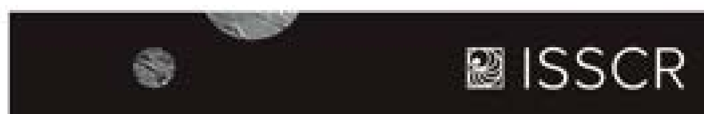

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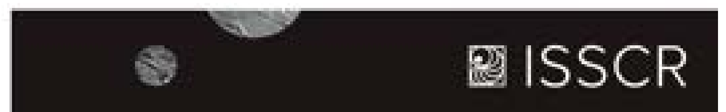

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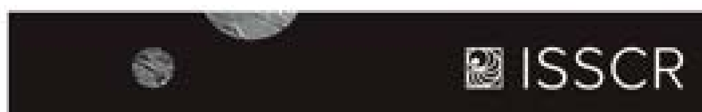

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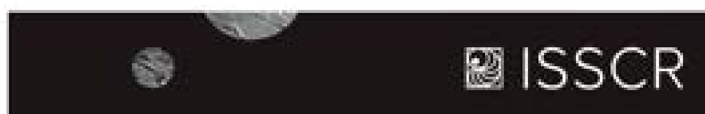

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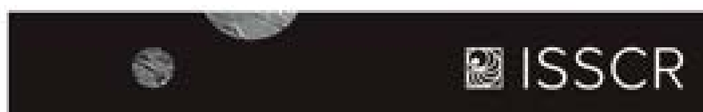
Tully Underhill
Achia Urbach
Ludovic Vallier
Nicole Van Bergen
Sara Vasconcelos
Gad Vatine
Carlos Villaescusa
Jane Visvader
Amy Wagers
Darcy Wagner
Jianlong Wang
Xiaoqun Wang
Claudia Waskow
Sara Wickström
Joseph Wu
Rongwen Xi
Wei Xie
Yasuhiro Yamada
Nan Yang
Zheng Yin
Jessica Young
Hui Zheng
Bin Zhou
Hao Zhu
Thomas Zwaka



AWARDS

JOIN US ON MONDAY, 21 JUNE FOR THE PRESENTATION OF THE 2021 ISSCR PUBLIC SERVICE AWARD

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 2021 recipient is Robin Lovell-Badge, PhD, FRS, The Francis Crick Institute, UK. Dr. Lovell-Badge is noted for his discovery, made in collaboration with Peter Goodfellow, of the SRY gene on the Y-chromosome that determines sex in mammals. They shared the 1995 Louis-Jeantet Prize for Medicine for their discovery. Dr. Lovell-Badge currently is a Senior Group Leader and Head of the Division of Stem Cell Biology and Developmental Genetics at the Francis Crick Institute in London. His work studies how stem cells that correspond to early embryo cells have the capacity to turn into many different cell types. His group also is studying how specific structures, such as the pituitary gland or the brain, develop as an embryo grows, and how these come to contain tissue-specific stem cells, and in turn what controls the fate of these stem cells.

For the last two years, Dr. Lovell-Badge has been leading a group of stem cell scientists, ethicists, and regulatory experts to update the ISSCR Guidelines for Stem Cell Research and Clinical Translation, driving international consensus around how to address rapid advances in the field.

The award will be presented during the Presidential Symposium on Monday, 21 June, 09:00-11:45 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 awards will be presented during the Presidential Symposium on Monday, 21 June, 09:00-11:45 EDT.

JOIN US ON TUESDAY, 22 JUNE FOR THE PRESENTATION OF THE 2021 ISSCR DR. SUSAN LIM AWARD FOR OUTSTANDING YOUNG INVESTIGATOR

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 and is supported by the Dr. Susan Lim Endowment Fund for Education and Research Ltd.

The 2021 recipient is Madeline Lancaster, PhD, The Medical Research Council (MRC) Laboratory of Molecular Biology, UK. Dr. Lancaster is a pioneer in the field of stem cell biology and was the first scientist to develop cerebral organoids as a model to study human brain development and disease. Her seminal breakthrough as a postdoctoral fellow inspired others and opened up a new area of research. Dr. Lancaster has published findings that demonstrate her combined knowledge of development, neuroscience, bioengineering, and application of new technology to generate more mature brain organoid models with impressive properties of long-distance connectivity. These models are needed in order to transition the field into investigation of human neuronal circuits and move the field forward in compelling ways.

Dr. Lancaster will present her research at a special lecture on Tuesday, 22 June, 13:15-13:45 EDT.

JOIN US ON WEDNESDAY, 23 JUNE FOR THE PRESENTATION OF THE 2021 ISSCR TOBIAS AWARD LECTURE

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 2021 recipient is Stuart H. Orkin, MD, Boston Children's Hospital, Dana-Farber Cancer Institute, and Harvard Medical School, USA.

Dr. Orkin, an Investigator of the Howard Hughes Medical Institute, is internationally recognized as a physician-scientist for his groundbreaking discoveries in many aspects of hematopoiesis and particularly as a pioneer in establishing the roles of transcription factors and genetic networks in hematopoietic cell differentiation. Over the past decade, he has used this knowledge to transform current understanding of erythroid differentiation and how fetal hemoglobin is silenced in development and can be experimentally reactivated via modulation of BCL11A function. This work forms the basis for novel gene therapy and gene editing approaches to provide cures for patients with the major hemoglobinopathies, sickle cell disease and ß-thalassemia.

Dr. Orkin will deliver the ISSCR Tobias Award Lecture on Wednesday, 23 June, 13:15-13:45 EDT.

JOIN US ON THURSDAY, 24 JUNE FOR THE PRESENTATION OF THE 2021 ISSCR ACHIEVEMENT AWARD

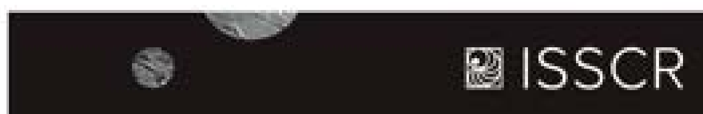
ISSCR ACHIEVEMENT AWARD

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 2021 recipient is Janet Rossant, PhD, FRS, FRSC, President and Scientific Director of Gairdner Foundation, Chief of Research and Senior Scientist, Research Institute, The Hospital for Sick Children, Toronto, Canada.

Janet Rossant is a leader in mammalian developmental and stem cell biology who has made seminal contributions to our understanding of early development and pioneered innovative technologies to manipulate the mouse genome. Dr. Rossant has been a leader in the ISSCR, including serving as president (2013-2014), and is a global influencer in stem cell research and its ethical implications for society.

Dr. Rossant will deliver the ISSCR Momentum Award Lecture at a special session on Thursday, 24 June, 13:15-13:45 EDT.



JOIN US ON FRIDAY, 25 JUNE FOR THE PRESENTATION OF THE 2021 ISSCR MOMENTUM AWARD

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 2021 recipient is Valentina Greco, PhD, Carolyn Walch Slayman Professor of Genetics at Yale School of Medicine and member of the Yale Stem Cell Center, USA. Studies from Dr. Greco's lab are redefining scientific understanding of the complex mechanisms that organize and regulate the skin stem cell niche and the behavior of normal and mutant cells in the epidermis under physiologic challenge and with aging. Her group's body of work exploring cell biology in vivo determined that the niche, rather than the stem cells, are required for tissue growth, that location in the niche dictates stem cell fate, that the niche exploits stem cell plasticity to maintain homeostasis, and that homeostatic correction battles disease emergence. These breakthroughs pave the way for new concepts in mammalian regenerative biology.

Dr. Greco will deliver the ISSCR Momentum Award Lecture at a special session on Friday, 25 June, 13:15-13:45 EDT.

CONGRATULATIONS TO THE 2021 ISSCR ZHONGMEI CHEN YONG AWARD WINNERS

2021 ISSCR ZHONGMEI CHEN YONG AWARDS FOR SCIENTIFIC EXCELLENCE

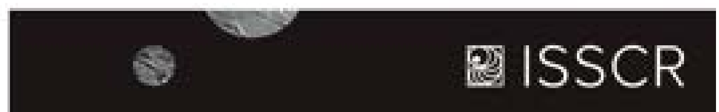
Supported by Chen Yong and the Zhongmei Group, the ISSCR Zhongmei Chen Yong Awards recognize scientific excellence and economic need for trainees who submit abstracts and present at the ISSCR Annual Meeting.

Alejandro Aguilera Castrejon
Nouraiz Ahmed
Juan Alvarez
Thomas Ambrosi
Madeline Andrews
Arianna Baggiolini
Marie Bannier-Hélaouët
Swarnabh Bhattacharya
Anine Crous
Olivia Cypris
Talya Dayton
Qiannan Deng
Laina Freyer
Kosuke Funato
Blair Gage
Laura Garcia Prat
Oliver Harschnitz
Luciana Isaja
Geraldine Jowett
Inseon Kim
Mirae Kim
Cody Kime
Azuma Kimura
Anja Knaupp
Milos Kostic
Shong Lau
Gabriel Linares
Kathryn Lye
Mona Mathews-Ajendra
Michela Milani
Yekaterina Miroshnikova
Fumika Moriya
Mika Nakayama
Toshiya Nishimura
Pascal Röderer
Iswarya Radhakrishnan
Sanjeev Ranade
Galina Schmunk
Irene Talon
Shoichiro Tani
Hiraku Tsujimoto
Rio Tsutsumi
Tomoya Uchimura
Adrian Veres
Holly Voges
Li-Tzu Wang
Lingyue Yang
Atilgan Yilmaz
Wenshu Zeng
Jing Zhao
Asmaa Zidan

LAWRENCE GOLDSTEIN POLICY FELLOWS

Fellows receiving abstract travel awards are:

Kirstin Matthews
Zubin Master



2021 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 2021 abstract reviewers.

Alejandro Aguilera Castrejon
Nouraiz Ahmed
Juan Alvarez
Thomas Ambrosi
Madeline Andrews
Arianna Baggiolini
Marie Bannier-Hélaouët
Swarnabh Bhattacharya
Sara Bizzotto
Olivia Cypris
Talya Dayton
Laina Freyer
Kosuke Funato
Blair Gage
Laura Garcia Prat
Oliver Harschnitz
Geraldine Jowett
Ava Keyvani Chahi
Inseon Kim
Cody Kime
Azuma Kimura
Anja Knaupp
Milos Kostic
Shong Lau
Gabriel Linares
Mona Mathews-Ajendra
Pascal Röderer
Iswarya Radhakrishnan
Sanjeev Ranade
Galina Schmunk
Hiraku Tsujimoto
Adrian Veres
Holly Voges
Atilgan Yilmaz
Jing Zhao

PRESIDENTIAL SYMPOSIUM

Sponsored by: BlueRock Therapeutics

Elaine Fuchs, Rockefeller University, USA

Dr. Elaine Fuchs, PhD is renowned for her research in skin biology, its stem cells and associated genetic disorders, particularly cancers. Following her Ph.D. from Princeton and postdoctorate at MIT, Fuchs joined the faculty at University of Chicago. In 2002, she relocated to Rockefeller University, where she is an Investigator of the Howard Hughes Medical Institute. Her awards include the National Medal of Science, L'Oreal-UNESCO Award, Albany Prize in Medicine, March of Dimes Prize, EB Wilson Award in Cell Biology, Vanderbilt Prize, AACR Clowes Award and ISSCR McEwen Award. Fuchs is an honorary member of the National Academy of Sciences, National Academy of Medicine, American Philosophical Society, Pontifical Academy of Sciences and the Royal Society. Her honorary doctorates include Harvard University. She is a past-President of the ISSCR.

Meritxell Huch, Max Planck Institute of Molecular Cell Biology & Genetics, Germany

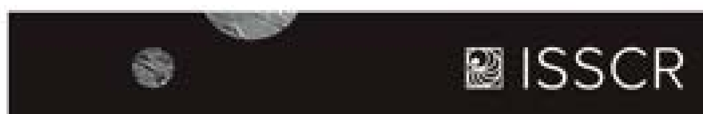
Dr Meritxell Huch, PhD is a Lise Meitner Max Planck Research Group Leader at the Max Planck-MPI-CBG, in Dresden. She obtained her PhD at the CRG in Barcelona and moved to the Netherlands to study with Professor Hans Clevers. She pioneered the work in organoids by obtaining the first gastric, liver and pancreas organoids from adult tissue-resident cells. In 2014 started her lab at the Gurdon Institute (Cambridge) and in 2019 moved to the MPI-CBG, in Dresden. Her research focuses on understanding tissue regeneration and its implication in disease. Her lab has established the first human liver cancer organoid model; described that hepatoblasts are heterogenous and can be clonally expanded as organoids and identified that global epigenetic remodelling is crucial to initiate cellular plasticity during regeneration.

Charles E. Murry, University of Washington, USA and Sana Biotechnology, USA

Dr. Chuck Murry received his MD-PhD at Duke University and residency training in Pathology at the University of Washington, followed by fellowships in vascular biology and diagnostic cardiovascular pathology. He is currently a professor in the departments of Laboratory Medicine & Pathology, Bioengineering, and Medicine/Cardiology at the University of Washington. Dr. Murry's research focuses on stem cell biology, with an emphasis on understanding differentiation of the human cardiovascular system and using these cells to study diseases and to regenerate damaged tissues. His group is a world leader in heart regeneration and is working toward a clinical trial of cardiomyocyte therapy. Murry is a member of the ISSCR's Board of Directors and serves on the Clinical Translation Committee. Most recently, Dr. Murry moved part of his research group to Sana Biotechnology, where he now serves as Senior VP and Chief Scientific Officer for Cell Therapy. His team at Sana is working to bring stem cell-based heart regeneration into clinical trials.

Lorenz Studer, Sloan-Kettering Institute for Cancer Research, USA

Lorenz P. Studer, MD, is the Director of the Center for Stem Cell Biology and a Member of the Developmental Biology Program at the Memorial Sloan Kettering Cancer Center. His lab has established techniques for turning human pluripotent stem cells into the diverse cell types of the nervous system. He has been among the first to realize the potential of patient-specific stem cell in modeling human disease and developed strategies to manipulate cellular age in pluripotent-derived lineages. Finally, he currently leads a multidisciplinary consortium to pursue the clinical application of human stem cell-derived dopamine neurons for the treatment of Parkinson's disease. Recent awards related to those studies include a Macarthur Fellowship, the Ogawa-Yamanaka Prize and the Jacob Heskel Gabbay award in Biotechnology and Medicine.



ANNE MCLAREN MEMORIAL LECTURE

Tuesday 22 June, Plenary II

Susana Chuva de Sousa Lopes, Leiden University Medical Center, Netherlands

Susana M. Chuva De Sousa Lopes, PhD is Full Professor Human Developmental Biology at the Department of Anatomy and Embryology, Leiden University Medical Center, the Netherlands and appointed Guest Professor at the Department of Reproductive Medicine, University Ghent, Belgium. Her lab is mainly focused on the urogenital system, in particular the germ cells, and how the (epi)genetic information is transmitted from generation to generation. She is engaged in the development of assays leading to the maturation of oocytes from ovarian material (artificial ovaries) and in vitro gametogenesis. She was awarded the de De Snoo-van't Hoogerhuijs prize and Aspasia awards, is funded by ERC and VICI grants and is the current coordinator of the Special Interest Group "Stem Cells" of the European Society of Human Reproduction and Embryology (ESHRE).

ERNEST MCCULLOCH MEMORIAL LECTURE

Tuesday 22 June, Plenary II

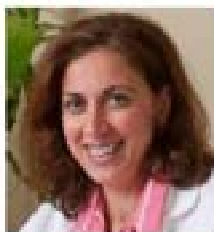
Sean J. Morrison, UT Southwestern, USA

Dr. Sean Morrison's laboratory studies the cellular and molecular mechanisms that regulate stem cell function and the role these mechanisms play in cancer. Dr. Morrison completed a B.Sc. in biology and chemistry at Dalhousie University (1991), a Ph.D. in immunology at Stanford University (1996), and a postdoctoral fellowship in neurobiology at Caltech (1999). Dr. Morrison is a Howard Hughes Medical Institute Investigator (since 2000), the founding Director of Children's Research Institute at the University of Texas Southwestern Medical Center (since 2011) and was elected to the National Academy of Medicine in 2018. Morrison served as president of the ISSCR and has been active in public policy issues surrounding stem cell research, testifying before the U.S. Congress, and serving as a leader in the successful "Proposal 2" campaign to protect and regulate stem cell research in Michigan's state constitution.

JOHN MCNEISH MEMORIAL LECTURE

Saturday 26 June, Plenary VII

Viviane Tabar, Memorial Sloan-Kettering Cancer Center, USA



Viviane Tabar, MD is the Chair of the Department of Neurosurgery and the Theresa C Feng Professor in Neurosurgical Oncology at Memorial Sloan Kettering Cancer Center in New York. She is a neurosurgeon with expertise in brain tumors, and a scientist with a focus on stem cell biology. Dr Tabar's research is focused on harnessing the regenerative potential of human stem cells towards repairing the brain, and as tools to dissect tumorigenesis mechanisms in brain tumors. Her lab portfolio includes the development of human pluripotent stem cell-derived dopamine neurons for Parkinson's disease, as well as therapies for chemotherapy and radiation therapy-induced brain injury. The lab has also pioneered the use of human embryonic stem cells to model brain tumors. Dr Tabar is a member of the National Academy of Medicine.

KEYNOTE ADDRESS

Saturday 26 June, Plenary VII

Sponsored by: bit.bio

Shinya Yamanaka, Gladstone Institutes, USA/CiRA, Kyoto University, Japan

Professor Shinya Yamanaka, MD, PhD, is the Director of the Center for iPS Cell Research and Application (CiRA), Kyoto University and a Senior Investigator at the Gladstone Institutes in San Francisco. He is also a Representative Director of Public Interest Incorporated Foundation, CiRA Foundation. He is most recognized for his original research on induced pluripotent stem (iPS) cells. Since his breakthrough finding, he has been the recipient of many prestigious awards, including the Nobel Prize in Physiology or Medicine jointly with Dr. John Gurdon (2012). Human iPS cells and their derivatives offer a new model for disease modeling, drug discovery, and regenerative medicine. His primary vision is to overcome diseases by delivering iPS cell-based innovative therapeutic options.

ISSCR

NEVER STOP

SUPPORTING LIFE SCIENCE RESEARCH

Uncovering the mechanisms of human disease requires years of dedication and access to the right tools. That's why we'll never stop encouraging therapeutic innovation by developing and supplying critical equipment and materials for researchers and academics. Our tissue-specific human cell products, cell culture media and unique laboratory reagents are designed to support life science research. By accelerating the availability of new treatments and diagnostics, we are helping to make the world a healthier place.

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Value from Innovation

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NETWORKING & EVENTS

What better way to nurture your research and career than networking? ISSCR 2021 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. The ISSCR understands our members' needs and offers various avenues to help scientists foster and strengthen their professional networks.

GAMIFICATION

Start earning points the moment you log into the event platform by completing your Profile. Some of the ways you can earn points include:

- Scheduling one on one meetings with colleagues
- Attend sessions
- Submit a question in a session
- Visit with exhibitors
- Interact with poster presenters in the Poster Theater
- Attend a Conversation Corner

The more you engage each day the more points you can earn. You can check your current point status by looking at the upper right corner of the platform page and clicking on the lightning bolt.

Prizes

The points leaderboard is sponsored by Astellas.

- 1st Place wins complimentary registration to the 2022 Annual Meeting and the opportunity to be featured in an ISSCR News Member Spotlight.
- 2nd & 3rd Place win complimentary registration to the 2022 Annual Meeting.
- Winners will be selected at the end of the program and will be notified via email.

EXHIBITORS AND SPONSORS

Network with industry professionals from over 50 partnering companies. Explore the possibilities on pages 68-79. Take advantage of networking with exhibitors at their virtual booths.

POSTER SESSIONS

Click Poster Theater in the virtual meeting platform to access posters and chat with poster presenters during their poster presentation time. All times are listed in EDT (Boston-USA).

Poster Session 1, 6/22/2021, 12:00- 13:00:

New Technologies
Tissue Stem Cells and Regeneration

Poster Session 2, 6/23/2021, 00:00- 01:00:

New Technologies
Tissue Stem Cells and Regeneration

Poster Session 3, 6/23/2021, 12:00- 13:00:

New Technologies
Tissue Stem Cells and Regeneration

Poster Session 4, 6/24/2021, 00:00- 01:00:

New Technologies
Tissue Stem Cells and Regeneration

Poster Session 5, 6/24/2021, 12:00- 13:00:
Cellular Identity
Modeling Development and Disease

Poster Session 6, 6/25/2021, 00:00- 01:00:
Cellular Identity
Modeling Development and Disease

Poster Session 7, 6/25/2021, 12:00- 13:00:
Cellular Identity
Modeling Development and Disease

Poster Session 8, 6/25/2021, 13:45- 14:45:
Clinical Applications

Poster Session 9, 6/26/2021, 00:00- 01:00:
Cellular Identity
Modeling Development and Disease

Poster Session 10, 6/26/2021, 01:45- 02:45:
Clinical Applications

CONVERSATION CORNER

ISSCR 2021 Virtual attendees will have the opportunity to engage in conversation through virtual discussion forums in ISSCR Central.

Sponsored by:

Meet the Editors of *Stem Cell Reports*

TUESDAY, 22 JUNE 07:00 – 07:30 EDT

Meet the editors of *Stem Cell Reports* to discuss new trends in scientific publishing, including the growth of open access, the increasing role of preprint servers, and the role of social media in disseminating research findings. There will be an opportunity for you to ask questions about *Stem Cell Reports* and publishing in your society's journal.

Early Career Scientists Networking

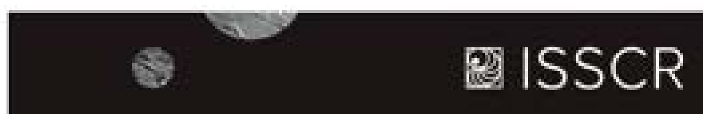
TUESDAY, 22 JUNE 15:45-16:15 EDT

Join us for a conversation around the impacts of COVID-19 and strategies for moving forward. How has this pandemic pushed you to reevaluate your priorities? What are some of the challenging decisions you have had to make? Join the Early Career Scientist Committee to chat about how your peers are navigating their research and career options in these challenging times. Tell us if and how COVID-related situations have changed your priorities and work life balance. Come hear how others have adapted their decision-making as we continue to push forward into post-pandemic times.

Computational Stem Cell Biology

WEDNESDAY, 23 JUNE 07:00 – 07:30 EDT

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.

Policy, Ethics, and Regulatory Issues

WEDNESDAY, 23 JUNE 15:45-16:15 EDT

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.

German Stem Cell Network and European Network of Networks (EuroNet)

THURSDAY, 24 JUNE 07:00 – 07:30 EDT

The German Stem Cell Network (GSCN) and the European Network of Networks (EuroNet) invite interested scientists to join the conversation corner to get information on new developments and discuss your needs and wishes.

Industry Scientists Networking

THURSDAY, 24 JUNE 15:45-16:15 EDT

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.

STEMCELL Technologies: Getting Started with Expansion and Scale-Up of hPSCs in 3D Suspension Culture

FRIDAY, 25 JUNE 15:45-16:15 EDT

Come and discuss your successes and challenges in 3D hPSC culture with Dr. Eric Jervis, who has 5+ years of experience developing suspension culture media for hPSCs. Bring your questions and let's discuss the common issues researchers face when moving their hPSC cultures into suspension as well as next steps for the field. This is a great opportunity to connect stem cell biologists with bioengineering solutions.

CAREER LAB

Interested in new career opportunities? Explore open positions in the ISSCR Career Lab. Meet virtually with fellow attendees currently hiring for academic and industry positions in stem cell science. Attendees can browse details of open positions and set up appointments to meet virtually via live video or message chat during ISSCR 2021. Open positions that match the tags in your profile will be highlighted for you.

Sponsored by:

CAREER PANEL

HOW HAS THE PANDEMIC CHANGED THE DECISION-MAKING PROCESS REGARDING YOUR EARLY CAREER GOALS AND OUTLOOK

Sponsored by:

These are certainly challenging times. This crisis has made it hard enough to navigate the daily routine, let alone deal with unpredictable slowdowns in research. The past year has changed the way we perceive and look at the future, leading many to reevaluate their paths. How do you make decisions about your career during a pandemic? Do you

change your goals, your timeline, the order of priorities to bear in mind when making THOSE decisions? Come listen to our panelists tell the stories about how they coped or drove changes in their career during this last crazy year. Find inspiration in how to make your next move, and have your questions answered on how you can shape your choices in a turbulent moment.

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

MODERATOR

Stephanie Luff
Icahn School of Medicine at Mount Sinai, USA
Member, ISSCR Early Career Scientist Committee

PANELISTS

Jonathan Hoggatt
Moderna Therapeutics, USA/Massachusetts General Hospital–Harvard Medical School, USA

Sruthi Purushothaman
The Hospital for Sick Children, Canada

Ting Xie
Hong Kong University of Science and Technology, Hong Kong

EARLY-CAREER GROUP LEADER PANEL

Sponsored by:

MOVING FORWARD: REGAINING MOMENTUM AND MAKING THE MOST OF SCIENCE POST-COVID

The last year has left deep impacts on the scientific enterprise, with especially strong effects on Early Career Researchers (ECRs) who are in the process of building their research programs toward publication, funding, and promotion. Join the Early Career Scientist Committee for a panel discussion about strategies to regain momentum, renew collaborations, and build a strong research community. Expect an interactive experience where participants can contribute questions through live Q&A and chat discussion with other early career group leaders.

This session is designed for ISSCR members who are early-career research group leaders (principal investigators or junior faculty for eight or fewer years, but any registrant is welcome to attend.

MODERATOR

Angela Wu
Hong Kong University of Science and Technology, Hong Kong
Member, ISSCR Early Career Scientist Committee

PANELISTS

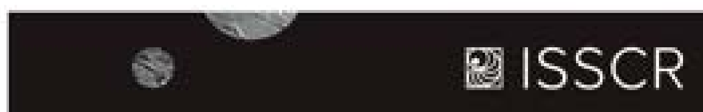
Katie Galloway
Massachusetts Institute of Technology, USA

Meritxell Huch
Max Planck Institute of Molecular Cell Biology & Genetics, Germany

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

PROMOTING EQUITY IN STEM CELL SCIENCE PANEL

Sponsored by:



DEFINING MOMENTS: ACHIEVING CAREER INDEPENDENCE

The ISSCR is dedicated to improving human health worldwide by advancing stem cell research and regenerative medicine. We are committed to supporting diversity and inclusion and promoting our entire community, which will accelerate scientific advancement and allow all people to realize the benefits of stem cell research. During this panel discussion, leading scientists will discuss how to promote equity in STEM, how to support scientists from historically underrepresented groups, and identify systemic changes that we can advocate for that will promote anti-racism in the field of stem cell science.

MODERATOR

Melissa Little
Murdoch Children's Research Institute, Australia

PANELISTS

Cato Laurencin
The University of Connecticut, USA

Shirley Malcom
American Association for the Advancement of Science, USA

Ubaka Ogbogu
University of Alberta, Canada

SCIENCE ADVOCACY AND COMMUNICATIONS SEMINAR

WEDNESDAY, 23 JUNE 12:00 – 13:00 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 J. Morrison
UT Southwestern, USA
Chair, ISSCR Public Policy Committee

SPEAKERS

Robin Lovell-Badge
The Francis Crick Institute, UK

Amander T. Clark
UCLA, USA

Jocelyn Kaiser
Science Magazine, USA

THE ISSCR GUIDELINES: 2021 UPDATES

WEDNESDAY, 23 JUNE 7:30 - 8:30

The ISSCR's updated Guidelines for Stem Cell Research and Clinical Translation provide new recommendations for advancing areas of research and application such as embryo research, embryo models, organoids, chimeras, mitochondrial replacement techniques, genome editing, and the development of therapies. The speakers will provide an overview of the new guidelines and discuss the rationale behind the recommendations.

CHAIR

Robin Lovell-Badge
The Francis Crick Institute, UK

SPEAKERS

Andy Greenfield
MRC Harwell Institute, UK

Melissa Carpenter
ElevateBio, USA

Janet Rossant
Gairdner Foundation & The Hospital for Sick Children, Canada

Insoo Hyun
Case Western Reserve University School of Medicine, USA

WOMEN IN SCIENCE PANEL

DEFINING MOMENTS: ACHIEVING CAREER INDEPENDENCE

Sponsored by:

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 third annual Women in Science Panel Discussion will be focused on transitioning to a leadership position. Hear diverse perspectives from an esteemed panel of successful female researchers who will share how they forged their own scientific path as principal investigator or company co-founder.

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

PANELISTS

Chee Yeun Chung
Yumanity Therapeutics, USA

Valentina Greco
Yale Stem Cell Center, USA

Madeline Lancaster
MRC Laboratory of Molecular Biology, UK

Janet Rossant
Gairdner Foundation/The Hospital for Sick Children, Canada

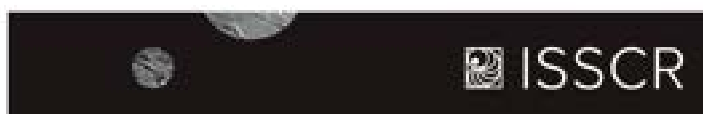
SPECIAL SESSIONS

SPECIAL SESSION 1: GERMAN STEM CELL NETWORK (GSCN) & BERLIN INSTITUTE OF HEALTH (BIH) SESSION 1

THURSDAY, 24 June 12:00- 13:00

Presented by German Stem Cell Network (GSCN) & Berlin Institute of Health (BIH)

Stem Cell Derivation by Directed Programming: Problems and Solutions
An important goal in the development and therapeutic application of stem cells is to program cells at will. One issue relates to whether programmed cells are derived directly from differentiated cells or first undergo a pluripotent state, as



pluripotent cell would have tumorigenic potential. We have shown that the neuro-specific factor Brn4, in combination with Klf4, Sox2, cMyc, can transduce fibroblasts directly into induced neural stem cells, without transiently acquiring pluripotency. Another potential issue with cell programming is that the epigenetic memory of the parental cell may not be completely erased. To test this, we generated induced oligodendrocyte progenitor cells (iOPCs) from various somatic sources. The iOPCs were then studied in vitro and applied as cell therapeutic agents in a myelin disorder. When fibroblasts were used, efficiency was low, and the resulting iOPCs had limited expansion and differentiation capacities. These limitations were overcome by transducing a permissive donor phenotype, the pericyte, with an optimized transcription factor combination. Pericyte-derived iOPCs were metastable, however, as they also reverted to their original identity in a context-dependent manner. Phenotypic reversion was closely associated with the memory of their original epigenome. As I will show, phenotypic reversion can be uncoupled from donor cell memory.

Presenters

Hans Schöler

Max Planck Institute for Molecular Biomedicine, Germany

Daniel Besser

German Stem Cell Network (GSCN), Germany

SPECIAL SESSION 2: GERMAN STEM CELL NETWORK (GSCN) & BERLIN INSTITUTE OF HEALTH (BIH) SESSION 2

FRIDAY 25 June 12:00- 13:00

Presented by German Stem Cell Network (GSCN) & Berlin Institute of Health (BIH)

Single-cell Proteo-genomic Reference Maps of the Hematopoietic System Enable the Purification and Massive Profiling of Precisely Defined Cell States

Single-cell genomics has transformed our understanding of complex cellular systems. However, excessive costs and a lack of strategies for the purification of newly identified cell types impede their functional characterization and large-scale profiling. Here, we have generated high content single-cell proteo-genomic reference maps of human blood and bone marrow that quantitatively link the expression of up to 197 surface markers to cellular identities and biological processes across all hematopoietic cell types in healthy aging and leukemia. These reference maps enable the automatic design of cost-effective high-throughput cytometry schemes that outperform state-of-the-art approaches, accurately reflect complex topologies of cellular systems, and permit the purification of precisely defined cell states. The systematic integration of cytometry and proteo-genomic data enables the interpretation of functional capacities of such precisely mapped cell states at the single-cell level. Our study serves as an accessible resource and paves the way for a data-driven era in cytometry.

Presenters

Simon Haas

Berlin Institute of Health (BIH), Germany

Daniel Besser

German Stem Cell Network (GSCN), Germany

FOCUS SESSIONS

Monday 21 June

13:00 – 16:00 EDT

Session agendas start at 0:00 for every session

TOOLS FOR BASIC AND APPLIED STEM CELL BIOLOGY

Organized by: Stem Cell COREdinates

Supported by: STEMCELL Technologies and Thermo Fisher Scientific

Stem Cell COREdinates (https://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". This year our Focus session will be joined by the Germany-based PluriCore network (https://gscn.org/en/RESOURCES/

GermanStemCellCores.aspx) that promotes the exchange of technologies, methods, and cooperation among iPSC Cores in Europe. The first part of the session will be presentations from COREdinate and PluriCore members and our sponsors that will cover advancements in different areas of expertise including reprogramming, gene editing, disease modeling, and laboratory robotic systems. The second part of the session will explore advancements in iPSC-derived organoid systems including SARS-CoV-2 research.

0:00 - 0:05 **Deborah French,** *The Children's Hospital of Philadelphia, USA*
Welcome from COREdinates

0:05 - 0:10 **Sebastian Diecke,** *Max-Delbrück-Centrum for Molecular Medicine (MDC) Berlin, Germany*
Welcome from Pluricore

0:10 - 0:25 **Valeria Fernandez-Vallone,** *Berlin Institute of Health at Charite - Universitätsmedizin Berlin, Germany*
Methods for Automated Single Cell Isolation and Sub-Cloning of Human Pluripotent Stem Cells

0:25 - 0:40 **Ilyas Singec,** *NIH Regenerative Medicine Program, USA*
Stem Cell Translation by Design

0:40 0:55 **Adam Hirst,** *STEMCELL Technologies, Canada*
Facilitating hPSC Single Cell Seeding Workflows Using CloneR™2

0:55 - 1:10 **Micha Drukker,** *Leiden University, Netherlands*
Deciphering the Logic of iPSC Manufacturing for Clinical Applications

1:10 - 1:25 **Erik Willems,** *ThermoFisher, USA*
Tools and Technologies for Facilitated Genome Editing in iPSCs

1:25 - 1:40 **Jared Churko,** *University of Arizona, USA*
SARS CoV-2 Infection of hPSC-Derived Cardiomyocytes

1:40 - 1:45 **Wenli Yang,** *University of Pennsylvania, USA*
Overview iPSC -Derived Organoid Development

2:00 - 2:15 **Patapia Zafeiriou,** *University of Gottingen, Germany*
Neuro-Cardiomyocyte Crosstalk in a Human iPSC-Derived Autonomically Innervated Cardiac Muscle Model

2:15 - 2:30 **Agnieszka Rybak-Wolf,** *Berlin Institute for Medical Systems Biology (BIMSB) Max-Delbrück-Centrum for Molecular Medicine (MDC) Berlin, Germany*
Disease/Viral Infection Modeling Using Brain Organoids

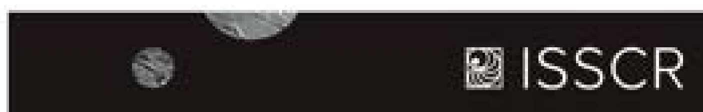
2:30 - 2:45 **Tim Blenkinsop,** *Icahn School of Medicine at Mount Sinai, NY, USA*
SARS CoV -2 Infection in The Human Eye and In an Eye Organoid Model

2:45 - 3:00 **Jessie Huang,** *Boston University, USA*
SARS CoV-2 Infection of Pluripotent Stem Cell-Derived Human Lung Alveolar Type 2 Cells Elicits A Rapid Epithelial-Intrinsic Inflammatory Response

DEVELOPMENTS TO SIMPLIFY AND ACCELERATE IPSC RESEARCH

Organized by: The European Bank for induced Pluripotent Stem Cells (EBiSC)

The European Bank for iPSCs (EBiSC) is a centralised repository, currently in a second project phase including both non-profit and commercial iPSC researchers (EBiSC2), working to make iPSC tools available and developing protocols which improve and simplify their use. This focus session will share how EBiSC2 partners are adapting and consolidating iPSC expansion, differentiation and cryopreservation approaches to help ease transition into high volume applications whilst also ensuring accessibility for non-expert users. We will discuss how the inclusion of iPSC tool lines in these protocol developments enables rapid generation of functionally mature derived cell types and how the associated iPSC datasets can be broadly shared in an ethically compliant manner. Lastly, common stumbling blocks will be discussed to raise awareness across the community.



0:00 - 0:05 **Julia Neubauer,** *Fraunhofer-IBMT, Germany*
Alfredo Cabrera-Socorro, *Janssen Pharmaceutica NV, Belgium*
Welcome and Overview

0:05 - 0:30 **Julia Neubauer,** *Fraunhofer-IBMT, Germany*
Approaches Towards Expansion, Differentiation and Banking of iPSCs at High Volume

0:30 - 0:55 **Mikkel Rasmussen,** *Bioneer, Denmark*
Emilie Lemesre, *Servier, France*
iPSC-Derived Hepatocytes in Drug Screening and Toxicology

0:55 - 1:20 **Alfredo Cabrera-Socorro,** *Janssen Pharmaceutica NV, Belgium*
Development of a Fully Human Neuronal and Astrocyte Co-Culture Assay Amenable for Electrophysiological Studies in Functionally Mature Neurons

1:20 - 1:45 **Benjamin Schmid,** *Bioneer, Denmark*
Gene-Editing in iPSCs - Unexpected Pitfalls: On-Target Effects

1:45 - 2:10 **Andreas Kurtz,** *Fraunhofer-IBMT, Germany*
Collection, Standardisation and Sharing of iPSC Associated Datasets Using Open Tools

2:10 - 2:35 **Eugenia Jones,** *Fujifilm Cellular Dynamics, USA*
Common Non-Scientific Challenges in the Generation, Use and Sharing of iPSC Lines

2:35 - 3:00 **Panel Discussion: Upcoming Challenges in iPSC Research from an Academic and Industry Perspective**

IMPROVING REPRODUCIBILITY AND SCALING-UP OF STEM CELL CULTURES

Organized by: Eppendorf AG

Technologies based on hiPSCs, MSCs and differentiated cells derived from them lay the basis for pioneering approaches in drug discovery and regenerative medicine. The routine use of stem cell-based applications requires high cell numbers in consistent quality. Standardized cell production requires a thorough understanding of how growth parameters impact cell growth and fate, and the ability to control these parameters. The supply of high cell numbers needs scalable culture systems. In this focus session Eppendorf will host experts from industry and academia to discuss methods for improving reproducibility and for scaling-up of stem cell cultivation. Our speakers will present examples for the benefits of process control using bioreactors. Experts will share their experience when translating cultivation from flasks and dishes to a three-dimensional bioreactor system. Furthermore, the session will offer insight into scale-up strategies using stirred-tank bioreactors.

0:00 - 0:05 **Philipp Nold,** *Eppendorf AG, Germany*
Welcome and Overview

0:05 - 0:08 **Leopold Koenig,** *TissUse GmbH, Germany*
My Motivation to Work with Stem Cells

0:08 - 0:33 **Leopold Koenig,** *TissUse GmbH, Germany*
Scalable Production of Neural Spheroids in a Suspension Bioreactor System For Multi-Organ-Chip Systems

0:33 - 0:36 **Margarida Serra,** *iBET, Portugal*
My Motivation to Work with Stem Cells

0:36 - 1:01 **Margarida Serra,** *iBET, Portugal*
Improving Differentiation and Maturation of hPSC into Functional Hepatocytes: A Biology-Inspired Approach

1:01 - 1:04 **Arie Reijerkerk,** *Ncardia Services BV, Netherlands*
My Motivation to Work with Stem Cells

1:04 - 1:29 **Arie Reijerkerk,** *Ncardia Services BV, Netherlands*
Large-Scale Manufacturing of hiPSC-Derived Cardiomyocytes For Cell Therapy

1:29 - 1:32 **Juline Guenat,** *Cell and Gene Therapy Catapult, UK*
My Motivation to Work with Stem Cells

1:32 - 1:57 **Juline Guenat,** *Cell and Gene Therapy Catapult, UK*
Development of An End-To-End Platform for the Controlled, Closed, Scalable And Cost-Effective Manufacture of Allogeneic Therapies Derived from Pluripotent Stem Cells in Aggregate-Based Cultures

1:57 - 2:00 **Ferdinand Biermann,** *Fraunhofer-Institut für Produktionstechnologie IPT, Germany*
My Motivation to Work with Stem Cells

2:00 - 2:25 **Ferdinand Biermann,** *Fraunhofer-Institut für Produktionstechnologie IPT, Germany*
Fully Automated Robotic Large-Scale Production and Analysis of Mesenchymal Stem Cells

2:25 - 3:00 **Panel: Ask the Experts: Strategies in Stem Cell Bioprocessing**

DRIVING CHANGE IN REGENERATIVE MEDICINE THROUGH GLOBAL PARTNERSHIPS

Organized by: Novo Nordisk A/S

In Stem Cell R&D our mission is to improve the lives of patients suffering from serious chronic diseases by developing innovative stem cell-based therapies. We want to be an industry leader in stem cell research and development – a rapidly developing area of far-reaching opportunity with the potential to address, or even cure, a number of serious chronic diseases. As a dedicated Transformational Research Unit, we combine the speed and agility of a small biotech with the quality of a major pharma company and we cover the full pharmaceutical value chain, often working in close collaboration with leading international scientists and with colleagues across Novo Nordisk. Novo Nordisk has been active in stem cell research for over 20 years in type 1 diabetes, and we are now expanding our project portfolio to include Parkinson's disease, dry age-related macular degeneration and chronic heart failure plus a number of early exploratory projects. At the session, we and some of our partners will present key projects we are working on and give an introduction to what it is like to work at Novo Nordisk and with Novo Nordisk in a partnership.

0:00 - 0:15 **Jacob Sten Petersen,** *Novo Nordisk, Denmark*
Welcome and Introduction to Novo Nordisk

0:15 - 0:35 **Agnete Kirkeby,** *Lund University, Sweden*
A Pluripotent Cell Therapy for Parkinson's Disease - Preparing for Clinical Trail

0:35 - 0:55 **Klearchos Papas,** *University of Arizona, USA*
Partnering for the Development of a Functional Cure for T1D

0:55 - 1:00 **Break**

1:00 - 1:20 **Fredrik Lanner,** *Karolinska Institute, Sweden*
CellThRPE1 - A Collaborative Path Towards Treatment of Age-related Macular Degeneration

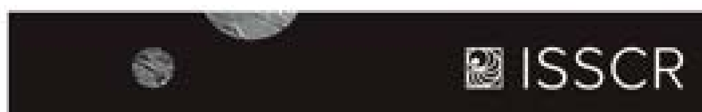
1:20 - 1:40 **Kikuo Yasui,** *Heartseed, Japan*
Innovative Cell Therapies to Treat Heart Failure

1:40 - 2:00 **Nico Lachmann and Robert Zweigerdt,** *Hannover Medical School, Germany*
Designer Macrophage Manufacturing & Therapeutic Applications in the Lung

2:00 - 2:25 **Melissa Little,** *Murdoch Children's Research Institute, CRI Australia*
Partnering to Drive Change in Stem Cell Based Therapies - Kidney, Engineering Collaboration

2:25 - 2:30 **Video**

2:30 - 3:00 **Q & A Panel discussion**



INNOVATIVE TECHNOLOGIES FOR DELIVERY OF CELL THERAPIES

Organized by: Sigilon Therapeutics, Inc.

Development of cell therapies, including stem cell-derived therapies, is a rapidly emerging field for treatment of a variety of diseases. Key considerations for cell-based products are shielding them from the immune system, mitigating the foreign body response, and maintaining viability and function. To address these considerations and leverage the potential of cell therapies, the session will focus on innovative approaches to enhance product delivery including encapsulation, genetic engineering, and modifications to improve the cellular niche.

0:00 - 0:15 **Olivia Kelly,** *Sigilon Therapeutics, Inc., USA*
Welcome and Overview

0:15 - 0:45 **Daniel Anderson,** *Massachusetts Insitute of Technology, USA*
Encapsulation Approaches for Cell Transplantation

0:45 - 1:15 **Omid Veiseh,** *Rice University, USA*
Functional Platforms of Implantable Devices for Clinical Applications

1:15 - 1:45 **Cherie Stabler,** *University of Florida, USA*
Development of Tissue Engineering Platforms for Improving Cell Therapies for Diabetes

1:45 - 2:00 **Break**

2:00 - 2:30 **Qizhi Tang,** *University of California, San Francisco, USA*
Co-Transplantation of Pancreatic Islets and Parathyroid Gland for Treatment Of Diabetes

2:30 - 3:00 **Tamir Rashid,** *Imperial College & King's College Hospital, UK*
Novel Platforms for Treating Liver Disease

THE SCIENCE AND ETHICS OF GENERATING GAMETES FROM STEM CELLS

Organized by: ISSCR Ethics Committee

Through in vitro gametogenesis (IVG) it is possible to create precursor cells to sperm and eggs in the lab using human stem cells. While it is yet to be established whether these cells can form fully functional human gametes, animal studies point to a future where this may be possible. As researchers refine IVG and its applications, it is important to consider what ethical boundaries ought to exist and whether researchers and regulators should define any limits. Join us to hear an expert panel discuss the latest scientific progress and important ethical and policy considerations. Topics will include challenges around evaluation of gamete functionality, ethical sources of cells and informed consent considerations, implications for nonhuman primate research, reproductive technologies, and future parentage arrangements. We will also discuss recent changes to the ISSCR Guidelines relevant to IVG. This is a timely opportunity to find out more about this fascinating area of stem cell research.

0:00 - 0:05 **Megan Munsie,** *University of Melbourne, Australia*
Welcome and Overview

0:05 - 0:20 **Katsuhiko Hayashi,** *Kyushu University, Japan*
Oogenesis In Vitro in Mice and Other Species

0:20 - 0:35 **Debra Mathews,** *Johns Hopkins Berman Institute of Bioethics, USA*
Reflections on the 2008 Hinxton Group Consensus Statement and a Decade of Scientific and Governance Evolution

0:35 - 0:50 **Amander T. Clark,** *University of California, Los Angeles, USA*
How Close Are We to Generating Human Sperm and Eggs in The Lab?

0:50 - 1:00 **Break**

1:00 - 1:15 **Heidi Mertes,** *Ghent University, Belgium*
Ethical Exploration of IVG: Between Innovation and Precaution

1:15 - 1:30 **Kyle Orwig,** *University of Pittsburgh, USA*
Spermatogonial Stem Cell Therapies for Male Infertility

1:30 - 1:45 **Insoo Hyun,** *Case Western Reserve University; Harvard Medical School, USA*
ISSCR Guidelines Revisions and What They Mean for Research and Policy Regarding IVG

1:45 - 2:40 **Panel Discussion**

2:40 - 2:45 **Megan Munsie,** *University of Melbourne, Australia*
Closing Remarks

WHAT TO KNOW BEFORE YOU GO: UNDERSTANDING CELL THERAPY PRODUCT DEVELOPMENT

Organized by The ISSCR Industry Committee

As new discoveries are quickly being translated into potentially transformative cell therapies, developing new treatments ultimately involves manufacturing stem cell-derived products. Manufacturing cell therapies, however, involves critical processes that are often unfamiliar to academic scientists driving research forward. This Focus Session will explore the important and necessary considerations for developing cell therapies, including key insights from seasoned cell therapy pioneers and regulators regarding what to know before you go, and how to get there.

0:00 - 0:05 **Felicia Pagliuca,** *Vertex Pharmaceuticals, USA*
Welcome and Overview: Part I | Clinical Cell Manufacturing: So You Think You Have a Cell Product?

0:05 - 0:25 **Melissa Carpenter,** *ElevateBio, USA*
End-to-End Product Development

0:25 - 0:45 **Derek Hei,** *Vertex Pharmaceuticals, USA*
Clinical Manufacturing

0:45 - 1:05 **Behnam Ahmadian Baghbaderani,** *Lonza, USA*
Process and Scaling

1:05 - 1:25 **Panel discussion**

1:25 - 1:35 **Break**

1:35 - 1:40 **Jennifer Moody,** *Pall Corporation, Canada*
Welcome and Overview: Part II | Navigating the Manufacturing and Regulatory Gauntlet: Lessons Learned

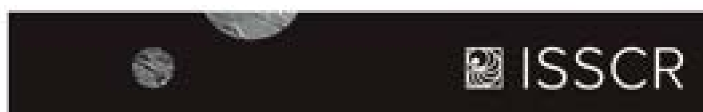
1:40 - 1:55 **Donald Fink,** *Dark Horse Consulting, USA*
Insights from Regulators- USA Perspective

1:55 - 2:10 **Jacqueline Barry,** *Cell and Gene Therapy Catapult, UK*
Insights from Regulators- UK/EU Perspective

2:10 - 2:25 **Jane Lebkowski,** *Regenerative Patch Technologies, USA*
Insights from A Stem Cell Therapy Pioneer - USA Perspective

2:25 - 2:40 **Jun Takahashi,** *CiRA & Kyoto University, Japan*
Insights from A Stem Cell Therapy Pioneer - Japan Perspective

2:40 - 3:00 **Panel discussion**



Come celebrate with us...

60 YEARS

ago, in 1961, **Drs. James Till and Ernest McCulloch** first identified hematopoietic stem cells in mouse bone marrow, in downtown Toronto. This work changed the landscape of scientific discovery in Canada and around the world.

20 YEARS

since the creation of Canada's **Stem Cell Network**. In that time the Network has built a strong and vibrant community, funding research, training next generation leaders, fostering partnerships and powering Canada's stem cell and regenerative medicine sector.

10 YEARS

of **Till & McCulloch Meetings (TMM)**, Canada's largest gathering for stem cell science. Bringing together Canada's leading stem cell scientists, clinicians, bioengineers and ethicists, as well as industry, government, health and NGO sector representatives from around the world.

Join the Stem Cell Network at Virtual TMM2021 – Nov. 15-17

www.tillandmcculloch.ca

DAY 1: MONDAY 21 JUNE (EDT)

07:30 – 08:30 **THE ISSCR GUIDELINES: 2021 UPDATES**

Chair: Robin Lovell-Badge
The Francis Crick Institute, UK

Speakers: Andy Greenfield
MRC Harwell Institute, UK
Melissa Carpenter
ElevateBio, USA
Janet Rossant
Gairdner Foundation & The Hospital for Sick Children, Canada
Insoo Hyun
Case Western Reserve University School of Medicine, USA

09:00 – 11:30 **PLENARY I: PRESIDENTIAL SYMPOSIUM**
Sponsored by: BlueRock Therapeutics

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

09:00 – 09:02 **Christine L. Mummery**
Leiden University Medical Center, Netherlands
WELCOMING REMARKS

09:02 – 09:12 **Leonard I. Zon**
Boston Children's Hospital, USA
TRIBUTE TO NANCY WITTY

09:12 – 09:22 **Christine L. Mummery**
Leiden University Medical Center, Netherlands
Sally Temple
Neural Stem Cell Institute, USA
2021 PROGRAM REMARKS

09:22 – 09:26 **Christine L. Mummery**
Leiden University Medical Center, Netherlands
ISSCR PRESIDENT'S ADDRESS

09:26 – 09:38 **Christine L. Mummery**
Leiden University Medical Center, Netherlands
Robin Lovell-Badge
The Francis Crick Institute, UK
ISSCR PUBLIC SERVICE AWARD PRESENTATION TO ROBIN LOVELL BADGE

09:38 – 09:39 **Christine L. Mummery**
Leiden University Medical Center, Netherlands
RECOGNITION OF ISSCR ZHONGMEI CHEN YONG AWARDS FOR SCIENTIFIC EXCELLENCE
RECOGNITION OF ISSCR MERIT ABSTRACT AWARDS

09:39 – 09:40 **Christine L. Mummery**
Leiden University Medical Center, Netherlands
INTRODUCTION OF PRESIDENTIAL SYMPOSIUM

09:40 – 10:05 **Charles Murry**
University of Washington, USA and Sana Biotechnology, USA
GENOME EDITING TO ELIMINATE ENGRAFTMENT ARRHYTHMIA DURING HEART REGENERATION

DAY 1: MONDAY 21 JUNE (EDT) *(Continued)*

10:05 – 10:30 **Meritxell Huch**
Max Planck Dresden, Germany
LIVER AND PANCREAS ORGANOIDS: THEIR APPLICATION TO THE STUDY OF TISSUE REGENERATION AND DISEASE

10:30 – 10:55 **Lorenz Studer**
Sloan-Kettering Institute for Cancer Research, USA
DERIVING AND REPAIRING THE ENTERIC NERVOUS SYSTEM FROM HUMAN PSCS

10:55 – 11:20 **Elaine Fuchs**
Rockefeller University, USA
STEM CELL ENCOUNTERS WITH STRESSFUL SITUATIONS: SURVIVAL OF THE FITTEST

11:45 – 12:45 **PLENARY ROUNDTABLE: FASCINATION WITH GASTRULATION**

Moderator: Janet Rossant
Gairdner Foundation/The Hospital for Sick Children, Canada

Panelists: Susana Chuva de Sousa Lopes
Leiden University Medical Center, Netherlands
Insoo Hyun
Case Western Reserve University School of Medicine and Harvard Medical School, USA
Matthias P. Lutolf
Ecole Polytechnique Fédérale de Lausanne (EPFL), Switzerland
Susanne Van Den Brink
Hubrecht Institute, Netherlands

FOCUS SESSIONS (See Page 28 for Details)

13:00 – 16:00 **IMPROVING REPRODUCIBILITY AND SCALING-UP OF STEM CELL CULTURES**
Organized by: Eppendorf AG

13:00 – 16:00 **INNOVATIVE TECHNOLOGIES FOR DELIVERY OF CELL THERAPIES**
Organized by: Sigilon Therapeutics, Inc.

13:00 – 16:00 **TOOLS FOR BASIC AND APPLIED STEM CELL BIOLOGY**
Organized by: Stem Cell COREdinates
Supported by: STEMCELL Technologies and Thermo Fisher Scientific

13:00 – 16:00 **DEVELOPMENTS TO SIMPLIFY AND ACCELERATE IPSC RESEARCH**
Organized by: The European Bank for induced Pluripotent Stem Cells

13:00 – 16:00 **THE SCIENCE AND ETHICS OF GENERATING GAMETES FROM STEM CELLS**
Organized by: The ISSCR Ethics Committee

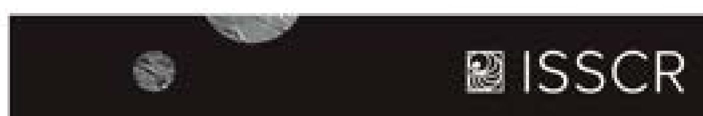
13:00 – 16:00 **DRIVING CHANGE IN REGENERATIVE MEDICINE THROUGH GLOBAL PARTNERSHIPS**
Organized by: Novo Nordisk A/S

13:00 – 16:00 **WHAT TO KNOW BEFORE YOU GO: UNDERSTANDING CELL THERAPY PRODUCT DEVELOPMENT**
Organized by: The ISSCR Industry Committee

16:30 - 17:30 **THE ISSCR GUIDELINES: 2021 UPDATES REBROADCAST**

19:30 - 20:30 **THE ISSCR GUIDELINES: 2021 UPDATES REBROADCAST**

21:00 – 23:45 **PLENARY I: PRESIDENTIAL SYMPOSIUM REBROADCAST**
Sponsored by: BlueRock Therapeutics



DAY 2: TUESDAY 22 JUNE (EDT)

07:00 – 07:30 **CONVERSATION CORNER: MEET THE EDITORS OF *STEM CELL REPORTS***

07:30 – 09:15 **THEME SESSION TSC 1: STEM CELLS AND CANCER**
Sponsored by: ROCHE and GENENTECH

Chairs: Ilaria Malanchi
Crick Institute, UK
Cédric Blanpain
Université Libre de Bruxelles, Belgium

07:32 – 07:37 **Ilaria Malanchi**
Crick Institute, UK
TOPIC OVERVIEW

07:37 – 07:57 **Cédric Blanpain**
Université Libre de Bruxelles, Belgium
MECHANISMS REGULATING TUMOR TRANSITION STATES

07:57 – 08:07 **Min Kyu Yum**
University of Cambridge, UK
TRACING ONCOGENE-DRIVEN PARACRINE REMODELING OF THE INTESTINAL STEM CELL NICHE

08:07 – 08:17 **Kaelyn Sumigray**
Yale School of Medicine, USA
CELLULAR MECHANISMS OF MOUSE INTESTINAL POLYP INITIATION

08:17 – 08:27 **Silvia Fontenete**
University of Copenhagen, Denmark
A MACROPHAGE – CANCER STEM CELL CROSSTALK VIA WNT LIGANDS GOVERNS SKIN CARCINOMA PROMOTION AND STEMNESS.

08:27 – 08:37 **Charlotte Nys**
KU Leuven, Belgium
STEM CELL BIOLOGY IN PITUITARY TUMORS AND DERIVED ORGANOIDS

08:37 – 08:47 **Alexandra Avgustinova**
Institut de Recerca Sant Joan de Deu, Spain
REPRESSION OF ENDOGENOUS RETROVIRUSES IS REQUIRED FOR MAMMARY GLAND DEVELOPMENT

08:47 – 09:07 **Ilaria Malanchi**
Crick Institute, UK
THE INSIDE OUT STEMNESS OF METASTASIS

07:30 – 09:15 **THEME SESSION NT 1: IMAGING**
Chairs: Scott E. Fraser
University of Southern California, USA
Prisca Liberali
FMI, Switzerland

07:30 – 07:35 **Scott E. Fraser**
University of Southern California, USA
TOPIC OVERVIEW

07:35 – 07:55 **Prisca Liberali**
FMI, Switzerland
SELF-ORGANISATION AND SYMMETRY BREAKING IN MULTICELLULAR SYSTEMS

DAY 2: TUESDAY 22 JUNE (EDT) *(Continued)*

07:55 – 08:05 **Nouraiz Ahmed**
ETH Zurich, Switzerland
LIVE SINGLE CELL QUANTIFICATION OF THE GATA SWITCH DYNAMICS DURING ADULT AND DEVELOPMENTAL ERYTHROPOIESIS

08:05 – 08:15 **Shu-Chi Yeh**
Massachusetts General Hospital, USA
QUANTIFYING INTERSTITIAL PH AND CALCIUM CONCENTRATION OF MOUSE BONE MARROW BY INTRAVITAL RATIOMETRIC IMAGING

08:15 – 08:25 **Krishnan Padmanaabhan**
University of Rochester School of Medicine, USA
IN VIVO IMAGING OF HUMAN NEURONAL DEVELOPMENT AT SINGLE CELL RESOLUTION IN CHIMERA MODELS

08:25 – 08:35 **Yolanda Markaki, University of California**
Los Angeles (UCLA), USA
XIST NUCLEATES LOCAL PROTEIN GRADIENTS TO PROPAGATE SILENCING ACROSS THE X CHROMOSOME DURING DEVELOPMENT

08:35 – 08:45 **Jose Martinez-Sarmiento**
University of Pennsylvania, USA
SUPER-RESOLUTION IMAGING REVEALS DYNAMIC CHANGES IN CHROMATIN STRUCTURE AND GENE ACTIVITY IN SINGLE CELLS AT THE ONSET OF HETEROKARYON REPROGRAMMING

08:45 – 09:05 **Scott E. Fraser**
University of Southern California, USA
MULTIMODAL, MULTIDIMENSIONAL AND MULTIPLEX IMAGING OF INTRINSIC AND EXTRINSIC SIGNALS EMPOWERS INTRAVITAL ANALYSES OF STEM CELLS, CELL LINEAGES AND TISSUE MORPHOGENESIS

09:30 – 11:05 **PLENARY II: STEM CELL NICHES**
Sponsored by: ROCHE and GENENTECH

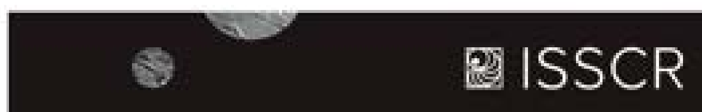
Session Chairs: Hans C. Clevers
Hubrecht Institute, Netherlands
Elaine Fuchs
Rockefeller University, USA

09:32 – 09:57 **Susana Chuva de Sousa Lopes**
Leiden University Medical Center, Netherlands
Anne McLaren Memorial Lecture: OOGENESIS SPOTLIGHTED: HOW FAR ARE WE FROM MAKING (MATURE) HUMAN OOCYTES?

09:57 – 10:17 **Ya-chieh Hsu**
Harvard University, USA
SKIN DEEP: STEM CELLS AT THE NEXUS OF THE NICHE, PHYSIOLOGY, AND THE EXTERNAL ENVIRONMENT

10:17 – 10:37 **Hans C. Clevers**
Hubrecht Institute, Netherlands
TISSUE STEM CELL-BASED ORGANOIDS TO MODEL HUMAN DISEASE

10:37 – 11:02 **Sean J. Morrison**
UT Southwestern, USA
Ernest McCulloch Memorial Lecture: NICHE BIOLOGY: BEYOND GROWTH FACTORS



DAY 2: TUESDAY 22 JUNE (EDT) *(Continued)*

11:15 – 12:00 **PLENARY ROUNDTABLE: STEM CELL ORGANOID MODELS AS EMPIRICAL TESTBEDS FOR PERSONALIZED MEDICINE DEVELOPMENT**

Moderator: Hans Clevers
Hubrecht Institute, Netherlands

Panelists: Jeffery Beekman
UMC Utrecht, Netherlands
Kors van der Ent
UMC Utrecht, Netherlands
Els van der Heijden
Stupers Consultancy, Netherlands

INNOVATION SHOWCASES (See Page 80 for Details)

12:00 – 13:00 **AXION BIOSYSTEMS**
Evangelos Kiskinis
Northwestern University, USA
David Belair
AbbVie, USA
NEURAL ACTIVITY IN A DISH: ADVANCING OUR UNDERSTANDING OF NERVOUS SYSTEM DISORDERS

12:00 – 13:00 **BIO-TECHNE**
Kevin Flynn
Bio-Techne, USA
Eun Joo Kim
University of Colorado, USA
OPTIMIZING ORGANOID AND STEM CELL CULTURE WORKFLOWS: APPLICATIONS FOR DISEASE MODELING

12:00 – 13:00 **EMULATE, INC.**
Luke Dimasi
Emulate Inc., USA
Sifis Pediaditakis
Emulate, Inc., USA
Michael Workman
Cedars-Sinai, USA
MICROENGINEERED HUMAN BRAIN-CHIP FOR DISEASE MODELING APPLICATIONS

12:00 – 13:00 **PHC CORPORATION OF NORTH AMERICA**
Carl Radosevich
PHC Corporation of North America, USA
Holly Hattaway
PHC Corporaton of North America, USA
ENHANCING PHYSIOLOGICAL RELEVANCE IN VITRO: TECH INNOVATIONS FOR STEM CELL SORTING, 3D MODELING, AND OXYGEN CONTROL

12:00 – 13:00 **SARTORIUS STEDIM ADVANCED THERAPIES**
Ibrahim Kassis
Hadassah Medical Center, Israel
Priya Baraniak
OrganaBio, LLC, USA
Xiangliang Lin
Esco Aster Pte Ltd, Singapore
MS, COGS AND CDMOS - MSCS ARE EVERYWHERE, AND FOR A GOOD CAUSE

DAY 2: TUESDAY 22 JUNE (EDT) *(Continued)*

12:00 – 13:00
TECAN TRADING AG
Lucia Bruzzone
Tecan Trading AG, Switzerland
Ole Pless
Fraunhofer ITMP ScreeningPort , Germany
Alejandro Hidalgo-Gonzalez
Murdoch Children's Research Institute, Australia
LEVERAGING AUTOMATION OF IPSC ASSAY TECHNOLOGY FOR DISEASE MODELLING, DRUG DISCOVERY & SAFETY

12:00 – 13:00
THERMO FISHER SCIENTIFIC
Cláudia Miranda
iBB – Institute for Bioengineering and Biosciences; IST – University of Lisbon, Portugal
Michael Akenhead
Thermo Fisher Scientific, USA
PAIRING 3D PLURIPOTENT STEM CELL SUSPENSION CULTURE WITH DOWNSTREAM DIFFERENTIATION TO ENABLE EFFICIENT GENERATION OF LARGE NUMBERS OF CELLS

12:00 – 13:00
POSTER SESSION 1: TISSUE STEM CELLS AND REGENERATION AND NEW TECHNOLOGIES

13:15 – 13:45
ISSCR DR. SUSAN LIM AWARD FOR OUTSTANDING YOUNG INVESTIGATOR SESSION

13:15 – 13:16
Allon Klein
Harvard Medical School, USA
WELCOME
Christina Tan, *Dr. Susan Lim Endowment for Education and Research, Singapore*
AWARD PRESENTATION

13:20 – 13:45
Madeline Lancaster
MRC Laboratory of Molecular Biology, UK
DECIPHERING MECHANISMS OF HUMAN BRAIN EXPANSION USING CEREBRAL ORGANOIDS

14:00 – 15:45
THEME SESSION TSC 2: TISSUE DEVELOPMENT AND MAINTENANCE

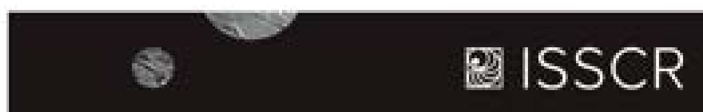
Chairs: Emma L. Rawlins
Gurdon Institute, University of Cambridge, UK
Salvador Aznar Benitah
IRB Barcelona, Spain

14:00 – 14:05
Emma L. Rawlins
Gurdon Institute, University of Cambridge, UK
TOPIC OVERVIEW

14:05 – 14:25
Salvador Aznar Benitah
IRB Barcelona, Spain
DISSECTING THE DAILY COMMUNICATION BETWEEN TISSUES TO MAINTAIN A COHERENT ORGANISMAL PHYSIOLOGY. HOW ITS LOSS CONTRIBUTES TO AGING AND PATHOLOGY

14:25 – 14:35
Shahragim Tajbakhsh
Pasteur Institute, France
UNIQUE REGULATORY MODULES UNDERLIE SKELETAL MUSCLE STEM CELL DIVERSITY AND FUNCTION

14:35 – 14:45
Maria Alcolea
University of Cambridge, UK
A BIOMECHANICAL SWITCH REGULATES THE TRANSITION TOWARDS HOMEOSTASIS IN MOUSE ESOPHAGEAL EPITHELIUM.



DAY 2: TUESDAY 22 JUNE (EDT) *(Continued)*

14:45 – 14:55 **Shiri Gur-Cohen**
The Rockefeller University, USA
SOCIALIZING WITH THE NEIGHBORS: LYMPHATIC NICHE SYNCHRONIZES STEM CELL FATE DECISION AND TISSUE REGENERATION

14:55 – 15:05 **Mika Nakayama**
The University of Tokyo, Japan
UNDERSTANDING A MECHANISM UNDERLYING BONE REPAIR BY COMBINATORIAL ANALYSIS OF LINEAGE TRACING AND SINGLE-CELL RNA SEQUENCING

15:05 – 15:15 **Irene Ylivinkka**
University of Helsinki, Finland
TISSUE AND CELL-SCALE MECHANICS DRIVE HAIR FOLLICLE MORPHOGENESIS

15:15 – 15:35 **Emma L. Rawlins**
Gurdon Institute, University of Cambridge, UK
BUILDING THE HUMAN LUNG: LESSONS FROM ORGANOIDS

14:00 – 15:45 **THEME SESSION NT 2: SINGLE-CELL OMICS**
Sponsored by: Burroughs Wellcome Fund

Chairs: Barbara Treutlein
ETH Zürich, Switzerland
Sten Linnarsson
Karolinska Institutet, Sweden

14:00 – 14:05 **Barbara Treutlein**
ETH Zürich, Switzerland
TOPIC OVERVIEW

14:05 – 14:25 **Sten Linnarsson**
Karolinska Institutet, Sweden
MOLECULAR ARCHITECTURE OF THE DEVELOPING HUMAN BRAIN

14:25 – 14:35 **Kyoung Jae Won**
University of Copenhagen, Denmark
MAPPING OF THE NICHE-SPECIFIC EXPRESSION USING PIC-SEQ ANALYSIS IN MOUSE EMBRYONIC DEVELOPMENT

14:35 – 14:45 **Hani Jieun Kim**
The University of Sydney, Australia
CEPO UNCOVERS CELL IDENTITY THROUGH DIFFERENTIAL STABILITY

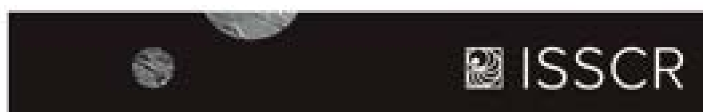
14:45 – 14:55 **Kalki Kukreja**
Harvard University, USA
DEVELOPMENT WITHOUT DIVISION IN ZEBRAFISH EMBRYOS

14:55 – 15:05 **Mo Li**
King Abdullah University of Science and Technology (KAUST), Saudi Arabia
SINGLE-CELL INDIVIDUAL COMPLETE MTDNA SEQUENCING UNCOVERS HIDDEN MITOCHONDRIAL HETEROGENEITY IN HUMAN AND MOUSE OOCYTES

15:05 – 15:15 **Martine Therrien**
Broad Institute of MIT and Harvard, USA
MODELING THE IMPACT OF ALZHEIMER'S DISEASE GENETIC RISK ON MICROGLIA STATES AND FUNCTIONS

DAY 2: TUESDAY 22 JUNE (EDT) *(Continued)*

15:15 – 15:35	**Barbara Treutlein** *ETH Zürich, Switzerland* LINEAGE DYNAMICS DURING BRAIN ORGANOID FORMATION
15:45 – 16:15	**CONVERSATION CORNER: EARLY CAREER SCIENTISTS NETWORKING**
16:30 – 18:15	**THEME SESSION TSC 1: STEM CELLS AND CANCER REBROADCAST** *Sponsored by: ROCHE and GENENTECH*
16:30 – 18:15	**THEME SESSION NT 1: IMAGING REBROADCAST**
19:30 – 21:15	**THEME SESSION TSC 1: STEM CELLS AND CANCER REBROADCAST** *Sponsored by: ROCHE and GENENTECH*
19:30 – 21:15	**THEME SESSION NT 1: IMAGING REBROADCAST**
21:30 – 23:00	**PLENARY II: STEM CELL NICHES REBROADCAST** *Sponsored by: ROCHE and GENENTECH*
0:00 – 1:00	**POSTER SESSION 2: TISSUE STEM CELLS AND REGENERATION AND NEW TECHNOLOGIES**
2:00 – 3:45	**THEME SESSION TSC 2: TISSUE DEVELOPMENT AND MAINTENANCE REBROADCAST**
2:00 – 3:45	**THEME SESSION NT 2: SINGLE-CELL OMICS REBROADCAST** *Sponsored by: Burroughs Wellcome Fund*



DAY 3: WEDNESDAY 23 JUNE (EDT)

07:00 – 07:30 **CONVERSATION CORNER: COMPUTATIONAL STEM CELL BIOLOGY**

07:30 – 09:15 **THEME SESSION TSC 3: WOUND HEALING, STRESS AND AGING**

Chairs: Kim B. Jensen
BRIC, Denmark
Emmanuelle Passegué
Columbia University Medical Center, USA

07:30 – 07:35 **Kim B. Jensen**
BRIC, Denmark
TOPIC OVERVIEW

07:35 – 07:55 **Emmanuelle Passegué**
Columbia University Medical Center, USA
EMERGENCY MYELOPOIESIS PATHWAYS

07:55 – 08:05 **Thomas Ambrosi**
Stanford University, USA
AGING OF MOUSE AND HUMAN SKELETAL STEM CELLS UNDERLIES LINEAGE SKEWING THAT ALTERS BONE MARROW NICHE DYNAMICS

08:05 – 08:15 **Yekaterina Miroshnikova**
University of Helsinki, Finland
NICHE MECHANICS CONTROLS STEM CELL POTENTIAL THROUGH REGULATING CHROMATIN ARCHITECTURE

08:15 – 08:25 **Maria Bejar**
University of Cambridge, UK
DEFINING THE TRANSCRIPTIONAL SIGNATURE OF ESOPHAGEAL-TO-SKIN LINEAGE CONVERSION

08:25 – 08:35 **Swarnabh Bhattacharya**
Technion – Israel Institute of Technology, Israel
SOFT BIOMECHANICAL PROPERTIES OF THE LIMBUS SUSTAIN YAP ACTIVITY TO PREVENT SMAD2/3 MEDIATED CELL DIFFERENTIATION IN THE MOUSE AND HUMAN CORNEAL EPITHELIUM

08:35 – 08:45 **Marie Bannier-Hélaouët**
Hubrecht Institute, Netherlands
EXPLORING THE HUMAN LACRIMAL GLAND USING ORGANOIDS AND SINGLE-CELL SEQUENCING

08:45 – 09:05 **Kim B. Jensen**
BRIC, Denmark
MAPPING CELL FATE CONTROL MECHANISMS DURING STATE TRANSITIONS

07:30 – 09:15 **THEME SESSION NT 3: CRISPR BASED TECHNOLOGIES**
Sponsored by: Horizon Discovery Ltd

Chairs: Martin Kampmann
University of California, San Francisco, USA
Kristen J. Brennand
Icahn School of Medicine, USA

07:32 – 07:37 **Martin Kampmann**
University of California, San Francisco, USA
TOPIC OVERVIEW

07:37 – 07:57 **Kristen J. Brennand**
Icahn School of Medicine, USA
USING STEM CELLS TO EXPLORE THE GENETICS UNDERLYING BRAIN DISEASE

DAY 3: WEDNESDAY 23 JUNE (EDT) *(Continued)*

07:57 – 08:07 **Michael Ward**
National Institutes of Health (NIH), USA
THE IPSC NEURODEGENERATIVE DISEASE INITIATIVE (INDI)

08:07 – 08:17 **Reto Eggenschwiler**
Hannover Medical School, Germany
A CRISPR PRIME EDITING PIGGYBAC TRANSPOSON ALLOWS FOR ENRICHMENT OF GENE EDITED CELLS IN HUMAN PLURIPOTENT STEM CELLS

08:17 – 08:27 **Atilgan Yilmaz**
The Hebrew University of Jerusalem, Israel
GENOME-WIDE FUNCTIONAL SCREENING OF HUMAN GENETIC DISORDERS IN PLURIPOTENT STEM CELLS AND THEIR NEURAL DERIVATIVES

08:27 – 08:37 **Alessandro Bertero**
University of Washington, USA
A PHENOTYPE-AGNOSTIC FUNCTIONAL SCREENING PLATFORM OPTIMIZED FOR HUMAN PLURIPOTENT STEM CELL-DERIVED LINEAGES

08:37 – 08:47 **Anja Knaupp**
Monash University, Australia
TINC – A METHOD TO DISSECT REGULATORY COMPLEXES AT SINGLE-LOCUS RESOLUTION – REVEALS AN EXTENSIVE PROTEIN COMPLEX AT THE NANOG PROMOTER

08:47 – 09:07 **Martin Kampmann**
University of California, San Francisco, USA
CRISPR-BASED FUNCTIONAL GENOMICS UNCOVER REGULATORS OF DISEASE-ASSOCIATED STATES OF GLIA AND NEURONAL PATHWAYS CONTRIBUTING TO NEURODEGENERATIVE DISEASE

09:30 – 11:00 **PLENARY III: EMERGING TECHNOLOGIES / ENABLING TECHNOLOGIES**
Sponsored by: T-CiRA Joint Research Program

Session Chairs: Martin Kampmann
University of California, San Francisco, USA
Timm Schroeder
ETH Zurich, Basel, Switzerland

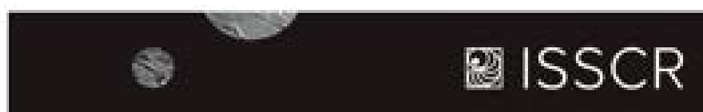
09:32 – 09:52 **Ruwanthi Gunawardane**
Allen Institute for Cell Science, USA
USING THE ALLEN CELL COLLECTION TO VISUALIZE AND MAP CELL STATES FROM PLURIPOTENCY THROUGH DIFFERENCIATION

09:52 – 10:12 **David R. Liu**
Harvard University, USA
BASE EDITING AND PRIME EDITING: GENOME EDITING WITHOUT DOUBLE-STRAND BREAKS

10:12 – 10:32 **Matthais Lutolf**
EFPL, Switzerland
ENGINEERING EPITHELIAL ORGANOIDS-ON-A-CHIP

10:32 – 10:52 **Timm Schroeder, ETH Zurich**
Basel, Switzerland
LONG-TERM SINGLE-CELL QUANTIFICATION: NEW TOOLS FOR OLD QUESTIONS

11:00 – 11:45 **PROMOTING EQUITY IN STEM CELL SCIENCE PANEL**
Sponsored by: Synthego



DAY 3: WEDNESDAY 23 JUNE (EDT) *(Continued)*

INNOVATION SHOWCASES (See Page 80 for Details)

12:00 – 13:00

BIOLEGEND
Vanda Lopes
BioLegend, USA
NOVEL TOOLS TO DIFFERENTIATE NEURAL AND LYMPHOID CELL LINEAGES

12:00 – 13:00

CORNING LIFE SCIENCES
Dennis Plenker
Cold Spring Harbor Laboratory, USA
Catherine Siler
Corning Life Sciences, USA
PATIENT DERIVED ORGANOIDS HELP GUIDE THERAPEUTIC DEVELOPMENT: MECHANISMS AND TOOLS FOR MODELING DISEASE AND THERAPEUTIC EFFICACY

12:00 – 13:00

HORIZON DISCOVERY, A PERKINELMER COMPANY
Amanda Haupt, Horizon Discovery
a PerkinElmer company, USA
Clarence Mills
Horizon Discovery, a PerkinElmer company, USA
Yasmin Paterson
Horizon Discovery, a PerkinElmer company, UK
Max Blanck
Horizon Discovery, a PerkinElmer company, UK
NEXT GENERATION CRISPR SOLUTIONS FOR IPSC GENE EDITING

12:00 – 13:00

MAXWELL BIOSYSTEMS
Thomas Hartung
Johns Hopkins Bloomberg School of Public Health, USA
Lena Smirnova
Johns Hopkins Bloomberg School of Public Health, USA
Maria Sundberg
Boston Children's Hospital, USA
David Pamies
University of Lausanne, Switzerland
Marie Obien
Maxwell Biosystems, Switzerland
Urs Frey
Maxwell Biosystems, Switzerland
EVERY CELL'S STORY: CHARACTERIZING THE ACTIVITY OF HUMAN IPSC DERIVED-NEURONS IN 2D AND 3D CULTURES AT HIGH RESOLUTION

12:00 – 13:00

MOLECULAR DEVICES
Oksana Sirenko
Molecular Devices, USA
AUTOMATED CULTURE AND HIGH-CONTENT IMAGING OF 3D LUNG AND CARDIAC ORGANOIDS FOR IN VITRO ASSESSMENT OF COMPOUND EFFECTS

12:00 – 13:00

YOKOGAWA GERMANY GMBH
Nader Pourmand
University of California Santa Cruz, USA
NANOPIPETTE TECHNOLOGY: A NEW TOOL FOR SINGLE-CELL ANALYSIS

12:00 – 12:30

STEMCELL TECHNOLOGIES
Philipp Kramer
STEMCELL Technologies, Canada
HPSC-DERIVED LUNG MODELS AS TOOLS FOR RESPIRATORY RESEARCH

DAY 3: WEDNESDAY 23 JUNE (EDT) *(Continued)*

12:30 – 13:00 **STEMCELL TECHNOLOGIES**
Leon Chew
STEMCELL Technologies, Canada
NEXT GENERATION BRAIN-REGION-SPECIFIC ORGANOID MODELS

12:00 – 13:00 **SCIENCE ADVOCACY AND COMMUNICATION SEMINAR**

12:00 – 13:00 **POSTER SESSION 3: TISSUE STEM CELLS AND REGENERATION AND NEW TECHNOLOGIES**

13:15 – 13:45 **ISSCR TOBIAS AWARD SESSION**

13:15 – 13:20 **Margaret A. Goodell**
Baylor College of Medicine, USA
AWARD PRESENTATION

13:20 – 13:45 **Stuart H. Orkin**
Harvard Medical School and Howard Hughes Medical Institute, USA
TURNING THE CLOCK BACK FOR THERAPY OF THE MAJOR HEMOGLOBIN DISORDERS

14:00 – 15:45 **THEME SESSION TSC 4: HEMATOPOIETIC STEM CELLS**
Sponsored by: Dana-Farber Cancer Institute

Chairs: Leonard I. Zon
Boston Children's Hospital, USA
Andreas Trumpp
DKFZ/HI-STEM, Germany

14:02 – 14:07 **Leonard I. Zon**
Boston Children's Hospital, USA
TOPIC OVERVIEW

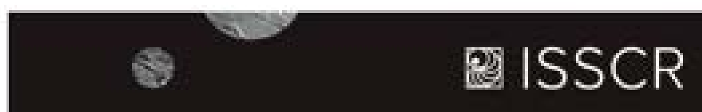
14:07 – 14:27 **Andreas Trumpp**
DKFZ/HI-STEM, Germany
REGULATION OF HEMATOPIETIC AND LEUKEMIC STEM CELLS

14:27 – 14:37 **Laura Garcia Prat**
University Health Network (UHN), Canada
DICHOTOMOUS REGULATION OF LYSOSOMES BY MYC AND TFEB CONTROLS HEMATOPOIETIC STEM CELL FATE

14:37 – 14:47 **Samuel Wattrus**
Harvard Stem Cell Institute, USA
ADULT HEMATOPOIETIC STEM CELL CLONAL CONTRIBUTION IS DETERMINED BY MACROPHAGE SENSING OF CALRETICULIN 3 ON HEMATOPOIETIC STEM CELLS DURING DEVELOPMENT

14:47 – 14:57 **Anna Beaudin**
University of Utah, USA
PRENATAL INFLAMMATION PERTURBS FETAL HEMATOPOIESIS AND DRIVE PERSISTENT CHANGES TO POSTNATAL IMMUNITY

14:57 – 15:07 **Carolina Petrillo**
Columbia University Medical Center, USA
MITOCHONDRIAL DYNAMICS REGULATE INTERFERON SIGNALING AND AGE-RELATED CHANGES IN HSPC



DAY 3: WEDNESDAY 23 JUNE (EDT) *(Continued)*

15:07 – 15:17 **Laina Freyer**
Institut Pasteur, France
OVERLAPPING DEFINITIVE PROGENITOR WAVES DIVIDE AND CONQUER TO BUILD A LAYERED HEMATOPOIETIC SYSTEM

15:17 – 15:37 **Leonard I. Zon**
Boston Children's Hospital, USA
STEM CELL CLONALITY AND THE NICHE

14:00 – 15:45 **THEME SESSION NT 4: BIOENGINEERING**
Sponsored by: Vertex Pharmaceuticals

Chairs: Jennifer Lewis
Harvard University, USA
Noo Li Jeon
Seoul National University, Korea

14:00 – 14:05 **Jennifer Lewis**
Harvard University, USA
TOPIC OVERVIEW

14:05 – 14:25 **Noo Li Jeon**
Seoul National University, Korea
IMPACT PLATFORM FOR VASCULARIZED MICROPHYSIOLOGICAL SYSTEMS

14:25 – 14:35 **Juan Alvarez**
Harvard University, USA
CYBORG ORGANOIDS: MEASURING HUMAN ISLET-WIDE CELL PHYSIOLOGY WITH SOFT IMPLANTED NANOELECTRONICS

14:35 – 14:45 **Evan Appleton**
Harvard Medical School, USA
MACHINE-GUIDED CELL-FATE ENGINEERING

14:45 – 14:55 **Eri Takematsu**
Stanford University, USA
BMP2 SURROGATE USING BISPECIFIC NANOBODIES FOR CARTILAGE REGENERATION

14:55 – 15:05 **David Sachs**
Icahn School of Medicine at Mount Sinai, USA
A MICROFLUIDIC ORGANOID PLATFORM FOR STUDYING HUMAN HEART DEVELOPMENT AND FUNCTION

15:05 – 15:15 **Ngan Huang**
Stanford University, USA
NANOPATTERNED SCAFFOLDS AUGMENT SURVIVAL OF HUMAN IPSC-DERIVED ENDOTHELIAL CELLS IN THE MURINE ISCHEMIC LIMB

15:15 – 15:35 **Jennifer Lewis**
Harvard University, USA
IN VITRO VASCULARIZATION OF HUMAN KIDNEY AND CARDIAC TISSUES

15:45 – 16:15 **CONVERSATION CORNER: POLICY, ETHICS, AND REGULATORY ISSUES**

16:30 – 18:15 **THEME SESSION TSC 3: WOUND HEALING, STRESS AND AGING REBROADCAST**

16:30 – 18:15 **THEME SESSION NT 3: CRISPR BASED TECHNOLOGIES REBROADCAST**
Sponsored by: Horizon Discovery Ltd.

DAY 3: WEDNESDAY 23 JUNE (EDT) *(Continued)*

19:30 – 21:15 **THEME SESSION TSC 3: WOUND HEALING, STRESS AND AGING REBROADCAST**

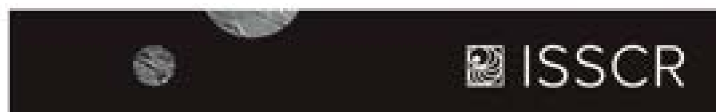
19:30 – 21:15 **THEME SESSION NT 3: CRISPR BASED TECHNOLOGIES REBROADCAST**
Sponsored by: Horizon Discovery Ltd.

21:30 – 23:00 **PLENARY III: EMERGING TECHNOLOGIES / ENABLING TECHNOLOGIES REBROADCAST**
Sponsored by: T-CIRA Joint Research Program

00:00 – 01:00 **POSTER SESSION 4: TISSUE STEM CELLS AND REGENERATION AND NEW TECHNOLOGIES GROUP 4**

02:00 – 03:45 **THEME SESSION TSC 4: HEMATOPOIETIC STEM CELLS REBROADCAST**
Sponsored by: Dana-Farber Cancer Institute

02:00 – 03:45 **THEME SESSION NT 4: BIOENGINEERING REBROADCAST**
Sponsored by: Vertex Pharmaceuticals



DAY 4: THURSDAY 24 JUNE (EDT)

07:00 – 07:30 **CONVERSATION CORNER: GERMAN STEM CELL NETWORK (GSCN) AND THE EUROPEAN NETWORK OF NETWORKS (EURONET)**

07:30 – 09:15 **THEME SESSION MDD 1: AGING**

Chairs: Marieke Essers
DKFZ, Germany
Anne Brunet
Stanford University, USA

07:30 – 07:35 **Marieke Essers**
DKFZ, Germany
TOPIC OVERVIEW

07:35 – 07:55 **Marieke Essers**
DKFZ, Germany
STEM CELL BASED HETEROGENEITY OF INTERFERON SIGNALING IN THE HEMATOPOIETIC SYSTEM

07:55 – 08:05 **Shong Lau**
Salk Institute for Biological Studies, USA
IDENTIFICATION OF AN AGE-RELATED PARKINSON'S DISEASE RISK FACTOR WHICH REGULATES SULFUR METABOLISM

08:05 – 08:15 **Wenshu Zeng**
Hong Kong University of Science and Technology, Hong Kong
CPEB4 REGULATES MOUSE MUSCLE STEM CELL FUNCTION DURING AGING BY MODULATING MITOCHONDRIAL PROTEOMIC LANDSCAPE AND ACTIVITY

08:15 – 08:25 **Christine Cheung**
Nanyang Technological University Singapore, Singapore
HYALURONIDASE-1-MEDIATED GLYCOCALYX IMPAIRMENT UNDERLIES ENDOTHELIAL ABNORMALITIES IN POLYPOIDAL CHOROIDAL VASCULOPATHY

08:25 – 08:35 **Mary Mohrin**
Genentech Inc, USA
INHIBITION OF LONGEVITY REGULATOR PAPP-A MODULATES TISSUE HOMEOSTASIS VIA RESTRAINT OF MESENCHYMAL STROMAL CELLS

08:35 – 08:45 **Foteini Mourkioti**
University of Pennsylvania, USA
PERSISTENT NF-KB ACTIVATION IN MUSCLE STEM CELLS INDUCES PROLIFERATION-INDEPENDENT TELOMERE SHORTENING

08:45 – 09:05 **Anne Brunet**
Stanford University, USA
MECHANISMS OF NEURAL STEM CELL AGING

07:30 – 09:15 **THEME SESSION CI 1: METABOLISM AND CELL IDENTITY**
Sponsored by: Agilent Technologies

Chairs: Alexander Aulehla
EMBL, Heidelberg, Germany
Erica Watson
University of Cambridge, UK

DAY 4: THURSDAY 24 JUNE (EDT) *(Continued)*

07:32 – 07:37 **Alexander Aulehla, EMBL**
Heidelberg, Germany
TOPIC OVERVIEW

07:37 – 07:57 **Erica Watson**
University of Cambridge, UK
HIRA AS A PHENOTYPE INHERITANCE BIOMARKER IN A MOUSE MODEL OF TRANSGENERATIONAL EPIGENETIC INHERITANCE

07:57 – 08:07 **Jing Zhao**
Zhejiang University, China
METABOLIC REPROGRAMMING DURING EARLY EMBRYOGENESIS REGULATES 2-HG/A-KG HOMEOSTASIS TO PROMOTE ERASURE OF HISTONE METHYLATION

08:07 – 08:17 **Jessica Garbern**
Harvard University, USA
SENESCENCE SUPPRESSION TO IMPROVE MATURATION OF STEM CELL-DERIVED CARDIOMYOCYTES

08:17 – 08:27 **Marlen Knobloch**
University of Lausanne, Switzerland
LIPID DROPLET AVAILABILITY INFLUENCES NEURAL STEM/PROGENITOR CELL PROLIFERATION AND DIFFERENTIATION INTO NEURONS

08:27 – 08:37 **Yan Yao**
Columbia University Medical Center, USA
REGULATION OF HEMATOPOIESIS BY MITOCHONDRIAL DYNAMICS

08:37 – 08:47 **Hua Yu**
Zhejiang University, China
RIBOSOMAL RNA BIOGENESIS REGULATES MOUSE 2C-LIKE STATE AND 2-CELL/4-CELL EMBRYO DEVELOPMENT BY NUCLEOLAR PHASE-SEPARATION-MEDIATED 3D CHROMATIN STRUCTURE REORGANIZATION

08:47 – 09:07 **Alexander Aulehla, EMBL**
Heidelberg, Germany
METABOLIC CONTROL OF MOUSE EMBRYONIC PATTERNING AND TIMING

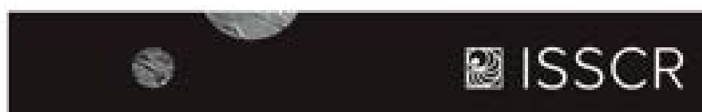
09:30 – 11:15 **PLENARY IV: SELF-ORGANIZATION OF DEVELOPMENTAL PROCESSES**
Sponsored by: Bio-Techne

Session Chairs: Meritxell Huch
Max Planck Institute of Molecular Cell Biology & Genetics, Germany
Elly Tanaka
IMP Research Institute of Molecular Pathology, Austria

09:30 – 09:50 **Anne Grapin-Botton**
Max Planck Institute of Molecular Cell Biology and Genetics, Germany
THREE DIMENSIONAL MODELS OF PANCREAS ORGANOGENESIS: FROM SELF-ORGANIZATION TO UNDERSTANDING DIABETES

09:50 – 10:10 **Nicolas Rivron**
IMBA, Austria
BLASTOID: MODELING MAMMALIANS BLASTOCYST DEVELOPMENT AND IMPLANTATION.

10:10 – 10:30 **Takanori Takebe**
Children's Hospital Cincinnati, USA and Dental University and Yokohama City University, Japan
PURSUING ORGANOID MEDICINE DURING GLOBAL PANDEMIC



DAY 4: THURSDAY 24 JUNE (EDT) *(Continued)*

10:30 – 10:50 **Yi Arial Zeng**
Shanghai Institute of Biological Sciences, China
GENERATION OF MOUSE PANCREATIC ISLET ORGANOIDS USING RESIDENT PROCR PROGENITORS

10:50 – 11:10 **Melissa Little**
Murdoch Children's Research Hospital, Australia
REBUILDING KIDNEY TISSUE FROM PLURIPOTENT STEM CELLS: THE CHALLENGES OF SCALING UP AND DOWN.

INNOVATION SHOWCASES (See Page 80 for Details)

12:00 – 13:00 **CELLINK**
Julian Riba
CYTENA, Germany

Itedale Namro Redwan
CELLINK, Sweden
THE QUEST FOR PERSONALIZED MEDICINE—TECHNOLOGICAL ADVANCEMENTS IN KEY WORKFLOWS

12:00 – 13:00 **IOTASCIENCES**
Katia Mattis
iotaSciences, UK
DELIVERING THE CELLS THAT MATTER: STREAMLINED SINGLE-CELL CLONING IN MINIATURE FLUID-WALLED CHAMBERS

12:00 – 13:00 **STEMCELL TECHNOLOGIES**
Martin Stahl
STEMCELL Technologies, Canada

Charis Segeritz-Walko
STEMCELL Technologies, Canada
HEPATIC AND INTESTINAL ORGANOIDS: IMPROVED TOOLS AND IN VITRO MODELS FOR DRUG DEVELOPMENT

12:00 – 13:00 **THERMO FISHER SCIENTIFIC**
David Kuninger
Thermo Fisher Scientific, USA

Alex Hannay
Thermo Fisher Scientific, USA
BEYOND GMP: CONSIDERATIONS FOR THE DEVELOPMENT OF PSC-BASED THERAPIES

12:00 – 12:30 **BIT.BIO**
Mark Kotter
bit.bio, UK
CHALLENGING BIOLOGY, CAN WE CODE HUMAN CELLS FOR HEALTH? REALISING THE POTENTIAL OF HIPSC REPROGRAMMING FOR RESEARCH, DISCOVERY AND THERAPEUTIC APPLICATIONS

12:30 – 13:00 **TREEFROG THERAPEUTICS**
Maxime Feyeux
TreeFrog Therapeutics, France
150 MILLION-FOLD CUMULATED EXPANSION OF ENCAPSULATED HIPS CELLS IN BIOREACTOR

DAY 4: THURSDAY 24 JUNE (EDT) *(Continued)*

12:00 – 13:00 **SPECIAL SHOWCASE: GERMAN STEM CELL NETWORK (GSCN) AND BERLIN INSTITUTE OF HEALTH (BIH) SESSION 1**
Sponsored by: BIH and GSCN

12:00 – 13:00 **EARLY CAREER GROUP LEADER PANEL**
Sponsored by: Synthego

12:00 – 13:00 **POSTER SESSION 5: MODELING DEVELOPMENT AND DISEASE AND CELLULAR IDENTITY**

13:15 – 13:45 **ISSCR ACHIEVEMENT AWARD SESSION**

13:15 – 13:20 **Fred H. Gage**
Salk Laboratory of Genetics, USA
AWARD PRESENTATION

13:20 – 13:45 **Janet Rossant**
Gairdner Foundation/The Hospital for Sick Children, Canada
FROM EMBRYOS TO STEM CELLS TO STEM CELL-BASED EMBRYO MODELS- WHY UNDERSTANDING EARLY DEVELOPMENT MATTERS

14:00 – 15:45 **THEME SESSION MDD 2: COMPARATIVE EARLY DEVELOPMENT**

Chairs: Katsuhiko Hayashi
Kyushu University, Japan
Hongmei Wang
Key State Laboratory Beijing, China

14:00 – 14:05 **Katsuhiko Hayashi**
Kyushu University, Japan
TOPIC OVERVIEW

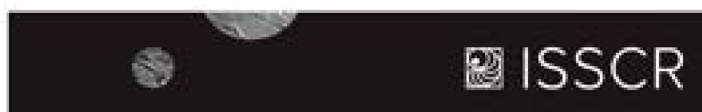
14:05 – 14:25 **Hongmei Wang**
Key State Laboratory Beijing, China
DECIPHERING THE MECHANISMS OF HOW PRIMATES ARE FORMED

14:25 – 14:35 **Cody Kime**
RIKEN, BDR, Japan
SYNTHETIC EMBRYOLOGY: REPROGRAMMING EPIBLAST STEM CELLS INTO PRE-IMPLANTATION BLASTOCYST CELL-LIKE CELLS

14:35 – 14:45 **Ivan Bedzhov**
Max Planck Institute for Molecular Biomedicine, Germany
3D BIOMIMETIC IMPLANTATION NICHE REVEALS THE FIRST INTERACTIONS OF THE EMBRYO AND THE MATERNAL BLOOD VESSELS

14:45 – 14:55 **Chen Dong**
Washington University School of Medicine, USA
A GENOME-WIDE CRISPR-CAS9 KNOCKOUT SCREEN IDENTIFIES ESSENTIAL AND GROWTH-RESTRICTING GENES IN HUMAN TROPHOBLAST STEM CELLS

14:55 – 15:05 **Kathryn Polkoff**
North Carolina State University, USA
TRANSGENIC PIG MODEL REVEALS CONSERVED LGR5 EXPRESSION IN HAIR FOLLICLE STEM CELLS IN POSTNATAL SKIN, BUT DIVERGENT EXPRESSION IN FETAL DEVELOPMENT ACROSS SPECIES



DAY 4: THURSDAY 24 JUNE (EDT) *(Continued)*

15:05 – 15:15 **Sara Bizzotto**
Boston Children's Hospital, USA
LANDMARKS OF HUMAN EMBRYONIC DEVELOPMENT INSCRIBED IN SOMATIC MUTATIONS

15:15 – 15:35 **Katsuhiko Hayashi**
Kyushu University, Japan
RECONSTITUTION OF OVARIAN FOLLICLES USING MOUSE PLURIPOTENT STEM CELLS

14:00 – 15:45 **THEME SESSION CI 2: CELL STATE TRANSITIONS IN DEVELOPMENT AND CANCER**
Sponsored by: The Allen Institute for Cell Science

Chairs: James Briscoe
The Crick Institute, UK
Allon Klein
Harvard Medical School, USA

14:02 – 14:07 **James Briscoe**
The Crick Institute, UK
TOPIC OVERVIEW

14:07 – 14:27 **Allon Klein**
Harvard Medical School, USA
INTEGRATING LINEAGE-TRACING WITH SINGLE CELL GENOMICS ACROSS EXPERIMENTAL DESIGNS

14:27 – 14:37 **Sanjeev Ranade**
The Gladstone Institutes, USA
SINGLE CELL CHROMATIN ACCESSIBILITY PROFILING OF MOUSE HEART DEVELOPMENT IDENTIFIES REGULATORY UNDERPINNING OF CARDIAC OUTFLOW TRACT ANOMALIES

14:37 – 14:47 **Arianna Baggiolini**
Memorial Sloan Kettering Cancer Center, USA
DEVELOPMENTAL CHROMATIN PROGRAMS DETERMINE ONCOGENIC COMPETENCE IN MELANOMA

14:47 – 14:57 **Inseon Kim**
ETH Zurich, Switzerland
INTEGRATIVE MOLECULAR ROADMAP FOR REPROGRAMMING MOUSE FIBROBLASTS INTO INDUCED MYOGENIC STEM AND PROGENITOR CELLS

14:57 – 15:07 **Carolyn Sangokoya**
UCSF, USA
ILLUMINATING POST-TRANSCRIPTIONAL REGULATION OF PLURIPOTENT CELL STATE TRANSITION AND CELL FATE AT SINGLE CELL RESOLUTION

15:07 – 15:17 **Eleonora Stronati**
Temple University, USA
YAP:NODAL SIGNALING AXIS REGULATES THE CELL FATE PATTERNING IN HUMAN GASTRULOIDS

15:17 – 15:37 **James Briscoe**
The Crick Institute, UK
QUANTITATIVE LANDSCAPES OF CELL FATE DECISIONS

15:45 – 16:15 **CONVERSATION CORNER: INDUSTRY SCIENTISTS NETWORKING**

16:30 – 18:15 **THEME SESSION MDD 1: AGING REBROADCAST**

16:30 – 18:15 **THEME SESSION CI 1: METABOLISM AND CELL IDENTITY REBROADCAST**
Sponsored by: Agilent Technologies

DAY 4: THURSDAY 24 JUNE (EDT) *(Continued)*

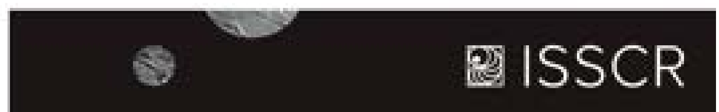
19:30 – 21:15 **THEME SESSION CI 1: METABOLISM AND CELL IDENTITY REBROADCAST**
Sponsored by: Agilent Technologies

21:30 – 23:15 **PLENARY IV: SELF-ORGANIZATION OF DEVELOPMENTAL PROCESSES REBROADCAST**
Sponsored by: Bio-Techne

0:00 – 01:00 **POSTER SESSION 6: MODELING DEVELOPMENT AND DISEASE AND CELLULAR IDENTITY**

02:00 – 03:45 **THEME SESSION MDD 2: COMPARATIVE EARLY DEVELOPMENT REBROADCAST**

02:00 – 03:45 **THEME SESSION CI 2: CELL STATE TRANSITIONS IN DEVELOPMENT AND CANCER REBROADCAST**
Sponsored by: The Allen Institute for Cell Science



DAY 5: FRIDAY 25 JUNE (EDT)

07:30 – 09:15 **THEME SESSION MDD 3: MODELING DISEASE**
Sponsored by: bit.bio

Chairs: Guo-li Ming
University of Pennsylvania, USA
Jeroen Bakkers
Hubrecht Institute, Netherlands

07:32 – 07:37 **Guo-li Ming**
University of Pennsylvania, USA
TOPIC OVERVIEW

07:37 – 07:57 **Jeroen Bakkers**
Hubrecht Institute, Netherlands
MECHANISMS DRIVING CARDIOMYOCYTE PROLIFERATION DURING ZEBRAFISH HEART REGENERATION

07:57 – 08:07 **Derek Tai**
Massachusetts General Hospital, USA
MOLECULAR AND FUNCTIONAL SIGNATURES ASSOCIATED WITH 16P11.2 RECIPROCAL GENOMIC DISORDER: INSIGHTS INTO NEURODEVELOPMENTAL DISORDERS

08:07 – 08:17 **Sarah Rockwood**
Gladstone Institute, USA
SARS-COV-2 INFECTION OF HUMAN IPSC-DERIVED CARDIAC CELLS PREDICTS NOVEL CYTOPATHIC FEATURES IN COVID-19 PATIENTS

08:17 – 08:27 **Talya Dayton**
Hubrecht Institute, Netherlands
ORGANOID MODELS OF NORMAL AND MALIGNANT PULMONARY NEUROENDOCRINE CELLS REVEAL PATHWAYS IMPORTANT FOR NEUROENDOCRINE CELL GROWTH, DIFFERENTIATION, AND TRANSFORMATION

08:27 – 08:37 **Kosuke Funato**
Memorial Sloan Kettering Cancer Center, USA
DISSECTING THE IMPACT OF REGIONAL IDENTITY IN A HUMAN ESC-BASED MODEL OF H3.3G34R-MUTANT HIGH-GRADE GLIOMA

08:37 – 08:47 **Malte Loos**
University Medical Center Hamburg-Eppendorf, Germany
CRISPR/CAS9-EDITED HUMAN IPSC-CM IN ENGINEERED HEART TISSUES REPRODUCE HALLMARKS OF PRIMARY CARNITINE DEFICIENCY

08:47 – 09:07 **Guo-li Ming**
University of Pennsylvania, USA
MODELING INTER- AND INTRA- TUMOR HETEROGENEITY USING PATIENT-DERIVED GLIOBLASTOMA ORGANOIDS

07:30 – 09:15 **THEME SESSION CI 3: EPIGENETIC REGULATION OF CELL IDENTITY**

Chairs: Maria Elena Torres-Padilla
Helmholtz Zentrum München, Germany
Edda G. Schulz
Max Planck Institute for Molecular Genetics, Germany

07:30 – 07:35 **Maria Elena Torres-Padilla**
Helmholtz Zentrum München, Germany
TOPIC OVERVIEW

DAY 5: FRIDAY 25 JUNE (EDT) *(Continued)*

07:35 – 07:55 **Edda G. Schulz**
Max Planck Institute for Molecular Genetics, Germany
DISTAL AND PROXIMAL CIS-REGULATORY ELEMENTS SENSE X-CHROMOSOMAL DOSAGE AND DEVELOPMENTAL STATE AT THE XIST LOCUS

07:55 – 08:05 **Ryan Geusz**
University of California, San Diego (UCSD), USA
DNA SEQUENCE LOGIC AT ENDODERMAL ENHANCERS DETERMINES CELL FATE ALLOCATION THROUGH REGULATION OF FOXA PIONEER FACTOR RECRUITMENT

08:05 – 08:15 **Grigorios Georgolopoulos**
KU Leuven, Belgium
DENSE CHROMATIN AND TRANSCRIPTIONAL PROFILING ALONG HEMATOPOIETIC DEVELOPMENT DELINEATES THE REGULATORY LANDSCAPE OF LINEAGE COMMITMENT AND DIFFERENTIATION

08:15 – 08:25 **Lauren Kuffler**
Jackson Laboratory/Tufts University, USA
GENETICALLY DIVERSE MOUSE EMBRYONIC STEM CELLS ENABLE INFERENCE OF GENETIC REGULATORY STRUCTURE

08:25 – 08:35 **M. Joaquina Delas**
The Francis Crick Institute, UK
TWO DISTINCT MODES OF CIS REGULATION CONTROL CELL SPECIFICATION IN RESPONSE TO SHH DURING MOUSE SPINAL CORD DEVELOPMENT

08:35 – 08:45 **Adam Bendall**
The Babraham Institute, UK
GENOME-WIDE CRISPR-CAS9 SCREENING UNCOVERS THE POLYCOMB COMPLEX PRC1.3 AS AN ESSENTIAL REGULATOR OF NAÏVE HUMAN PLURIPOTENT CELL REPROGRAMMING

08:45 – 09:05 **Maria Elena Torres-Padilla**
Helmholtz Zentrum München, Germany
EPIGENETIC MECHANISMS OF CELLULAR PLASTICITY AND REPROGRAMMING TO TOTIPOTENCY

09:30 – 11:00 **PLENARY V: CELLULAR IDENTITY**
Sponsored by: Vision Care, Inc.

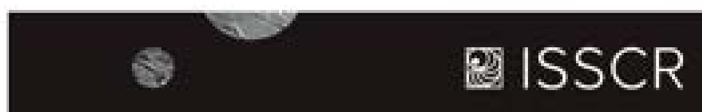
Session Chairs: Edith Heard
EMBL, Germany
Austin Smith
University of Exeter, UK

09:32 – 09:52 **Amanda Fisher**
UKRI-MRC & Imperial College London, UK
PRESERVING CELLULAR IDENTITY AND EPIGENETIC MEMORY THROUGH MITOSIS

09:52 – 10:12 **John Ngai**
NIH Brain Initiative, USA
ILLUMINATING NEURAL STEM CELL TRAJECTORIES AT SINGLE CELL RESOLUTION

10:12 – 10:32 **Samantha Morris**
Washington University School of Medicine, USA
NEW SINGLE-CELL TECHNOLOGIES TO DISSECT REPROGRAMMING AND DEVELOPMENT

10:32 – 10:52 **Ramesh Shivdasani**
Dana Farber Cancer Institute and Harvard Medical School, USA
TRANSCRIPTIONAL AND EPIGENETIC BASIS OF INTESTINAL CRYPT CELL PLASTICITY



DAY 5: FRIDAY 25 JUNE (EDT) *(Continued)*

11:00 – 11:45 **WOMEN IN SCIENCE PANEL: DEFINING MOMENTS: ACHIEVING CAREER INDEPENDENCE**
Sponsored by: T-CiRA Joint Research Program

12:00 – 13:00 **POSTER SESSION 7: MODELING DEVELOPMENT AND DISEASE AND CELLULAR IDENTITY GROUP 3**

12:00 – 13:00 **CAREER PANEL**
Sponsored by: Notch Therapeutics

12:00 – 13:00 **SPECIAL SESSION: GERMAN STEM CELL NETWORK (GSCN) & BERLIN INSTITUTE OF HEALTH (BIH) SESSION 2**
Sponsored by: BIH and GSCN

INNOVATION SHOWCASES (See Page 80 for Details)

12:00 – 13:00 **AMS Biotechnology**
AN IPSC AND ORGANOID MODEL FOR SARS-COV-2 INFECTION: FROM INDIVIDUAL VARIATION TO DRUG DISCOVERY

12:00 – 13:00 **PROTEINTECH GROUP**
Fiona Bellot
Proteintech Group, UK
Jeff Papp
Proteintech Group, UK

Joel Watkinson
Proteintech Group, UK
THE PROTEINTECH ADVANTAGE IN STEM CELL RESEARCH; FROM EXPANSION AND DIFFERENTIATION TO FLOW AND IF

12:00 – 13:00 **YAMAHA MOTOR**
Kazunori Kochiya
Yamaha Motor Co., Ltd., Japan

Raymond Price
PhenoVista Biosciences, USA
APPLICATIONS OF CELL PICKING AND HIGH CONTENT IMAGING IN 3D MODELS OF DISEASE

12:00 – 13:00 **STEMCELL TECHNOLOGIES**
Lynn Csontos
STEMCELL Technologies, Canada

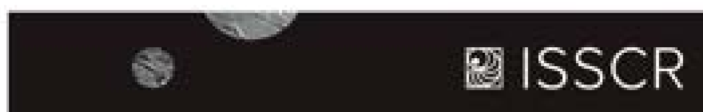
Kimberly Snyder
STEMCELL Technologies, Canada
QUALITY BY DESIGN: REAGENTS AND SUPPORT FOR HPSC-DERIVED CELL AND GENE THERAPIES

12:00 – 12:30 **SOLENTIM LTD**
Ian Taylor
Solentim Ltd., UK
ROBUST WORKFLOW FOR SINGLE CELL CLONING OF IPSCS FOR MAKING CGMP MASTER CELL BANKS

12:30 – 13:00 **STEMBIOSYS**
Travis Block
StemBioSys, Inc., USA
CELL-DERIVED EXTRACELLULAR MATRIX SUPPORTS RAPID MATURATION OF IPSC-DERIVED CELLS IN 2-D CULTURE

DAY 5: FRIDAY 25 JUNE (EDT) *(Continued)*

13:15 – 13:45	**ISSCR MOMENTUM AWARD SESSION**
13:15 – 13:20	**Mitinori Saitou** *Kyoto University, Japan* AWARD PRESENTATION
13:20 – 13:45	**Valentina Greco** *Yale Stem Cell Center, USA* PRINCIPLES OF REGENERATION CAPTURED BY IMAGING THE SKIN OF LIVE MICE
13:45 – 14:45	**POSTER SESSION 8: CLINICAL APPLICATIONS**
14:00 – 15:45	**THEME SESSION MDD 4: MODELING DEVELOPMENT** *Sponsored by: MaxWell Biosystems*
	Chairs: Matt Blurton-Jones *University of California, Irvine, USA* **Grayson Camp** *Institute of Molecular and Clinical Ophthalmology Basel (IOB), Switzerland*
14:02 – 14:07	**Matt Blurton-Jones** *University of California, Irvine, USA* TOPIC OVERVIEW
14:07 – 14:27	**Grayson Camp** *Institute of Molecular and Clinical Ophthalmology Basel (IOB), Switzerland* CHARTING HUMAN DEVELOPMENT USING A MULTI-ORGAN ENDODERMAL ATLAS AND ORGANOID MODELS
14:27 – 14:37	**Alejandro Aguilera Castrejon** *Weizmann Institute of Science, Israel* EX UTERO DEVELOPMENT OF MOUSE EMBRYOS FROM PRE-GASTRULATION TO ADVANCED ORGANOGENESIS
14:37 – 14:47	**Galina Popova** *University of California, San Francisco, USA* NEURO-IMMUNE ORGANOIDS FOR MODELING EARLY BRAIN DEVELOPMENT AND DISEASE
14:47 – 14:57	**Sasha Mendjan** *IMBA, Austria* CARDIOIDS REVEAL SELF-ORGANIZING PRINCIPLES OF HUMAN CARDIOGENESIS
14:57 – 15:07	**Hiraku Tsujimoto** *Center for iPS Cell Research and Application (CiRA), Kyoto University, Japan* GENERATION OF FUNCTIONAL HUMAN KIDNEY ORGANOIDS FROM METANEPHRIC NEPHRON PROGENITORS AND URETERIC BUD CELLS SEPARATELY DIFFERENTIATED FROM HUMAN IPS CELLS
15:07 – 15:17	**Holly Voges** *Murdoch Children's Research Institute, Australia* VASCULARIZATION OF CARDIAC ORGANOIDS CONTROL THE EXTRACELLULAR MATRIX ENVIRONMENT AND REGULATES FUNCTIONALITY
15:17 – 15:37	**Matt Blurton-Jones** *University of California, Irvine, USA* USING HUMAN IPSC-MICROGLIA AND CHIMERIC MICE TO STUDY THE GENETICS OF ALZHEIMER'S DISEASE



DAY 5: FRIDAY 25 JUNE (EDT) *(Continued)*

14:00 – 15:45 **THEME SESSION CI 4: PLURIPOTENCY DYNAMICS**
Sponsored by: NanoString Technologies

Chairs: Amander T. Clark
University of California, Los Angeles, USA
Duanqing Pei
Westlake University, China

14:02 – 14:07 **Amander T. Clark**
University of California, Los Angeles, USA
TOPIC OVERVIEW

14:07 – 14:27 **Duanqing Pei**
Westlake University, China
INTERCONVERSIONS BETWEEN NAIVE AND PRIMED PLURIPOTENCY AS MODELS TO STUDY CELL FATE CONTROL

14:27 – 14:37 **Vincent Pasque**
KU Leuven – University of Leuven, Belgium
COMPREHENSIVE MULTI-OMIC PROFILING REVEALS THE POLYCOMB REPRESSOR COMPLEX PRC2 RESTRICTS HUMAN NAIVE EPIBLAST TO TROPHOBLAST STEM CELL FATE INDUCTION

14:37 – 14:47 **Maria Vega Sendino**
National Institutes of Health, USA
THE ETS TRANSCRIPTION FACTOR ERF CONTROLS THE EXIT FROM THE NAÏVE PLURIPOTENT STATE

14:47 – 14:57 **Ivana Vasic**
Gladstone Institutes, University of California San Francisco, USA
EPITHELIAL TISSUE STRUCTURE REGULATES NAIVE AND PRIMED STATES IN HUMAN PLURIPOTENT STEM CELLS

14:57 – 15:07 **Marta Shahbazi**
MRC Laboratory of Molecular Biology, UK
CAPTURING PLURIPOTENT CELLS IN 3D SELF-RENEWING EPITHELIAL SPHEROIDS

15:07 – 15:17 **Hidemasa Kato**
Ehime University, Graduate School of Medicine, Japan
AMNION-SPECIFIC MARKERS DEMARCATE THE REALM OF VARIOUS HUMAN PLURIPOTENT STATES

15:17 – 15:37 **Amander T. Clark**
University of California, Los Angeles, USA
PLURIPOTENCY IN THE HUMAN GERMLINE

16:30 – 18:15 **THEME SESSION MDD 3: MODELING DISEASE REBROADCAST**
Sponsored by: bit.bio

16:30 – 18:15 **THEME SESSION CI 3: EPIGENETIC REGULATION OF CELL IDENTITY REBROADCAST**

19:30 – 21:15 **THEME SESSION MDD 3: MODELING DISEASE REBROADCAST**
Sponsored by: bit.bio

19:30 – 21:15 **THEME SESSION CI 3: EPIGENETIC REGULATION OF CELL IDENTITY REBROADCAST**

21:30 – 23:00 **PLENARY V: CELLULAR IDENTITY REBROADCAST**
Sponsored by: Vision Care, Inc.

DAY 6: SATURDAY 26 JUNE (EDT)

00:00 – 01:00 **POSTER SESSION 9: MODELING DEVELOPMENT AND DISEASE AND CELLULAR IDENTITY**

01:45 – 02:45 **POSTER SESSION 10: CLINICAL APPLICATIONS**

02:00 – 03:45 **THEME SESSION MDD 4: MODELING DEVELOPMENT REBROADCAST**
Sponsored by: MaxWell Biosystems

02:00 – 03:45 **THEME SESSION CI 4: PLURIPOTENCY DYNAMIC REBROADCAST**
Sponsored by: NanoString Technologies

07:30 – 09:15 **THEME SESSION CA 1: COMPLEX 3D SYSTEMS FOR THERAPY AND DRUG DISCOVERY**
Sponsored by: T-CiRA Joint Research Program

Chairs: Ben M. Maoz
Tel Aviv University, Israel
Misao Fujita
Center for iPS Cell Research and Application, Kyoto University, Japan

07:32 – 07:37 **Ben M. Maoz**
Tel Aviv University, Israel
TOPIC OVERVIEW

07:37 – 07:57 **Misao Fujita**
Center for iPS Cell Research and Application, Kyoto University, Japan
ETHICS OF HUMAN BRAIN ORGANOID RESEARCH FROM THE PERSPECTIVE OF SOCIAL SCIENCE SURVEY

07:57 – 08:07 **Rowan Karvas**
Washington University in St. Louis, USA
3D ORGANOIDS GENERATED FROM HUMAN TROPHOBLAST STEM CELLS MODEL EARLY PLACENTAL DEVELOPMENT AND SUSCEPTIBILITY TO EMERGING VIRAL INFECTIONS

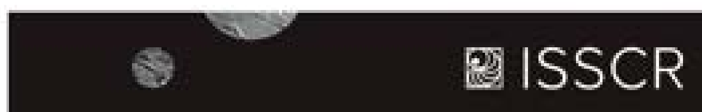
08:07 – 08:17 **Verena Charwat**
University of California, Berkeley, USA
PREDICTING ARRHYTHMOGENIC DRUG RISK IN A METABOLICALLY MATURED CARDIAC MICROPHYSIOLOGICAL SYSTEM

08:17 – 08:27 **Russell Quinn**
National Institutes of Health (NIH), USA
BIOPRINTED 3D HUMAN OUTER BLOOD RETINAL BARRIER UNCOVERS RPE-DEPENDENT CHOROIDAL PHENOTYPE IN ADVANCED MACULAR DEGENERATION

08:27 – 08:37 **Tatsuya Osaki**
The University of Tokyo, Japan
COMPLEX ACTIVITY AND SHORT-TERM MEMORIES IN RECIPROCALLY CONNECTED CEREBRAL ORGANOIDS

08:37 – 08:47 **Michael O'Connor**
Western Sydney University, Australia
HUMAN LENS REGENERATION VIA TRANSPLANTATION OF PLURIPOTENT STEM CELL-DERIVED LENS EPITHELIAL CELLS; A POTENTIAL NEW TREATMENT FOR CHILDHOOD CATARACT

08:47 – 09:07 **Ben M. Maoz**
Tel Aviv University, Israel
ORGANS-ON-A-CHIP: A NEW TOOL FOR THE STUDY OF HUMAN PHYSIOLOGY



DAY 6: SATURDAY 26 JUNE (EDT) *(Continued)*

07:30 – 09:15 **THEME SESSIONS CA 2: ENGINEERING TISSUE AND ORGANS**
Sponsored by: BlueRock Therapeutics

Chairs: James Hudson
University of Queensland; QIMR Berghofer, Australia
Randolph S. Ashton
University of Wisconsin, USA

07:32 – 07:37 **James Hudson**
University of Queensland; QIMR Berghofer, Australia
TOPIC OVERVIEW

07:37 – 07:57 **Randolph S. Ashton**
University of Wisconsin, USA
STANDARDIZATION OF A SCALABLE HUMAN NEURAL ROSETTE ASSSAY FOR ASSESSMENT OF NEURAL TUBE DEFECT RISK & DEVELOPMENTAL NEUROTOXICICTY

07:57 – 08:07 **Adrian Veres**
Harvard University, USA
IDENTIFYING GENETIC REGULATORS OF ENDOCRINE AND BETA CELL IN VITRO DIFFERENTIATION VIA GENOME-WIDE LOSS-OF-FUNCTION SCREENING

08:07 – 08:17 **Toshiya Nishimura**
The University of Tokyo, Japan
IGF1R DELETION IN A HOST EMBRYO AUGMENTS DONOR CONTRIBUTION TO HOST TISSUES IN BOTH INTRA- AND INTER-RODENT CHIMERAS

08:17 – 08:27 **Catherine Lee**
Brigham and Women's Hospital, Harvard Medical School, USA
3D-PRINTED ABCB5-POSITIVE STEM CELLS FOR TREATING BILATERAL LIMBAL STEM CELL DEFICIENCY

08:27 – 08:37 **Matteo Ghiringhelli**
Technion-Israel Institute of Technology, Israel
ELECTRICAL PROPERTIES AND OPTOGENETIC STIMULATION OF HUMANIZED CHAMBER-SPECIFIC ENGINEERED HEART TISSUES COMBINING DECELLULARIZED HEARTS WITH INDUCED PLURIPOTENT STEM CELL DERIVED CARDIOMYOCYTES

08:37 – 08:47 **Tobias Deuse**
University of California, San Francisco, USA
HYPOIMMUNE IPSC-DERIVED CELL PRODUCTS TREAT CARDIOVASCULAR DISEASES IN IMMUNOCOMPETENT ALLOGENEIC MICE

08:47 – 09:07 **James Hudson**
University of Queensland; QIMR Berghofer, Australia
BROMODOMAIN AND EXTRATERMINAL INHIBITION BLOCKS INFLAMMATION-INDUCED CARDIAC DYSFUNCTION

09:30 – 11:00 **PLENARY VI: CELLULAR THERAPY AND TISSUE ENGINEERING**
Sponsored by: STEMCELL Technologies

Session Chairs: Lorenz Studer
Sloan-Kettering Institute for Cancer Research, USA
Shinya Yamanaka
Gladstone Institutes, USA/CiRA, Kyoto University, Japan

09:32 – 09:52 **Paul Tesar**
Case Western Reserve University, USA
MECHANISMS OF OLIGODENDROCYTE REGENERATION

DAY 6: SATURDAY 26 JUNE (EDT) *(Continued)*

09:52 – 10:12 **Shuibing Chen**
Weill Cornell Medical College, USA
HPSC-DERIVED ORGANOIDS FOR COVID-19 DISEASE MODELING AND DRUG SCREENING

10:12 – 10:32 **Wolfram Zimmermann**
University Medical Center Goettingen, Germany
TISSUE ENGINEERED HEART REPAIR: FROM NON-HUMAN PRIMATES TO A FIRST-IN-PATIENT CLINICAL TRIAL

10:32 – 10:52 **Sonja Schrepfer**
UCSF and Sana Biotechnology Inc., USA
IDENTIFYING AND OVERCOMING THE IMMUNOLOGICAL HURDLE IN CELLULAR THERAPY FOR REGENERATIVE MEDICINE

11:10 – 11:55 **PLENARY ROUNDTABLE: ENGINEERED TISSUES: CHALLENGES TO BRING TO CLINIC**
Sponsored by: Astellas

Moderator: Lorenz Studer
Sloan-Kettering Institute for Cancer Research, USA

Panelists:
Kapil Bharti
National Institutes of Health (NIH), USA
Deborah Hursh
Food and Drug Administration, USA
Jennifer Lewis
Harvard University, USA
Wolfram Zimmermann
University Medical Center Goettingen, Germany

12:00 – 13:10 **THEME SESSIONS CA 3: ROAD TO CLINIC I (REGENERATIVE MEDICINE)**
Sponsored by: BlueRock Therapeutics

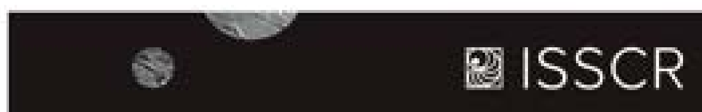
Chairs: Elena Cattaneo
University of Milan and National Institute of Molecular Genetics, Italy
Timothy Kieffer
University British Columbia, Canada

12:02 – 12:07 **Elena Cattaneo**
University of Milan and National Institute of Molecular Genetics, Italy
TOPIC OVERVIEW

12:07 – 12:27 **Timothy Kieffer**
University British Columbia, Canada
STEM CELL DERIVED ISLETS TO TREAT DIABETES

12:27 – 12:37 **Tae Wan Kim**
Memorial Sloan Kettering Cancer Center, USA
TWO STEP WNT SIGNALLING ACTIVATION FACILITATES THE INDUCTION OF HUMAN PLURIPOTENT STEM CELL DERIVED MIDBRAIN DOPAMINERGIC NEURONS FOR TRANSLATIONAL USE

12:37 – 12:47 **Kathryn Lye**
University of Toronto, Canada
CELL FUSION TO COMBINE THERAPEUTIC PROPERTIES



DAY 6: SATURDAY 26 JUNE (EDT) *(Continued)*

12:47 – 13:07 **Elena Cattaneo**
University of Milan and National Institute of Molecular Genetics, Italy
INFORMING IN VITRO STEM CELL DIFFERENTIATION THROUGH SINGLE-CELL RNASEQ ANALYSIS OF THE DEVELOPING HUMAN FETAL STRIATUM

12:00 – 13:10 **THEME SESSIONS CA 4: ROAD TO CLINIC II (DRUG DISCOVERY)**
Sponsored by: Surrozen

Chairs: Junya Toguchida
Center for iPS Cell Research and Application, Kyoto University, Japan
Jane S. Lebkowski
Regenerative Patch Technologies, USA

12:02 – 12:07 **Junya Toguchida**
Center for iPS Cell Research and Application, Kyoto University, Japan
TOPIC OVERVIEW

12:07 – 12:27 **Jane S. Lebkowski**
Regenerative Patch Technologies, USA
PHASE 1/2A CLINICAL ASSESSMENT OF A BIOENGINEERED, RPE CELL-BASED IMPLANT FOR THE TREATMENT OF ADVANCED DRY AGE-RELATED MACULAR DEGENERATION

12:27 – 12:37 **Pei-Hsuan Chu**
National Center for Advancing Translational Sciences (NCATS), USA
COMBINED GENETIC AND CHEMICAL SCREENS USING HUMAN NEURAL STEM CELLS IDENTIFY ZIKA VIRUS RESISTANCE FACTORS AND NEW DRUG CANDIDATES

12:37 – 12:47 **Shinichi Takahashi**
Keio University School of Medicine, Japan
ROPALS TRIAL: PHASE 1/2A, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY OF ROPINIROLE HYDROCHLORIDE FOR ALS PATIENTS BASED ON THE IPSC DRUG REPOSITIONING

12:47 – 13:07 **Junya Toguchida**
Center for iPS Cell Research and Application, Kyoto University, Japan
APPLICATION OF DISEASE-SPECIFIC IPS CELLS FOR DISCLOSING THE PATHOMECHANISM AND DISCOVERING THERAPEUTIC CHEMICALS FOR INTRACTABLE DISEASES

13:15 – 15:15 **PLENARY VII: BREAKTHROUGHS IN THERAPY DEVELOPMENT**
Sponsored by: bit.bio

Session Chairs: Christine L. Mummery
Leiden University Medical Center, Netherlands
Sally Temple
Neural Stem Cell Institute, USA

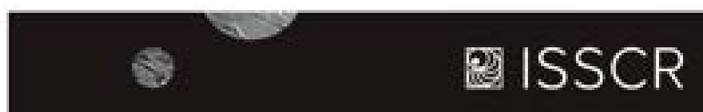
13:18 – 13:26 **Melissa Little**
Murdoch Children's Research Hospital, Australia
PRESIDENT ELECT ADDRESS

13:26 – 13:36 **Jacquelien Noordhoek**
Dutch CF Foundation, Netherlands
PATIENT ADVOCATE ADDRESS

13:36 – 14:01 **Viviane Tabar**
Memorial Sloan-Kettering Cancer Center, USA
JOHN MCNEISH MEMORIAL LECTURE: THE GENESIS OF A PHASE 1 CLINICAL TRIAL OF HUMAN ES-DERIVED MIDBRAIN DOPAMINE NEURON GRAFTS FOR PARKINSON'S DISEASE

DAY 6: SATURDAY 26 JUNE (EDT) *(Continued)*

14:01 – 14:21	**Sergiu Pasca** *Stanford University, USA* FROM STEM CELLS TO BRAIN ASSEMBLOIDS: CONSTRUCTING AND DECONSTRUCTING THE HUMAN NERVOUS SYSTEM
14:21 – 14:41	**James E. Bradner** *Novartis Institute for BioMedical Research, USA* DELIVERING ON THE PROMISE OF CELL AND GENE THERAPY
14:41 – 15:06	**Shinya Yamanaka, CiRA** *Kyoto University, Japan* KEYNOTE ADDRESS: RECENT PROGRESS IN IPS CELL RESEARCH AND APPLICATION
15:06 – 15:10	**Christine L. Mummery** *Leiden University Medical Center, Netherlands* CLOSING REMARKS
16:30 – 18:15	**THEME SESSION CA 1: COMPLEX 3D SYSTEMS FOR THERAPY AND DRUG DISCOVERY REBROADCAST** *Sponsored by: T-CiRA Joint Research Program*
16:30 – 18:15	**THEME SESSION CA 2: ENGINEERING TISSUE AND ORGANS REBROADCAST** *Sponsored by: BlueRock Therapeutics*
19:30 – 21:15	**THEME SESSION CA 1: COMPLEX 3D SYSTEMS FOR THERAPY AND DRUG DISCOVERY REBROADCAST** *Sponsored by: T-CiRA Joint Research Program*
19:30 – 21:15	**THEME SESSION CA 2: ENGINEERING TISSUE AND ORGANS REBROADCAST** *Sponsored by: BlueRock Therapeutics*
21:30 – 23:00	**PLENARY VI: CELLULAR THERAPY AND TISSUE ENGINEERING REBROADCAST** *Sponsored by: STEMCELL Technologies*
00:00 – 01:10	**THEME SESSION CA 3: ROAD TO CLINIC I (REGENERATIVE MEDICINE) REBROADCAST** *Sponsored by: BlueRock Therapeutics*
00:00 – 01:10	**THEME SESSION CA 4: ROAD TO CLINIC II (DRUG DISCOVERY) REBROADCAST** *Sponsored by: Surrozen*
01:15 – 03:20	**PLENARY VII: BREAKTHROUGHS IN THERAPY DEVELOPMENT REBROADCAST** *Sponsored by: bit.bio*



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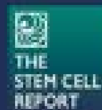

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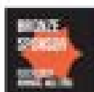

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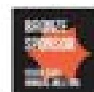

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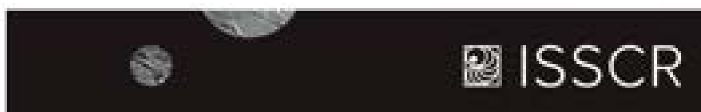

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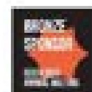

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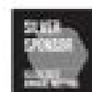

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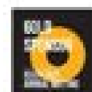

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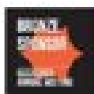

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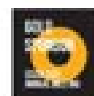

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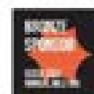

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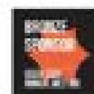

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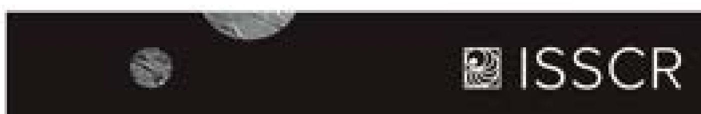

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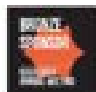

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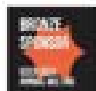
ELEVATEBIO



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Suite 500
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617-433-2600
info@elevate.bio
https://www.elevate.bio/

ElevateBio is a cell and gene therapy technology company built to power the development of transformative cell and gene therapies today and for many decades to come. The company has assembled industry-leading talent, built world-class facilities, and integrated diverse technology platforms necessary for rapid innovation and commercialization of cell, gene, and regenerative therapies.

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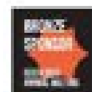

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Emulate Inc. creates advanced in vitro models for understanding how diseases, medicines, chemicals, and foods affect human health. Each Organ-Chip model contains tiny hollow channels lined with thousands of living human cells and tissues — offering researchers the ability to predict human response with greater precision than conventional cell culture or animal-based experimental testing.

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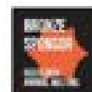

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bioprocess-info@eppendorf.de
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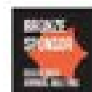
Large cell numbers are needed for the development of cell therapies, stem cell-based drug research and future applications such as lab grown meat. By utilizing its strong synergies in cell culture expertise, bioreactor technology, and polymer manufacturing, Eppendorf has emerged as an expert partner for the cultivation of stem cells at large scale.

EUROPEAN BANK FOR INDUCED PLURIPOTENT STEM CELLS (EBISC2) PHE



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Salisbury SP4 0JG
United Kingdom
+44 (0)1980-612512
https://ebisc.org/

The European Bank for Induced Pluripotent Stem Cells is a centralised, not-for-profit iPSC bank providing researchers across academia and industry with access to an extensive range of high-quality, research-grade, and fully consented iPSCs for use in disease modelling and other forms of pre-clinical research. The collection currently holds iPCs generated from a wide range of donors representing specific disease backgrounds and healthy normal donors.

EUROPEAN NETWORK OF NETWORKS (EURONET)

c/o Max Delbrück Center
Robert-Rössle-Str. 10
13125 Berlin
Germany
+49-30-9406 2488
d.besser@mdc-berlin.de
www.gscn.org

The German Stem Cell Network (GSCN) aims at creating synergies between all areas of basic and applied stem cell research and to provide an interface between science, education, politics and society as a whole.

FATE THERAPEUTICS

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bob.valamehr@fatetherapeutics.com
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Fate Therapeutics is a clinical-stage biopharmaceutical company dedicated to the development of first-in-class cellular immunotherapies for patients with cancer. The Company has established a leadership position in the clinical development and manufacture of universal, off-the-shelf cell products using its proprietary induced pluripotent stem cell (iPSC) product platform.

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Fluicell is an innovative life science company dedicated to providing single-cell microfluidic research tools and services for scientists worldwide. We deliver easy-to-use solutions for your research needs in single-cell biology, electrophysiology, single-cell bioprinting and tissue modelling.

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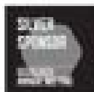

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From research to therapy, Horizon Discovery drives the application of gene editing and gene modulation. Innovative tools and services enable scientists to gain a greater understanding of the genetic drivers behind disease, develop and validate diagnostic workflows, and deliver new therapies for precision medicine.

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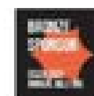

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iotaSciences offers compact automation for highly efficient single-cell cloning. Our cloning platform will accelerate your genome-editing projects with powerful automation, assurance of monoclonality and high cloning efficiency for diverse cell lines, including iPSCs. The platform uniquely automates all the fluid-handling steps of the single-cell cloning workflow and enables easy clone tracking which ensures consistency in every cloning experiment.

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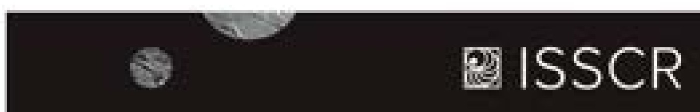

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MaxWell Biosystems develops and markets high-content electrophysiology platforms. The MaxOne (single-well) and MaxTwo (multi-well) high-density microelectrode array (HD-MEA) systems allow label-free recording and stimulation of every active cell on a dish at unprecedented spatio-temporal resolution, facilitating the detailed investigation of cells. The powerful MaxLab Live all-in-one software enables live visualization, recording, and analysis of extracellular HD-MEA signals from different biological preparations such as iPSC-derived neurons, organoids, retina, or brain slices. MaxWell Biosystems' solutions will advance scientific breakthroughs and accelerate drug discovery. Every cell has a story to tell. MaxWell Biosystems aims to equip everyone with tools to easily observe, track, and discover cells functionality, health, maturity, and response to compounds.

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Mill Creek Life Sciences is a leading provider of human platelet lysate products for the manufacture of cell, gene, and tissue-engineered products.

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Miltenyi Biotec provides products that advance biomedical research and cellular therapy. Our innovative tools support research from basic research to translational research to clinical application. Our 30 years of expertise includes immunology, stem cell biology, neuroscience, and cancer. Miltenyi Biotec has about 3,000 employees in 28 countries.

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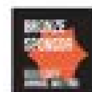

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Notch is developing renewable, stem cell-derived immunotherapies, with an initial focus on cancer. We believe that cell therapies can bring value to patients and healthcare systems by being safe, effective, and accessible. The Notch Engineered Thymic Niche (ETN) provides developing hematopoietic cells the right signals at the right time to predictably direct their development.

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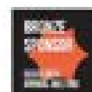

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On-chip Biotechnologies is the manufacturer of the world's first microfluidic chip-based flow cytometer and cell sorter. The cell sorter, "On-chip Sort", has an exchangeable and disposable microfluidic cartridge as its core. This innovative technology allows for damage-free cell and large cell cluster (e.g. spheroids) recovery in sterile condition which is not attainable with conventional sorters.

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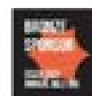

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Protein Fluidics is commercializing biological assay automation using our novel microfluidic technologies. By combining microfluidics and proprietary design, the Pu·MA System® has been designed to run automated immunoassays and 3D cell-based assays for different applications such as oncology, cellular stress, inflammation and metabolic disease research. We in the SF Bay Area and run by an experienced team of scientists, engineers, and industry executives.

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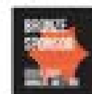

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Proteintech's HumanKine product line of human cell expressed cytokines and growth factors was established in 2005. The purpose-built GMP, ISO 13485 certified, production facility near Chicago was opened in 2020. In addition, Proteintech has the largest proprietary portfolio of self-manufactured antibodies covering 2/3 of the human proteome and has acquired ChromoTek, a leading producer of Nanobody-based reagents.

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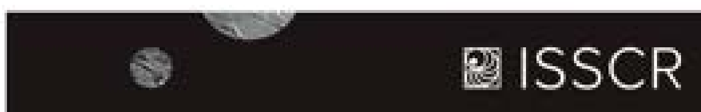

SANA BIOTECHNOLOGY, INC.

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Sana Biotechnology, Inc. is focused on creating and delivering engineered cells as medicines for patients. We share a vision of repairing and controlling genes, replacing missing or damaged cells, and making our therapies broadly available to patients. We are more than 250 people working together to create an enduring company that changes how the world treats disease. Sana has operations in Seattle, Cambridge, and South San Francisco. For more information about Sana Biotechnology, please visit https://sana.com/.

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Sartorius is a partner of life science research and the biopharmaceutical industry. With laboratory instruments and consumables, the Lab Products & Services Division concentrates on serving the needs of laboratories performing research and quality control at pharma and biopharma companies and those of academic research institutes. The Bioprocess Solutions Division with its product portfolio focusing on single-use solutions helps customers to manufacture biotech medications and vaccines.

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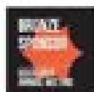

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SCREEN is a leader in the areas of imaging and electronics technology. Scientists at SCREEN have implemented their technology in the Life Sciences by developing robust, versatile imaging platforms with a broad spectrum of applications for 2D and 3D in vitro and ex vivo assays. Additionally, SCREEN's MED64 electrophysiology platforms provide the most-sensitive microelectrode arrays for label-free functional assays with stem cell-derived cardiomyocytes or/and neurons.

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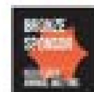

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https://sigilon.com/

Sigilon Therapeutics seeks to develop functional cures for chronic diseases through its Shielded Living Therapeutics™ platform. Sigilon's product candidates are non-viral engineered cell-based therapies designed to produce the crucial proteins, enzymes or factors needed by patients living with chronic diseases such as hemophilia, lysosomal disorders and diabetes.

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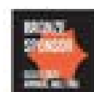
General electrical manufacturer. Develops automated cell culture equipment.

SOLENTIM INC.



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Solentim is the trusted global leader in workflows for antibody and cell based therapies. Our assurance rich technologies enable the isolation, growth and characterization of high value cells while our data driven platform enables smarter decision earlier in the process. Together, our customers experience faster workflows, confidently designed for regulatory environments.

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Sony Biotechnology Inc. is dedicated to helping researchers working across different life science disciplines to achieve the best scientific results. By leveraging Sony's vast know- how in electronics innovation and design, we offer next-generation cell analysis systems to accelerate your discoveries. Our goal is to bring a unique perspective to the flow cytometry tools required for single-cell isolation and in-depth analysis. With our core expertise in automation and software development, we hope to enable discovery research across immunology, oncology, cell biology, and microbiology.

STEM CELL REPORTS

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Stem Cell Reports, the official journal of the ISSCR, is an online, open access forum communicating basic discoveries in stem cell research, in addition to translational and clinical studies. The journal also produces a podcast, The Stem Cell Report, a Podcast with Martin Pera available on iTunes, Spotify and other podcast platforms.

STEMBIOSYS, INC.

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www.stembiosys.com

StemBioSys, Inc., a privately held, San Antonio-based biomedical company, manufactures and develops innovative, advanced stem cell technologies to meet the promise of regenerative medicine in a surging global market. Its patented and proprietary technology platforms – licensed from the University of Texas System – overcome key obstacles to creating clinically useful cell therapies. StemBioSys markets its products to the global research community under the CELLvo™ brand name.

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At STEMCELL, science is our foundation. Driven by our mission to advance research globally, we offer over 2,500 tools and services supporting discoveries in stem cell research, regenerative medicine, immunotherapy and disease research. By providing access to innovative techniques like gene editing and organoid cultures, we're helping scientists accelerate the pace of discovery. Inspired by knowledge, innovation and quality, we are Scientists Helping Scientists.

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Stem Genomics provides specific services and kits to assess the genetic integrity of stem cells. We offer a range of tests that use digital PCR and a panel of specific probes to detect most of the recurrent genetic abnormalities observed in stem cells. These tests are fast (1-3 days), robust, and cost-effective. They are particularly useful for frequently monitoring cultured stem cell lines.

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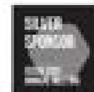
Surrozen is a biotechnology company discovering and developing drug candidates to selectively modulate the Wnt pathway. We are pioneering the selective activation of Wnt signaling, designing and engineering Wnt pathway mimetics and advancing tissue-specific candidates. Our lead product candidates are multi-specific, antibody-based therapeutics that mimic the roles of naturally occurring Wnt or R-spondin proteins, both of which are involved in activation of the Wnt pathway.

SYNTHEGO CORPORATION



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Synthego is a genome engineering company that enables the acceleration of life science research and development in the pursuit of improved human health. The company leverages machine learning, automation, and gene editing to build platforms for science at scale. With its foundations in engineering disciplines, the company's full-stack platform vertically integrates proprietary hardware, software, bioinformatics, chemistries, and molecular biology to advance both basic research and therapeutic development programs. By providing both commercial and academic researchers and therapeutic developers with unprecedented access to cutting-edge genome engineering products and services, Synthego is at the forefront of innovation in engineered biology.

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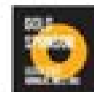

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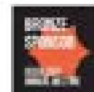

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Tecan is a leading global provider of automated laboratory instruments and solutions. Our systems and components help people working in clinical diagnostics, basic and translational research and drug discovery bring their science to life.
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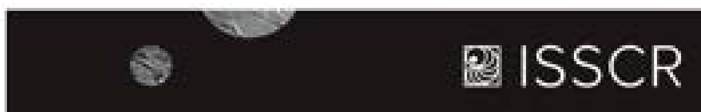
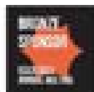


TENAYA THERAPEUTICS



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Tenaya is shaping the future of heart disease treatment driven by a bold mission: to discover, develop, and deliver curative therapies that address the underlying drivers of disease, including rare genetic disorders as well as more prevalent heart conditions. Tenaya is using biological insights from our team and our collaborators to chart new paths to treat and mitigate the progression of heart disease, the leading cause of mortality worldwide. Using the vastly more sophisticated tools now available in drug development – including gene therapy techniques, advances in cellular modeling of disease, and high content imaging and machine learning algorithms – we are pioneering a new class of medicines to target these disease processes and advance therapies that have the potential to transform treatment.

THE NYSCF RESEARCH INSTITUTE

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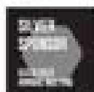
www.nyscf.org

The New York Stem Cell Foundation (NYSCF) Research Institute accelerates cures for the major diseases of our time through stem cell research and technology innovation. Our fully automated iPSC reprogramming platform has built a vast community repository of lines used by laboratories worldwide. We take a "team science" approach, collaborating with and funding leading stem cell scientists to advance translational research.

THERMO FISHER SCIENTIFIC



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Thermo Fisher Scientific is the world leader in serving science. Our mission is to enable our customers to make the world healthier, cleaner and safer. Through our Gibco and Invitrogen brands, we help customers accelerate innovation and enhance productivity.

TREEFROG THERAPEUTICS (CONTRIBUTING SPONSOR)

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contact@treefrog.fr
https://treefrog.fr/

TreeFrog Therapeutics is a biotech company aiming at providing access to cell therapies for millions of patients. TreeFrog Therapeutics has developed C-Stem, a high-throughput cell encapsulation technology allowing the mass-production and differentiation of stem cells in industrial bioreactors. This proprietary technology platform provides an end-to-end and scalable solution that dramatically improves the quality of therapeutic cells and reduce treatment costs.

UNION BIOMETRICA, INC.

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Holliston, MA 01746
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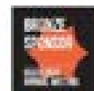
+1 508-893-3115
Sales@unionbio.com
www.unionbio.com

Union Biometrica COPAS FP™ and BioSorter® Large Particle Flow Cytometers automate analysis and sorting of objects too big/fragile for traditional cytometers, e.g., large cells/clusters, cells in/on beads and small model organisms (5-1500 micron diameter). The COPAS VISION cytometer adds brightfield image capture on the fly for convenient identification of objects.

VERTEX PHARMACEUTICALS, INC.



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Union Biometrica COPAS FP™ and BioSorter® Large Particle Flow Cytometers automate analysis and sorting of objects too big/fragile for traditional cytometers, e.g., large cells/clusters, cells in/on beads and small model organisms (5-1500 micron diameter). The COPAS VISION cytometer adds brightfield image capture on the fly for convenient identification of objects.

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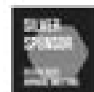

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Vision Care Inc. is a leading company in stem cell therapy in ophthalmology. Our focus is on transplantation of stem cell-derived Retinal Pigment Epithelial cells and Photoreceptor cells. We innovate and accelerate production processes by Robotics and AI technologies, to provide high-quality cells for clinical use. Our vision is "Any and all means for all patients".

WICELL

504 South Rosa Road, Suite 101
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www.wicell.org

As a recognized world leader in pluripotent stem cell banking and characterization, WiCell provides the stem cell community with high-quality cell lines as well as accurate and reliable characterization testing. WiCell Stem Cell Bank offerings include human pluripotent cell lines (ES, iPS, disease model, cGMP, and modified). Characterization services include karyotype, FISH, SKY, SNP microarray, mycoplasma testing, and identity by STR. In addition, long-term liquid nitrogen storage and customizable services such as quality control testing and cell banking are offered, allowing laboratories to fully optimize their limited resources.

WORTHINGTON BIOCHEMICAL CORPORATION

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Worthington is an ISO9001 Certified primary supplier of enzymes and biochemicals for primary & stem cell isolation, bioprocessing, biopharm and related applications. New! Animal/Xeno Free Collagenases, DNAses, RNAses and STEMxyme® Collagenase/Neutral Protease Blends. Connect with us for our latest Catalog, Manuals and Cell Isolation/Tissue Guide and FREE Collagenase Sampling Program.

YAMAHA MOTOR CO., LTD.

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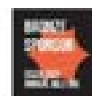
https://global.yamaha-motor.com/business/hc/?isscr2021

Yamaha Motor has developed a cell picking and imaging system CELL HANDLER. The integration of sophisticated imaging and picking technology enables confirming monoclonality and isolating 3D-organoids/spheroids or single cells in a gentle manner even from gels. The system can enhance the efficiency of stem cell and single-cell research, drug discovery through the expansion of options in cell-based screening, cell quality management and cell line development.

YOKOGAWA GERMANY GMBH



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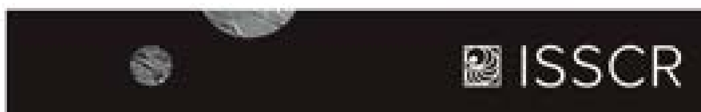
Yokogawa launched its life innovation business in FY2018 with the aim of contributing to the achievement of well-being for all. The company provides platforms for the effective utilization of human intelligence in research related to life sciences, biotech, pharmaceutical and foodstuff production processes through high-level linking with information from observation and measurement, and autonomous control utilizing the results of analysis of this information.



INNOVATION SHOWCASES

TUESDAY, 22 JUNE

12:00 to 13:00

ENHANCING PHYSIOLOGICAL RELEVANCE IN VITRO: TECH INNOVATIONS FOR STEM CELL SORTING, 3D MODELING, AND OXYGEN CONTROL

PRESENTED BY PHC CORPORATION OF NORTH AMERICA

As the fields of stem cell research and regenerative medicine continue to gain clinical importance, the standards for physiological relevance in cultures will continue to heighten. Conventional platforms for incubation, cell sorting and 2D cultures often inhibit differentiation potential, lack lineage control and skew phenotypes for a variety of stem cell characteristics. Conventional cell sorters inflict shear stress, electrostatic charges and high collision rates that lead to loss of function and stem cell death. 2-dimensional cell cultures limit true in vivo replication of the extracellular matrix and stem cell morphology. Stem cells at atmospheric oxygen give rise to chromosomal abnormalities as well as genomic and epigenomic aberrations.

Technology innovations in microfluidic-based cell sorting, 3-D cell cultures and oxygen-controlled incubation platforms allow more robust control of stem cell fate within the laboratory workflow. Join PHC Corporation of North America, a unique solutions provider of the On-chip Sort cell sorter, PrimeSurface spheroid-forming platform & Cell-IQ™ series incubation line. Hear how we have integrated cutting-edge technology into stem cell instrumentation that is changing the convention on "typical" stem cell drawbacks.

PRESENTERS:

Carl Radosevich, *PHC Corporation of North America, USA*

Holly Hattaway, *PHC Corporaton of North America, USA*

12:00 to 13:00

LEVERAGING AUTOMATION OF IPSC ASSAY TECHNOLOGY FOR DISEASE MODELLING, DRUG DISCOVERY & SAFETY

PRESENTED BY TECAN TRADING AG

Induced pluripotent stem cells (iPSCs) are a very powerful tool to build biologically relevant cellular models for drug discovery & develo ent. Automation of iPSCs assay technologies enables assay standardization and increased throughput, two essential aspects for their use in a drug discovery setting. In this webinar, automation of iPSC based assays to assess drug potency and safety/toxicology will be presented. The session will be articulated in 3 segments:

1) Brief presentation of Tecan's next generation liquid handler, Fluent®, and multimode reader with imaging, Spark® Cyto
2) Automated hiPSC based drug screening to assess safety/potency/toxicology by Ole Pless, Fraunhofer ITMP Screenport, Hamburg, Germany.
3) Automated drug screening on iPSC-derived organoids by Alejandro Hidalgo Gonzalez, Murdoch Children's Research Institute, Melbourne, Australia.

PRESENTERS:

Lucia Bruzzone, *Tecan Trading AG, Switzerland*

Ole Pless, *Fraunhofer ITMP ScreeningPort , Germany*

Alejandro Hidalgo-Gonzalez, *Murdoch Children's Research Institute, Australia*

12:00 to 13:00

MICROENGINEERED HUMAN BRAIN-CHIP FOR DISEASE MODELING APPLICATIONS

PRESENTED BY EMULATE, INC.

The need for human-relevant systems that can recreate key aspects of brain physiology and pathophysiology are being driven by the challenges of using the currently available animal models for translational research. An improved understanding of the neurovascular unit function and its alterations in disease should lead to new strategies for therapeutic intervention in neurological and neurodegenerative disorders. Here we present a human Brain-Chip system engineered to recapitulate critical aspects of the complex neurovascular unit. Our human organotypic microphysiological system includes endothelial cells, pericytes, glia, and neurons and maintains blood-brain barrier permeability at in vivo relevant levels. You will also hear from the Board of Governors Regenerative Medicine Institute on how they have created an induced pluripotent stem cell (iPSC) derived model of the human gut-brain axis and are beginning to use this platform to interrogate the contribution of various gut microbes and microbial metabolites in promoting CNS disease pathology. Progress in developing a blood-brain barrier (BBB)-Chip and an Intestine-Chip from iPSCs which recapitulate several in vivo molecular functions and can be fluidically linked to simulate the gut-brain axis will be presented.

PRESENTERS:

Luke Dimasi, *Emulate, Inc., USA*

Sifis Pediaditakis, *Emulate, Inc., USA*

Michael Workman, *Cedars-Sinai, USA*

12:00 to 13:00

MS, COGS AND CDMOS - MSCS ARE EVERYWHERE, AND FOR A GOOD CAUSE

PRESENTED BY SARTORIUS LAB INSTRUMENTS GMBH & CO. KG

Bone marrow derived MSC hold immunomodulatory and neuroprotective features. In Multiple Sclerosis, the use of MSC

might be useful in term of diseases amelioration and progression. An open-label clinical study (Phase I) was conducted in our center and positive results regarding safety, feasibility and efficacy were reported. Recently, our center has finished a large placebo-controlled double-blinded clinical trial (Phase II) in progressive MS patients determining the best dose and administration method of MSCs. For allogeneic cell therapies to be successful, manufacturing challenges related to scalability and reproducibility need to be addressed. Particularly the greatest driver of COGs is typically the media required for manufacturing large cells lots. Thus, finding robust, cost-effective bioprocess media solutions and engineering them for scalability is paramount to the success of any therapeutic program. This talk will focus on considerations and approaches for large scale cell therapy manufacturing and sustained manufacturing success.Esco Aster Pte Ltd is a CDMO running primarily on patented Tide Motion Adherent Cell Platform. The founder and CEO of Esco Aster will be presenting findings from studies with the tide motion bioreactors for large scale manufacturing of mesenchymal stromal cells, and exosome-derived from stem cell culture using the Nutristem XF media.

PRESENTERS:

Ibrahim Kassis, *Hadassah Medical Center, Israel*

Priya Baraniak, *OrganoBio, LLC, USA*

Xiangliang Lin, *Esco Aster Pte Ltd , Singapore*

12:00 to 13:00

NEURAL ACTIVITY IN A DISH: ADVANCING OUR UNDERSTANDING OF NERVOUS SYSTEM DISORDERS

PRESENTED BY AXION BIOSYSTEMS

Using electrodes embedded in the culture surface of multiwell plates, Axion BioSystems' Maestro Pro MEA system measures real-time neural activity from iPSC-derived neurons cultured in the wells.

What you will learn in this webinar:

- Epilepsy – Dr Evangelos Kiskinis' lab (Northwestern University) isolated cells from patients with KCNQ2 epilepsy and used these cells to create iPSC-derived neurons with or without the mutation. When the electrical activity of these neurons was recorded using the Maestro MEA system, it was highly reminiscent of the patterns seen on electroencephalograms (EEGs) of patients with the mutation. Targeting the dyshomeostatically altered ion currents caused by the KCNQ2 mutation might offer an alternative therapeutic strategy for the cognitive and develo ental deficits in KCNQ2 epilepsy.
- Neuropathy – Chemotherapy-induced peripheral neuropathy (CIPN) is a common and debilitating adverse event that can alter patient treatment options and halt candidate drug develo ent. However, translating preclinical neuropathy findings to humans proves challenging as no robust in vitro models of CIPN exist. Using commercially available hiPSC-derived peripheral neurons (PN) and the Maestro MEA system, Dr David Belair and colleagues at Bristol Myers Squibb demonstrated the utility of the hiPSC-PN MEA assay for evaluating CIPN in vitro that could serve as a suitable counter-screen to de-risk CIPN liability.

PRESENTERS:

Evangelos Kiskinis, *Northwestern University, USA*

David Belair, *AbbVie, USA*

12:00 to 13:00

OPTIMIZING ORGANOID AND STEM CELL CULTURE WORKFLOWS: APPLICATIONS FOR DISEASE MODELING

PRESENTED BY BIO-TECHNE

Organoids and stem cell culture systems are emerging as powerful tools in basic, translational and clinical research. These physiologically relevant models have revolutionized the way researchers are able to investigate develo ental biology, disease mechanisms, discover drugs and develop therapeutics for regenerative and personalized medicine. In this session we present Bio-techne's innovative solutions for culturing organoids derived from pluripotent stem cells and adult stem cells. In our first presentation, we will introduce our new matrix, the Cultrex™ UltiMatrix, and pluripotent stem cell expansion medium as well as other reagents to optimize organoid and stem cell culture workflows. Our second presentation will focus on the application of organoids for disease modeling. Dr. Eun Joo Kim will describe the develo ent and optimization of mouse- and human-derived lung organoid models of idiopathic pulmonary fibrosis. Through recapitulating pulmonary fibrosis in organoids, Dr. Kim is able to characterize the underlying pathology and investigate molecular mechanisms that may lead to the develo ent of diagnostics and therapeutic targets.

PRESENTERS:

Kevin Flynn, *Bio-Techne, USA*

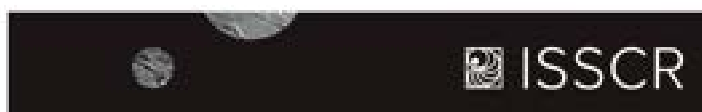
Eun Joo Kim, *University of Colorado, USA*

12:00 to 13:00

PAIRING 3D PLURIPOTENT STEM CELL SUSPENSION CULTURE WITH DOWNSTREAM DIFFERENTIATION TO ENABLE EFFICIENT GENERATION OF LARGE NUMBERS OF CELLS

PRESENTED BY THERMO FISHER SCIENTIFIC

To achieve large numbers of pluripotent stem cells (PSCs) necessary for therapeutic and screening applications, three-dimensional (3D) suspension cultures offer key advantages over two-dimensional (2D) adherent cultures. Overall cost and reduced consumption of plastics makes suspension cultures more desirable for scale-up. The recent launch of our 3D culture medium – Gibco™ StemScale™ PSC Suspension Medium – offers the ability to rapidly and efficiently scale to large numbers of cells. StemScale medium promotes the self-aggregation of singularized PSCs into spheroids and subsequent expansion over a 4-5 day culture period, while maintaining highly viable and pluripotent cells. This versatile system supports efficient scale-up in a variety of vessel sizes,



from small-scale (well-plates and shake flasks) to large-scale (3L bioreactors). PSC spheroids also can be taken directly into various differentiation workflows while maintained in suspension. Here, we will demonstrate the ability of StemScale PSC Suspension Medium to sustain scalable cultures of human pluripotent stem cells (hPSCs) at different scales. We will compare culture systems for neural induction of hPSC in different neural lineages using spinner flasks to obtain large numbers of cells while also discussing the considerations for adapting 2D differentiation protocols to 3D applications and turning long workflows into more manageable and controllable steps.

PRESENTERS:

Cláudia Miranda, *iBB - Institute for Bioengineering and Biosciences; IST - University of Lisbon, Portugal*

Michael Akenhead, *Thermo Fisher Scientific, USA*

WEDNESDAY, 23 JUNE

12:00 to 13:00

AUTOMATED CULTURE AND HIGH-CONTENT IMAGING OF 3D LUNG AND CARDIAC ORGANOIDS FOR IN VITRO ASSESSMENT OF COMPOUND EFFECTS

PRESENTED BY MOLECULAR DEVICES, LLC

Stem cell-derived and 3D cell models are becoming increasingly popular for studying complex biological effects, tissue functionality, and diseases. While complexity cell models remains a hurdle for the wider adoption in research and drug develo ent, industry leaders are removing barriers with innovative hardware and software solutions that automate cell culture, monitoring, and cell analysis. Simultaneously, advances in high-content imaging technology are helping to reveal significant information from these complex biological systems, inviting scientists to push the boundaries of their research.

We describe an integrated workflow that allows us to automate processes of cell culture, imaging, and cell maintenance to monitor the develo ent of stem cells and organoids, as well as characterize the complex responses to test compounds. The method was applied for automation of three complex workflows: iPSC maintenance, lung toxicity assay using 3D organoids, and monitoring compound effects on functional activity in 3D cardiac cultures.

High-content imaging and analysis methods allowed visualization and quantitative characterization of organoid phenotypes and cell content in 3D, and also characterization of functional activity of cardiac organoids using analysis of calcium oscillations. The methods were used to test multiple compounds and demonstrated predicted effects of compounds known to cause lung damage or cardiac toxicity.

PRESENTER:

Oksana Sirenko, *Molecular Devices, USA*

12:00 to 13:00

EVERY CELL'S STORY: CHARACTERIZING THE ACTIVITY OF HUMAN IPSC DERIVED-NEURONS IN 2D AND 3D CULTURES AT HIGH RESOLUTION

PRESENTED BY MAXWELL BIOSYSTEMS

Information not avialable at time of printing.

12:00 to 13:00

NANOPIPETTE TECHNOLOGY: A NEW TOOL FOR SINGLE-CELL ANALYSIS

PRESENTED BY YOKOGAWA GERMANY GMBH

Approaching sub-cellular biological problems requires the incorporation of new technologies and readouts. Due to their low invasiveness, nanotechnology-based tools hold great promise for single-cell manipulation. In this Innovation Showcase we discuss the incorporation of electrical measurements into nanopipette technology and present results showing the rapid and reversible response of these subcellular sensors to different analytes such as antigens, ions and carbohydrates. During the talk I will introduce a newly developed single-cell manipulation platform (SU-10) using a nanopipette for single-cell injection into living cells. This newly developed technology positions its nanopipette with nanoscale precision allowing injection and/or aspiration of minute amounts of material into and from individual cells without comprising cell viability. Furthermore, I will display develo ent of new application for single-cell-omics and how this nanopipette technology can be used to analyze multiple analytes including DNA, RNA, proteins and other small molecules in basic research or drug discovery.

PRESENTER:

Nader Pourmand, *University of California Santa Cruz, USA*

12:00 to 13:00

NEXT GENERATION CRISPR SOLUTIONS FOR IPSC GENE EDITING

PRESENTED BY HORIZON DISCOVERY LTD

Gene editing technology has rapidly evolved over the past several years as researchers search for tools and methods to simplify interrogation of biologically relevant cell types to model and treat human disease. These new methods have begun a shift in research requirements to enable work in more flexible, yet sometimes difficult to manipulate cellular systems such as induced pluripotent stem cells. Horizon is proud to support researchers' ever-changing needs with its advanced gene editing and modulation reagents. When coupled with world-class cell line engineering and screening services, Horizon has a solution for allowing researchers to move quickly from discovery to breakthrough.

Recently, Horizon launched a new suite of gene modulation products that allow researchers to harness the power of CRISPR, without the cut. Here we will demonstrate how

Horizon's CRISPRi reagents allow for gene inactivation with PAM-anchored targeting in iPSCs. Further, we will demonstrate Horizon's CRISPRko gene editing reagents as powerful tools that allow researchers to easily modify the genome and create functional knockout or knock-in models within a human iPSC line. Lastly, because gene editing can be complex, we will highlight Horizon's iPSC Cell Line Engineering capabilities and Screening services.

PRESENTERS:

Amanda Haupt, *Horizon Discovery, a PerkinElmer company, USA*

Clarence Mills, *Horizon Discovery, a PerkinElmer company, USA*

Yasmin Paterson, *Horizon Discovery, a PerkinElmer company, UK*

Max Blanck, *Horizon Discovery, a PerkinElmer company, UK*

12:00 to 13:00

NOVEL TOOLS TO DIFFERENTIATE NEURAL AND LYMPHOID CELL LINEAGES

PRESENTED BY BIOLEGEND

Both primary cells and stem cells have become powerful tools in basic and translational studies. In this workshop, we will demonstrate how our cytokine and antibody reagents can be successfully used to grow and characterize the induced pluripotent stem cell line ASE-9109 and drive differentiation into neural lineages. ASE-9109 cells were propagated and cell pluripotency was assessed by several semi-quantitative techniques using anti-SOX2, OCT4 and Nanog antibodies. Cells were then differentiated into neural progenitor cells and neurons with our cytokine reagents. We will also introduce our first GMP cell culture media supplement, Cell-Vive™ T-NK Xeno-Free Serum Substitute, and demonstrate its performance. Human PBMC-derived T and NK cells were activated, expanded and characterized with BioLegend's reagents, including our serum-free GMP recombinant human IL-2 and GMP anti-CD3 antibody. Our serum substitute was a suitable replacement for human AB serum in our cultures, both in terms of cell numbers and cell characteristics. In summary, BioLegend tools can effectively differentiate and characterize iPS cells into neural lineages, and consistently generate and characterize T and NK cells. Our reagents provide a complete workflow to progress basic research discovery, while our GMP materials can provide a smooth transition from discovery to cure.

PRESENTER:

Vanda Lopes, *BioLegend, USA*

12:00 to 13:00

PATIENT DERIVED ORGANOIDS HELP GUIDE THERAPEUTIC DEVELOPMENT: MECHANISMS AND TOOLS FOR MODELING DISEASE AND THERAPEUTIC EFFICACY

PRESENTED BY CORNING LIFE SCIENCES

Stem cell research has a broad reach across applications, from studying disease mechanisms, toxicity, and regeneration to personalized medicine. Building relevant cellular models is crucial to predictive therapeutic responses. For disease models, patient derived organoids, which more closely mimic in vivo behavior of tissues and organs, prove especially useful. Whether iPSC or adult stem cell-derived, these models are gaining importance as clinical tools because they offer the ability to study mechanisms of disease progression and therapeutic efficacy. Here we will discuss how these organoid models are being utilized to advance personalized medicine for pancreatic ductal adenocarcinoma patients in the context of clinical trials examining chemotherapeutic efficacy. As a further supplement to personalized medicine workflows, we present a unique capability to facilitate patient derived diseased models, including tools to streamline cell isolation as well as enablement of bioprinting stem cell culture environments.

Dr. Plenker will discuss the development of clinically relevant models of pancreatic cancer, focusing on patient-derived organoids and their utility in predicting chemotherapeutic response, including molecular analysis of human Pancreatic Ductal Adenocarcinoma organoids and patient data.

Dr. Siler will present novel organoid technologies for use in personalized medicine, including healthy and diseased models, in addition to cell isolation technology.

PRESENTERS:

Dennis Plenker, *Cold Spring Harbor Laboratory, USA*

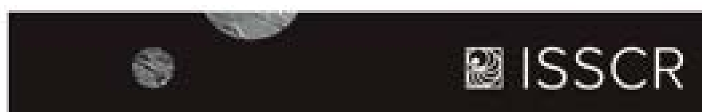
Catherine Siler, *Corning Life Sciences, USA*

12:00 to 12:30

HPSC-DERIVED LUNG MODELS AS TOOLS FOR RESPIRATORY RESEARCH

PRESENTED BY STEMCELL TECHNOLOGIES

Functional lung or region-specific airway organoids derived from human pluripotent stem cells (hPSCs) provide valuable in vitro models for studying lung develo ent and respiratory diseases. These model systems would also facilitate future regenerative cell-based or gene therapies for complex respiratory disorders. However, current protocols for the derivation of hPSC-derived lung models are highly variable and lack standardization between different labs. To standardize these differentiation protocols, STEMCELL Technologies recently developed cell culture media and streamlined protocols for the reproducible generation of lung progenitors and lung organoids across multiple human embryonic stem (ES) and induced pluripotent stem (iPS) cell lines. In this session, I will discuss the use of these hPSC-derived lung models, their characterization, and potential applications.



PRESENTER:

Philipp Kramer, *STEMCELL Technologies, Canada*

12:30 to 13:00

NEXT GENERATION BRAIN-REGION-SPECIFIC ORGANOID MODELS

PRESENTED BY STEMCELL TECHNOLOGIES

Advancements in brain organoid technologies have enabled the generation of regionalized organoids representing specific areas of the brain in a dish. Here I will present on the recently launched STEMdiff™ Dorsal and Ventral Forebrain Organoid Differentiation Kits, which can be used to generate models of the early developing human forebrain. Each kit can robustly and efficiently generate hundreds of neural organoids for downstream applications. I will discuss characterization of the resulting organoids by gene expression and activity using single-cell sequencing and microelectrode array recording, respectively. I will further show that these organoids can be fused to generate Assembloids™. These next-generation models help investigators to understand how different cell types and brain regions interact, highlighting the utility of brain region-specific organoids in disease modeling.

PRESENTER:

Leon Chew, *STEMCELL Technologies, Canada*

THURSDAY, 24 JUNE

12:00 to 13:00

BEYOND GMP: CONSIDERATIONS FOR THE DEVELO ENT OF PSC-BASED THERAPIES

PRESENTED BY THERMO FISHER SCIENTIFIC

As the use of pluripotent stem cells (PSCs) transitions from research to allogeneic therapeutic applications, several unique challenges arise in translating research-scale cell expansion and differentiation to the clinical manufacturing environment. During discovery, experiments are usually small scale and reagent selection is typically driven by performance, such as maintenance of pluripotency and efficiency of differentiation to the cell type of interest. When transitioning to a clinical workflow, considerations must be made to maintain protocol robustness at scale to drive efficiency and cost effectiveness, while selecting reagents which will not hinder or delay regulatory approval. Specifically, this involves (1) the transition from adherent to suspension PSC culture in order to achieve high-fold expansion and ultimately reduce cost, (2) the use of reagents which either do not contain animal-origin components or contain animal origin components which have been properly risk-assessed, and (3) the use of reagents which are manufactured in a GMP environment, which ensures products are traceable, safe, pure, and effective.

PRESENTERS:

David Kuninger, *Thermo Fisher Scientific, USA*

Alex Hannay, *Thermo Fisher Scientific, USA*

12:00 to 13:00

DELIVERING THE CELLS THAT MATTER: STREAMLINED SINGLE-CELL CLONING IN MINIATURE FLUID-WALLED CHAMBERS

PRESENTED BY IOTASCIENCES

Expansion of single cells into clonal iPSC lines is an important aspect of diverse research applications, including genome editing, disease modelling and toxicity testing.

Traditional single-cell cloning methods such as manual colony picking or limiting dilution lack reliable verification of monoclonality and carry a substantial risk of selecting and propagating non-clonal cells.

iotaSciences' single-cell cloning platform circumvents this bottleneck by automatically dispensing and culturing cells into novel small-scale culture chambers (GRIDs), which are fabricated on polystyrene dishes utilising a novel fluid-shaping technology. Due to the optical properties and small chamber size, individual cells and their outgrowth into colonies are clearly visible and easy to track allowing reliable verification of single-cell clonality directly after cell plating. Clonally-derived iPSC lines are genomically stable and retain pluripotency.

PRESENTER:

Katia Mattis, *iotaSciences, UK*

12:00 to 13:00

HEPATIC AND INTESTINAL ORGANOIDS: IMPROVED TOOLS AND IN VITRO MODELS FOR DRUG DEVELO ENT

PRESENTED BY STEMCELL TECHNOLOGIES

Organoid and organotypic cultures model human tissues with greater physiological relevance than traditional cell culture platforms, expanding our tools for in vitro research and drug develo ent. The liver and the intestine are two key organs involved in drug absorption and metabolism in the body, making them target tissues for evaluating drug efficacy and toxicity. These tissues can now be effectively modeled in vitro using organoid-based technologies, enhancing the precision and speed of metabolic research and drug develo ent. This session will provide an overview of cell culture reagents developed by STEMCELL Technologies for establishing, maintaining and differentiating hepatic and intestinal organoid cultures derived from human tissue. We will explore how these organoids can be used as cutting-edge tools for drug develo ent, highlighting new assays and protocols optimized to work with both 3D and 2D cultures. Finally, we will compare the use of human hepatic and intestinal organoids with common intestinal and hepatic cell lines for analyzing drug response.

PRESENTERS:

Martin Stahl, *STEMCELL Technologies, Canada*

Charis Segeritz-Walko, *STEMCELL Technologies, Canada*

12:00 to 13:00

THE QUEST FOR PERSONALIZED MEDICINE - TECHNOLOGICAL ADVANCEMENTS IN KEY WORKFLOWS

PRESENTED BY CELLINK

Join us for a discussion on how advancements in technologies across critical steps in cell line develo ent and bioprinting are bringing us closer to developing personalized therapies and treatments. Dr. Julian Riba, CSO at CYTENA, will delve into isolating stem cells for colony develo ents with high yield. Then, Dr. Itedale Namro Redwan, CSO at CELLINK, will lead a discussion on regenerative medicine and how bioprinting and stem cells are essential to breakthroughs to come.

PRESENTERS:

Julian Riba, *CYTENA, Germany*

Itedale Namro Redwan, *CELLINK, Sweden*

12:00 to 12:30

CHALLENGING BIOLOGY, CAN WE CODE HUMAN CELLS FOR HEALTH? REALISING THE POTENTIAL OF HIPSC REPROGRAMMING FOR RESEARCH, DISCOVERY AND THERAPEUTIC APPLICATIONS

PRESENTED BY BIT.BIO

Cellular reprogramming has challenged traditional concepts of cellular identity, suggesting a more fluid landscape not constrained by the principles of developmental biology. This frontier has achieved protocols for generating sub-cell types and approaches for deterministic conversion of cells into a new cellular identity - opening up new doors for studying human health and disease, drug discovery, and the development of cell therapies.

Widespread use of human-induced, pluripotent stem cell (iPSC)-derived, mature cell types however, is restricted by complex differentiation protocols and inefficient reprogramming methods. A novel reprogramming technology, opti-ox, overcomes these restrictions by enabling the precisely controlled expression of transcription factors, and as a result, deterministic induction of a new cell identity. The resulting mature hiPSC-derived cells are functional within days and provide high quality cellular models with simple protocols.

This talk will explore the practical applications of cellular reprogramming and present human cell types that have been generated using opti-ox, including human iPSC-derived immune, CNS and muscle cells and associated disease models for disease modelling, drug discovery and cell therapy.

PRESENTER:

Mark Kotter, *bit.bio, UK*

12:30 to 13:00

150 MILLION-FOLD CUMULATED EXPANSION OF ENCAPSULATED HIPS CELLS IN BIOREACTOR

PRESENTED BY TREEFROG THERAPEUTICS

Here, we present the results of an extensive benchmark of cell culture technologies for the large-scale expansion of human pluripotent stem cells. We compared standard 2D culture in flasks and agitated spheroid culture in bioreactors with the C-Stem technology, which combines high-throughput stem cell encapsulation and large-scale bioreactors. With C-Stem, encapsulated pluripotent stem cells self-organize in 3D to form a biomimetic epiblast-like stem cell colony, protected from bioreactor mechanical stressors. Results demonstrate :

- A very robust weekly amplification factor: >100x in 7 days
- Straightforward scale-up from 3mL static culture to 1L bioreactor (10L pending)
- Proven maintenance of stemness: >92% OCT4/NANOG co-expression in 4 iPS cell lines
- Less than 2% total cell mortality over 7 days
- Serial encapsulation capacity: 4 encapsulations in a row, 151 000 000X cumulated amplification factor over 28 days of dynamic suspension culture in capsulo, >99 % OCT4/NANOG coexpression at day 28 in IMG005 line

In summary, high throughput production of scale-independent micro-environments ensures consistency of cellular parameters throughout the scale-up process. This integrated platform might de-risk and accelerate the clinical translation of iPS-derived cell therapies.

PRESENTER:

Maxime Feyeux, *TreeFrog Therapeutics, France*

FRIDAY, 25 JUNE

12:00 to 13:00

PRESENTED BY AMS BIOTECHNOLOGY

AN IPSC AND ORGANOID MODEL FOR SARS-COV-2 INFECTION: FROM INDIVIDUAL VARIATION TO DRUG DISCOVERY

12:00 to 13:00

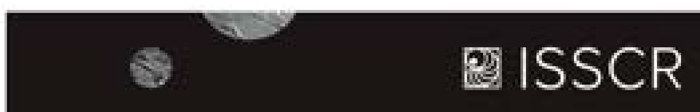
PRESENTED BY PROTEINTECH GROUP INC.

THE PROTEINTECH ADVANTAGE IN STEM CELL RESEARCH; FROM EXPANSION AND DIFFERENTIATION TO FLOW AND IF

12:00 to 13:00

PRESENTED BY YAMAHA MOTOR CO., LTD.

APPLICATIONS OF CELL PICKING AND HIGH CONTENT IMAGING IN 3D MODELS OF DISEASE



12:00 to 13:00

QUALITY BY DESIGN: REAGENTS AND SUPPORT FOR HPSC-DERIVED CELL AND GENE THERAPIES

PRESENTED BY STEMCELL TECHNOLOGIES

Each year, more human pluripotent stem cell-derived (hPSC-derived) therapies move towards the clinic, and STEMCELL is committed to supporting researchers from discovery to clinical application. This talk will focus on how we can enable you on your path to the clinic, from support and guidance to highly qualified reagents.

Engaging in early discussions with your ancillary material suppliers about product qualification, sustainability, and traceability is critical to success on your journey to the clinic. STEMCELL has a proven track record, as demonstrated by successfully supporting 46 clinical trials. We will walk you through how we build quality into our products, processes and policies to support your project timing, product quality, and overall vision.

We have a comprehensive portfolio of cGMP-manufactured products for expansion of high-quality hPSCs, integrated into complete workflows ensuring reproducibility. To further address the needs of the field, a novel animal origin-free (AOF) hPSC maintenance medium, TeSR™-AOF, made with animal-free raw materials to the secondary level of manufacturing was developed. TeSR™-AOF was designed with quality and safety in mind and formulated to support optimized cell quality, improved performance and reproducibility across all cell lines. With high-quality regulated products, we can support research from discovery to the clinic.

PRESENTERS:

Lynn Csontos, *STEMCELL Technologies, Canada*

Kimberly Snyder, *STEMCELL Technologies, Canada*

12:00 to 12:30

ROBUST WORKFLOW FOR SINGLE CELL CLONING OF IPSCS FOR MAKING CGMP MASTER CELL BANKS

PRESENTED BY SOLENTIM

High efficiency and documented single cell cloning of iPSCs will be a pre-requisite for allogeneic iPSC-derived cell therapies

This talk will include the following:

- Discuss the importance of the matrix, MatriClone
- Illustrate the timeline improvements using the VIPS for single cell cloning iPSCs
- Discuss cGMP requirements and considerations in choice of platform
- Presentation of new data for gene-edited iPSCs

PRESENTER:

Ian Taylor, *Solentim Ltd., UK*

12:30 to 13:00

CELL-DERIVED EXTRACELLULAR MATRIX SUPPORTS RAPID MATURATION OF IPSC-DERIVED CELLS IN 2-D CULTURE

PRESENTED BY STEMBIOSYS INC.

In theory, pluripotent stem cells are capable of differentiating into any cell type in the body. In practice, however, obtaining mature phenotypes from iPSCs remains an elusive goal. Many of the recent advances in obtaining more realistic phenotypes from iPSC-derived cells, have centered around 3-D cultures. While promising, these approaches still struggle with reproducibility and manufacturability, and do not represent an attractive approach for large scale cell manufacturing. Recently, we have produced a novel extracellular matrix from perinatal stem cells that support self-renewal of induced pluripotent stem cells and their differentiation into cardiomyocytes. More importantly, it supports the rapid maturation of iPSC-derived cardiomyocytes. Cardiomyocytes cultured on our matrix naturally align and spontaneously beat. Importantly, they also express other hallmarks of mature cardiomyocytes, including polarized N-cadherin expression, cardiac troponin-I expression, multinucleation, aligned sarcomeres that are visible in brightfield microscopy, rod-like morphology, etc. Critically, these differences in maturation state manifest themselves in more biologically-relevant responses to stimuli. The ability to produce a mature cell phenotype from iPSC-derived cells represents an important step forward for the field. Moreover, this work provides proof-of-concept for ongoing efforts to develop a suite of tissue-specific matrices for achieving and/or maintaining mature phenotypes of differentiated cells in culture.

PRESENTER:

Travis Block, *StemBioSys, Inc., USA*

MONDAY, JUNE 21

PLENARY I: PRESIDENTIAL SYMPOSIUM 9:00 - 11:45 EDT REBROADCAST WITH LIVE CHAT 21:00 - 23:45 EDT

GENOME EDITING TO ELIMINATE ENGRAFTMENT ARRHYTHMIA DURING HEART REGENERATION

Murry, Charles

Institute for Stem Cell and Regenerative Medicine, University of Washington, Seattle, WA, USA and Sana Biotechnology

New developments in cell therapy for the heart will be presented.

Keywords: Heart regeneration, Cardiac Repair

LIVER AND PANCREAS ORGANOIDS; THEIR APPLICATION TO THE STUDY OF TISSUE REGENERATION AND DISEASE

Huch, Meritxell

Max Planck Institute of Molecular Cell Biology and Genetics, Max Planck, Dresden, Germany

In vitro 3D cultures are emerging as novel systems to study tissue development, organogenesis and stem cell behavior ex-vivo. We have developed organoid cultures from healthy and diseased, human and mouse, adult and embryonic tissues for a range of organs including stomach, liver and pancreas. These have allowed, for the first time, the long-term expansion of adult (stomach, liver and pancreas) and embryonic (liver) tissue into 3D-epithelial structures that we have termed organoids, since these (1) self-assemble and can be clonally expanded, (2) resemble the corresponding tissues-of-origin and (3) allow the study of some aspects of tissue physiology in a dish. Here, I will present our liver and pancreas organoid work and summarize our findings on how this culture system is amenable for disease modeling and for the study of adult tissue regeneration in a dish. Briefly, our mouse and human liver and pancreas organoid culture system enables the long-term expansion of liver cells in vitro. The expanded cells are highly stable at the chromosome and genomic structure level, while single base changes occur at very low rates. By modifying the system, we recently established the first human liver cancer organoid culture system for modeling liver cancer ex-vivo and demonstrated its applicability for drug testing. Additionally, we have recently found that our organoid culture system enables the study of some aspects of liver regeneration in a dish, specially the activation of adult differentiated liver cells into bi-potent cells that contribute to regeneration. We have found that the transition from the differentiated state to an active progenitor state involves global, yet transient, genome-wide epigenetic reprogramming in the form of DNA-methylation changes that are required for organoid formation and tissue regeneration. In summary, clonal long-term expansion of liver and pancreas primary cells as organoid cultures opens up experimental avenues for disease modeling, toxicology studies, regenerative medicine and gene therapy.

Funding Source: Max Planck Gesellschaft, Wellcome Trust, H2020-Horizon LSMF4LIFE

Keywords: Organoids, regeneration, disease

DERIVING AND REPAIRING THE ENTERIC NERVOUS SYSTEM FROM HUMAN PSCS

Studer, Lorenz

Sloan-Kettering Institute for Cancer Research, USA

There has been considerable progress in realizing the potential of human pluripotent stem cells (PSCs) in regenerative medicine with first-in-human clinical studies initiated recently for Parkinson's disease and other human central nervous system (CNS) disorders. Repair of the peripheral nervous system (PNS) represents another major goal for the field. The enteric nervous system (ENS) is the largest and most complex component of the PNS and plays an essential role in controlling gastro-intestinal (GI) function such as gut motility and secretion. Defects of the ENS are associated with a broad range of human disorders including Hirschsprung's disease, a congenital disorder characterized by the lack of enteric ganglia in distal portions of the gut.
The ENS is established from the neural crest, a transient developmental structure, with both the vagal and the sacral portions of the neural crest contributing to ENS development. Our lab has previously reported the derivation of ENS precursors from human PSCs via a vagal neural crest intermediate. Here, I will present our progress on deriving the sacral portion of the neural crest and our work determining the relative contribution of vagal versus sacral neural crest to human ENS development and repair. Interestingly, the sacral neural crest appears to originate from a distinct, caudal progenitor that can be captured in vitro. Furthermore, we observe that sacral versus vagal-derived ENS lineages exhibit unique phenotypic, migratory and functional properties. The ability to generate sacral-derived neural crest lineages and the possibility of combined vagal/sacral grafts enables us to achieve a more complete ENS repair and partial rescue even of very severe models of Hirschsprung's disease. Those results indicate considerable translational potential, and I will also discuss current efforts to translate our ENS work towards the future application in human patients. After nearly two decades of human PSC research the field is at an exciting stage with emerging applications in regenerative medicine for both CNS and PNS disease.

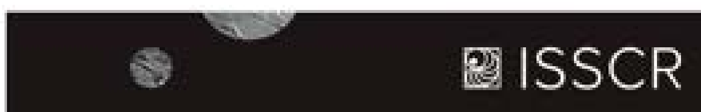
STEM CELL ENCOUNTERS WITH STRESSFUL SITUATIONS: SURVIVAL OF THE FITTEST

Fuchs, Elaine

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

Barrier epithelial tissues such as the skin, lung and gut are the first line of defense between our body and the external environment. As such, their stem cells must take on multifaceted tasks, self-renewing, protecting themselves, rejuvenating and repairing their tissues and calling for help from the immune system when their barrier has been breached. These stem cells reside in protected niches and together they undergo complex crosstalk to coordinate the stem cells' behavior and tasks. When the tissue is challenged by environmental stresses, such as allergens, pathogens or wounding, the stem cells must be poised to cope. 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 stresses 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



TUESDAY, JUNE 22

THEME SESSION NT 1 (NEW TECHNOLOGIES) IMAGING
7:30 - 9:15 EDT
REBROADCAST WITH LIVE CHAT 16:30 - 18:15 AND 19:30 - 21:15 EDT

SELF-ORGANISATION AND SYMMETRY BREAKING IN MULTICELLULAR SYSTEMS

Liberali, Prisca

Quantitative Biology, Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

Multicellular organisms are composed of cells and tissues with identical genomes but different properties and functions. They all develop from one cell to form multicellular structures of astounding complexity. During development, in a series of spatio-temporal coordinated steps, cells differentiate into different cell types and establish tissue-scale architectures and functions. Throughout life, continuous tissue renewal and regeneration is required for tissue homeostasis, which also requires fine-tuned spatio-temporal coordination of cells. I will discuss how cellular interactions generate the specific contexts and spatio-temporal coordination underlying development and regeneration and how we specifically investigate what are the molecular and physical mechanisms that allow a cell, in a tissue, to sense its complex environment, to take individual coordinated decisions. Moreover, I will discuss the molecular mechanisms of intestinal organoid self-organization and the role of cell-to-cell variability in populations of differentiating cells during symmetry breaking.

Keywords: Organoids and Gastruloids, Imaging and single cells, cell-to-cell variability and heterogeneity

LIVE SINGLE CELL QUANTIFICATION OF THE GATA SWITCH DYNAMICS DURING ADULT AND DEVELOPMENTAL ERYTHROPOIESIS

Ahmed, Nouraiz, Hoppe, Philipp, Kull, Tobias, Loeffler, Dirk, Schroeder, Timm

Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland

Transcription factor (TF) networks control mammalian developmental programs. Red blood cell (RBC) development is assumed to be driven by the 'GATA switch', where positive and negative auto- and cross-regulation of the TFs GATA1 and GATA2 in hematopoietic stem and progenitor cells (HSPCs) lead to their correctly timed up- and down-regulation required for normal RBC differentiation. However, this prominent long-standing model is mostly based on RNA expression data and chromatin binding measurements from population-averaged snapshot analyses, leaving its underlying regulation disputed. Here, we generate a novel GATA2VENUS/GATA1mCHERRY reporter mouse line and used long-term quantitative time-lapse microscopy for the simultaneous live single-cell quantification of GATA1 and 2 protein levels in HSPCs. Quantification of GATA dynamics, RBC differentiation and cellular kinship over many cell generations for thousands of GATA switch events during fetal and adult erythropoiesis revealed several unexpected aspects of its regulation. For example, in contrast to current assumptions, GATA cross-regulation does not correlate with GATA1 and 2 expression in single cells. GATA2 downregulation in adult erythropoiesis is not initiated by a GATA1-induced stop of GATA2 production, but by a shortening of cell cycle length. These findings correct current assumptions about the GATA switch and demonstrate a role of regulated cell cycle dynamics in regulating RBC differentiation.

Keywords: GATA2 - GATA1 switch, Hematopoiesis, Erythropoiesis

QUANTIFYING INTERSTITIAL PH AND CALCIUM CONCENTRATION OF MOUSE BONE MARROW BY INTRAVITAL RATIOMETRIC IMAGING

Yeh, Shu-Chi[1], Hou, Jue[1], Wu, Juwell W.[1], Camargo, Fernando D.[2], Lin, Charles P.[1]

[1]*The Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA, USA,* [2]*Stem Cell Program, Boston Children's Hospital, Boston, MA, USA*

Hematopoietic stem cells (HSCs) reside in the bone marrow (BM), long considered to be a compartment that is rich in calcium, particularly near the endosteal surface bordering the bone and the BM. In contrast to the tightly regulated calcium concentration in the blood serum with a setpoint near 2 mM, the local extracellular calcium concentration can vary significantly with bone remodeling and was reported to reach as high as 40 mM at sites of bone resorption. Adams et al. demonstrated that fetal liver HSCs rely on the calcium sensing receptor for homing to the BM, suggesting that high calcium is required for HSC engraftment in vivo. On the other hand, cultured HSCs showed improved maintenance with low calcium media (< 0.2 mM) in vitro. These findings, though highly divergent, suggest the importance of environmental calcium in directing the fate and functions of HSCs. Our recent finding that BM cavities are heterogeneous, undergoing distinct stages of bone remodeling, with HSC expansion restricted to a subset of cavities exhibiting bone resorption activities further highlight the need for a live imaging technique that can measure the distribution of extracellular calcium in the BM and around individual HSCs with high spatial resolution. Here we developed an intravital ratiometric imaging approach to quantify the absolute pH and calcium concentration in the mouse calvarial bone marrow, taking into account the pH sensitivity of the calcium probe. We achieved robust ratiometric quantification through the highly scattering bone tissue by implementing corrections for wavelength-dependent attenuation of the fluorescence signal and precise image segmentation of the interstitial space. We then uncovered distinct BM calcium distribution tied to local bone remodeling, with the lowest concentration (0.3 mM) found in BM cavities predominated by bone formation. Notably, steady-state HSCs are not found in these low calcium locations; instead, they reside in locations with varying calcium levels (1.0-3.6 mM) that is significantly higher than in vitro culture conditions reported to enforce HSC maintenance (< 0.2 mM). This work also established a tool to further investigate pH and calcium in the stem cell niche under malignant or stressed conditions.

Funding Source: National Institute of Health (R01 DK123216 and P01 HL142494 to C.P.L)

Keywords: intravital calcium and pH imaging, Bone remodeling, Hematopoietic stem cells

IN VIVO IMAGING OF HUMAN NEURONAL DEVELOPMENT AT SINGLE CELL RESOLUTION IN CHIMERA MODELS

Padmanaabhan, Krishnan

Department of Neuroscience, University of Rochester School of Medicine, Rochester, NY, USA

While the structural changes that human neurons undergo during development are key to understanding neural circuit function, there are currently limited ways to study these dynamic processes. To address this challenge, we developed a novel method for time-lapse in vivo two-photon imaging of GFP-labeled human

neural precursor cells (hNPCs) derived from induced Pluripotent Stem Cells (iPSCs) transplanted into the visual cortex of a mouse. By imaging over both short periods (~1 hour) and long intervals (~1 week), we were able to uncover features of hNPC proliferation, migration and growth dynamics across different times scales of development for up to 8 months. In transplants with large numbers of engrafted hNPCs, we observed extensive cell proliferation and migration. Migrating cells tended to travel towards and accumulate around blood vessels, with neurites surrounding and ultimately ensheathing these vessels. By contrast, in transplants with small numbers of engrafted hNPCs, we could identify individual neurites that were tracked for up to 8 months. In these engrafts, we observed extensions and retractions of neurites, often as great as 100s of um within a 24-hour period. Furthermore, neurites tended to form fascicles, with extensions favoring the direction of established neurites, suggesting that cells that extend together, stabilize together. Interestingly, these processes often grew along blood vessels, suggesting that the host vasculature plays a critical role in guiding development, possibly as an energy source to supporting both migration and neurite maturation. Taken together, our data allowed us to track the diversity of developmental programs, including proliferation, migration, and neurite maturation from the hNPC state to putative cortical neurons. Our new method thus provides a platform for modeling both normal human development as well as developmental and psychiatric disorders.

Funding Source: NSF CAREER (1749772), NIMH (R01MH11392), the Schmitt Foundation, and the Cystinosis Research Foundation

Keywords: human iPSC-derived neural precursor cells, mouse transplantation, 2-photon in vivo imaging

XIST NUCLEATES LOCAL PROTEIN GRADIENTS TO PROPAGATE SILENCING ACROSS THE X CHROMOSOME DURING DEVELOPMENT

Markaki, Yolanda[1], Gan Chong, Johnny[1], Luong, Christy[1], Wang, Yuying[1], Tan, Shawn[1], Jacobson, Elsie C.[1], Mistry, Bhaven A.[2], Banerjee, Abhik[3], Dror, Iris[1], Schöneberg, Johannes[4], Guttman, Mitchell[3], Chou, Tom[5], Plath, Kathrin[1]

[1]BSRB, University of California, Los Angeles, CA, USA, [2]Quantitative and Computing Lab, Claremont McKenna College, Claremont, CA, USA, [3]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, USA,[4]Departments of Pharmacology and Chemistry and Biochemistry, University of California, San Diego, CA, USA, [5]Departments of Computational Medicine and Mathematics, University of California, Los Angeles, CA, USA

Female eutherians inactivate one of their two X-chromosomes during early development to achieve dosage compensation of X-linked genes between sexes. Transcriptional silencing of ~1000 genes is induced by the long non-coding RNA Xist through the recruitment of effector proteins. Xist is expressed from and localises on the inactive X-chromosome in a small number of diffraction-limited foci. How a small number of foci can silence a much larger number of genes is unknown. To explore this question, we applied super-resolution microscopy during initiation of X-chromosome inactivation (XCI) in differentiating murine embryonic stem cells, live-cell imaging and kinetic modelling. We discover that ~50 locally confined Xist foci, each containing ~2 RNA molecules, induce protein crowding to nucleate supra-molecular complexes of interacting proteins, including many copies of the major XCI silencing protein SPEN. Intrinsically disordered domains are required for partitioning SPEN into these protein compartments and completion of transcriptional silencing. Accumulation of silencing proteins in the supra-molecular complexes, combined with rapid cycling, generates local protein gradients that extend silencing to broad X-chromosome regions in their vicinity. Polycomb-mediated compaction brings chromatin regions towards the supra-molecular complexes, facilitating chromosome-wide silencing. Our findings provide novel insights into the mechanism of gene regulation by RNA-seeded nuclear compartments, where protein crowding of transcriptional and chromatin architecture regulators enables silencing of a large target space.

Keywords: X-chromosome inactivation, long-non coding RNA, super-resolution microscopy

SUPER-RESOLUTION IMAGING REVEALS DYNAMIC CHANGES IN CHROMATIN STRUCTURE AND GENE ACTIVITY IN SINGLE CELLS AT THE ONSET OF HETEROKARYON REPROGRAMMING

Martinez-Sarmiento, Jose A.

Physiology, University of Pennsylvania, Philadelphia, PA, USA

Reprogramming to pluripotency holds a great promise for regenerative medicine, and is characterized by extensive chromatin reorganization, involving the repression of somatic genes and establishment of the pluripotency gene regulatory network; however, intrinsic limitations of the iPSC systems such as low efficiency and long kinetics complicate the study of the dynamic chromatin changes occurring in individual cells undergoing a correct reprogramming trajectory. Here, we take advantage of the highly efficient heterokaryon reprogramming system (i.e. cell fusion between somatic and pluripotent cells) to study the spatial changes to the global chromatin organization at the onset of pluripotency conversion of the somatic nucleus using super-resolution microscopy, a technology that allows us to study specific molecular events at nano-scale resolution at the single-cell level. Our results revealed that, following fusion of human fibroblasts with mouse ESCs, the somatic chromatin undergoes a progressive de-condensation and acquisition of a more pluripotent-like open chromatin configuration by 48h. This occurred concomitantly with changes in the localization of chromatin at the nuclear periphery as well as changes in histone modification marks such as H3K9me3 and H3K27me3, among others. Finally, by performing RNA-FISH, we quantified the expression levels of pluripotency genes at the single-cell level, showing that more than 70% of heterokaryons at 24h expressed endogenous OCT4 but only 10% expressed NANOG, suggesting distinct reactivation kinetics. Altogether, our findings show significant changes in chromatin compaction and spatial organization of the nucleus during early reprogramming that correlate with the reactivation of certain pluripotency genes, highlighting the potential of super-resolution microscopy coupled with single-molecule RNA FISH in dissecting chromatin nano-structure.

Funding Source: Consejo Nacional de Ciencia y Tecnologia (CONACYT) Scholarship for PhD studies (709402) Linda Pechenik Montague Investigator Award

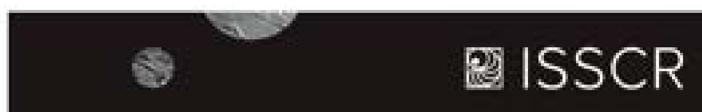
Keywords: Reprogramming, Pluripotency, Super-resolution microscopy

MULTIMODAL, MULTIDIMENSIONAL AND MULTIPLEX IMAGING OF INTRINSIC AND EXTRINSIC SIGNALS EMPOWERS INTRAVITAL ANALYSES OF STEM CELLS, CELL LINEAGES AND TISSUE MORPHOGENESIS

Fraser, Scott E.

Biology; Biomedical Engineering; Stem Cell Biology and Regenerative Medicine, University of Southern California (USC), Los Angeles, CA, USA

Imaging of living specimens can animate the wealth of high-throughput molecular data to better understand complex events ranging from embryonic development to disease pro-



cesses. We are advancing this approach despite the unavoidable tradeoffs - between spatial & temporal resolution, field of view, limited photon budget - by constructing faster and more efficient light sheet microscopes that maintain subcellular resolution. Our two-photon light-sheet microscope combines the deep penetration of two-photon microscopy and the speed of light sheet microscopy to generate images with more than 10x improved imaging speed and sensitivity. Two-photon excitation light is far less scattered, permitting subcellular resolution to be maintained better than conventional light sheet microscopes, resulting in 4D (3D over time) cell and molecular imaging with sufficient speed and resolution to unambiguous trace cell lineages, movements and signals in intact systems. To increase the 5th Dimension (number of simultaneous labels), we are refining new multispectral image analysis tools that exceed the performance of our previous work on Linear Unmixing by orders of magnitude in speed, error propagation and accuracy. Novel denoising strategies permit imaging at far lower light levels, yielding rapid and unambiguous analyses without perturbing even fragile multiplex-labeled specimens. Parallel refinements in label-free approaches extend imaging to patient-derived tissues and even human subjects. The low concentrations of these intrinsic labels required us to refine fluorescence lifetime imaging (FLIM), and combine it with multispectral and advanced denoising tools, to perform intravital imaging in such challenging settings. Combined, these imaging and analysis tools offer the multi-dimensional imaging required to follow key events in intact systems as they take place, and allow us to use noise and variance as experimental tools rather than experimental limitations.

Keywords: Multispectral imaging, Fluorescence Lifetime Imaging, Denoising and image processing

THEME SESSION TSC 1 (TISSUE STEM CELLS AND REGENERATION) STEM CELLS AND CANCER 7:30 - 9:15 EDT REBROADCAST WITH LIVE CHAT 16:30 - 18:15 AND 19:30 - 21:15 EDT

MECHANISMS REGULATING TUMOR TRANSITION STATES

Blanpain, Cédric

Faculty of Medicine, Université Libre de Bruxelles, Belgium

Different theories have been proposed to explain tumour heterogeneity including the cell of origin of cancer. Here, using new genetically engineered mouse models allowing lineage tracing together with oncogenic activation in different cell lineages, I will present evidence that the cancer cell of origin controls tumour heterogeneity, stemness, EMT, and metastasis. We identify the existence of multiple tumor subpopulations associated with different EMT stages. Although all EMT subpopulations presented similar tumor-propagating cell capacity, they displayed differences in cellular plasticity, invasiveness and metastatic potential. We identify the transcriptional and epigenetic landscapes and the underlying gene regulatory networks, transcription factors and signaling pathways that control these different EMT transition states. Finally, I will present new study demonstrating that Fat1 loss of function, one of the most frequently cancer drivers, also control EMT transition states, stemness and metastasis. These results have important implications for our understanding of the mechanisms controlling tumor heterogeneity and metastasis as well as for the development of new strategies to block tumor progression and metastasis.

Funding Source: This work is supported by the ERC, WELBIO, FNRS, TELEVIE, and the WWCR.

Keywords: tumour heterogeneity, EMT, metastasis

TRACING ONCOGENE-DRIVEN PARACRINE REMODELING OF THE INTESTINAL STEM CELL NICHE

Yum, Min Kyu[1], Han, Seungmin[1], Fink, Juergen[2], Dabrowska, Catherine[2], Wu, Szu-Hsien[3], Chatzeli, Lemonia[1], Kim, Jong Kyung[4], Stange, Daniel[5], Philpott, Anna[2], Lee, Joo-Hyeon[2], Koo, Bon-Kyoung[3], Simons, Benjamin[1]

[1]*The Gurdon Institute, University of Cambridge, UK,* [2]*WT-MRC Stem Cell Institute, University of Cambridge, UK,* [3]*Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Vienna, Austria,* [4]*Department of New Biology, DGIST, Daegu, Korea,* [5]*Technische Universität (TU) Dresden, Germany*

Interactions between tumor cells and the surrounding microenvironment contribute to tumor progression, metastasis and recurrence. Although mosaic analyses in Drosophila have advanced our understanding of such cellular interactions during tumor initiation, parallel approaches have remained challenging to engineer in vertebrate systems. Here, we present an oncogene-associated, multicolor reporter mouse model, the Red2Onco system, that allows differential tracing of mutant and wild-type cells in the same tissue. Applied to the small intestine, we show that oncogene-expressing mutant crypts alter the cellular organization of neighboring wild-type crypts, driving accelerated clonal drift. Using the capacity of the Red2Onco system as a platform for comparative single-cell transcriptomics, we show how environmental changes in the shared niche, mediated by direct and indirect signals from oncogene-expressing clones, promote the accelerated differentiation of wild-type stem cells. These findings illustrate how the Red2Onco system can be used to detect and dissect mechanisms of cell competition and field transformation.

Keywords: Mosaic genetics, Intestinal stem cell, niche remodeling

CELLULAR MECHANISMS OF MOUSE INTESTINAL POLYP INITIATION

Sumigray, Kaelyn, Li, Mei Lan, McDonald, Elizabeth

Genetics, Yale School of Medicine, New Haven, CT, USA

Intestinal epithelial proliferation is maintained by the intestinal stem cells in the crypt. Heterogeneous fibroblasts surrounding the intestinal epithelium tightly regulate stem cell behavior and proliferation by secreting ligands, including Bmp antagonists. Alterations in signaling within fibroblasts have been shown to result in uncontrolled epithelial proliferation and subsequent polyp formation. The mechanisms by which stromal cells influence epithelial cells during the early events in polyp formation have not been elucidated. Previous findings have revealed that loss of Bmp signaling in fibroblasts is sufficient to drive polyp formation, suggesting that fibroblasts alter intestinal epithelial cell behavior and stem cell dynamics in a developing polyp. These findings challenge the prevailing model of polyp formation in which epithelial mutations lead to uncontrolled proliferation. We use existing mouse models to generate an inducible Bmp loss-of-function system through overexpression of the Bmp inhibitor Noggin. Unlike previous reports that polyps formed within four weeks of Bmp inhibition, we observed changes to the intestinal epithelium within three days of Noggin overexpression, which then became more dramatic over the next several weeks. Strikingly, these morphological changes were strictly architectural and did not involve epithelial hyperproliferation. Rather, epithelial hyperproliferation occurred after several weeks of Bmp inhibition. Thus, the primary

consequence of Bmp signaling loss in fibroblasts is a change in tissue architecture such that the intestinal epithelium and stroma form an ectopic internal tissue fold. Interestingly, these architectural changes coincide with changes in fibroblast localization, particularly to PDGFRa-high telocytes. Our data support a model in which Bmp signaling within fibroblasts is necessary to maintain proper fibroblast localization and epithelial architecture. We hypothesize architectural changes alter stem cell behavior, resulting in unregulated epithelial cell proliferation in a developing polyp. Current studies are focused on understanding the cellular mechanisms that drive this initial architectural change and how these architectural changes support subsequent hyperproliferation.

Keywords: intestinal stem cell niche, fibroblast, epithelial-mesenchymal crosstalk

A MACROPHAGE - CANCER STEM CELL CROSSTALK VIA WNT LIGANDS GOVERNS SKIN CARCINOMA PROMOTION AND STEMNESS.

Fontenete, Silvia[1], Christensen, Johan[1], Martinez-Silgado, Adriana[2], Peña-Jiménez, Daniel[2], Zarzuela, Eduardo[3], Muñoz, Javier[3], Megias, Diego[3], Castellana, Donatello[2], Loewe, Robert[4], Perez-Moreno, Mirna[1]

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The Wnt/β-catenin signalling pathway exerts a prominent role in maintaining cancer stem cells in cutaneous squamous cell carcinoma (SCC). However, the cellular sources of the Wnt ligands sustaining the activation of β-catenin in cutaneous cancer stem cells are not fully defined. Cancer stem cells reside in a niche where numerous cell types, including tumour-associated macrophages (TAMs), establish a unique tumour-supporting microenvironment. We aimed to investigate whether TAM-derived Wnt ligands promote tumour progression and SCC maintenance. To this end, we conducted comprehensive analyses using human SCC tissue arrays, multistage chemically induced mouse skin tumours and mouse genetic approaches. Our results showed that TAMs expressing high levels of a cohort of Wnt ligands distribute in the cancer stem cell niche. Interestingly, the deletion of Wls in TAMs, and thereby Wnt secretion, prevented skin tumour promotion by reducing the cancer stem cell pool and proliferation. Furthermore, the conditional deletion of Wls in TAMs led to tumour regression. Mechanistically, we identified that cell adhesion receptors, such as CD99, mediate the Wnt-dependent TAM-cancer stem cell interaction. The reduction of CD99 levels in cancer stem cells precludes the TAM-CSC interaction. Overall, these results expose for the first time that Wnt signals arising from TAMs are essential microenvironmental cues at the cancer stem cell niche to promote SCC development.

Funding Source: This work was supported by the Worldwide Cancer Research UK Foundation, the Novo Nordisk Foundation, NEYE-Fonden, Tømmerhandler Vilhelm Bangs Fond, Toyota-Fonden Denmark and the Candys Foundation (to M.P.M).

Keywords: Cancer stem cell niche, Squamous cell carcinoma, Wnt/beta-catenin

STEM CELL BIOLOGY IN PITUITARY TUMORS AND DERIVED ORGANOIDS

Nys, Charlotte, Laporte, Emma, Lee, Luyun, Vankelecom, Hugo, Vennekens, Annelies

Department of Development and Regeneration, KU Leuven, Heverlee, Belgium

The pituitary gland represents the regulatory centre of our hormonal system and steers fundamental processes like growth, metabolism, sexual maturation, reproduction and stress. Pituitary dysfunction, as caused by local tumors overproducing hormones or damaging healthy pituitary tissue by compression, can therefore lead to severe and life-threatening health problems. Not much is known on the mechanisms of tumorigenesis in the pituitary. Our group discovered that the tissue stem cells are activated during tumor development in the gland, as explored using the dopamine receptor D2 (Drd2) knockout (KO) mouse model in which prolactinoma tumors develop. Stem cells show increased proliferative activity and expanded numbers in the Drd2 KO pituitary. This activated phenotype is further supported by bulk RNA-sequencing of the pituitary stem cell population, showing upregulated expression of stemness markers and associated pathways. In addition, gene ontology analysis revealed enrichment of chemokine and cytokine signaling processes in the stem cell compartment of the tumorous gland. Recently, our lab established organoids from mouse pituitary as novel and powerful model to study pituitary stem cell biology. Organoids are 3D self-forming cell constructs that develop from tissue stem cells and that mimic key features of the native tissue epithelium. Organoids cannot only be developed from healthy, but also diseased tissue (including tumors). Here, we started to generate organoids from pituitary tumors. When compared to wildtype mouse pituitary organoids, organoids derived from the tumorous Drd2 KO gland were more proliferative and larger in size, thereby supporting the activated state of the underlying stem cells. Moreover, the organoids from the tumorous pituitary showed upregulated expression of several chemokine and cytokine factors. We also developed organoids from human pituitary tumor samples, although further technological optimization is needed. Taken together, we are deciphering the behavior and role of pituitary stem cells during tumorigenesis in the gland. A better understanding will lead to more efficient and targeted therapies, at present still inadequate.

Funding Source: FWO

Keywords: Pituitary tumor, Pituitary stem cell, Organoid

REPRESSION OF ENDOGENOUS RETROVIRUSES IS REQUIRED FOR MAMMARY GLAND DEVELOPMENT

Avgustinova, Alexandra[1], Laudanna, Carmelo[2], Pascual-García, Mónica[2], Rovira, Quirze[3], Djurec, Magdolna, Castellanos, Andres[2], Urdiroz-Urricelqui, Uxue[2], Marchese, Domenica[4], Prats, Neus[5], Van Keymeulen, Alexandra[6], Heyn, Holger[7], Vaquerizas, Juan M.[3], Aznar Benitah, Salvador[2]

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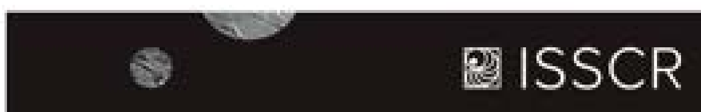
Epigenetic regulation of euchromatin is critical for cell fate specification during organ development, yet whether this is also true for heterochromatin is unclear. Here, we investigate the effects of increased chromatin accessibility on cell fate specification during mammary gland development in mice. We demonstrate that loss of the H3K9 methyltransferase G9a in the mammary epithelium results in de novo chromatin opening, which leads to severely impaired development of the mammary ductal tree, concomitant with impaired stem cell potential and disrupted intraductal polarity. Intriguingly, these phenotypes are not due to alterations in basal or luminal lineage specification or fidelity but rather to increased chromatin opening, which derepresses long terminal repeat (LTR) retroviral sequences, most prominently from the ERVK family. These transcriptionally activated endogenous retroviruses



generate double-stranded DNA (dsDNA) that triggers an antiviral innate immune response in mammary fat pads. Consequently, knockdown of the cytosolic dsDNA sensor Aim2 in G9a-knock out (G9acKO) mammary epithelium rescues mammary ductal invasion, functionally linking LTR retroviral derepression with aberrant mammary gland development. Importantly, using mammary stem cell transplantation into immunocompromised or G9acKO-conditioned hosts, we show partial dependence of the G9acKO mammary morphological defects on the inflammatory milieu of the host mammary fat pad. Thus, altering chromatin accessibility of retroviral elements severely disrupts functional mammary gland development and mammary stem cell activity, through both cell autonomous and cell non-autonomous mechanisms.

Keywords: Chromatin accessibility, Endogenous retroviruses, Mammary stem cells

THE INSIDE OUT STEMNESS OF METASTASIS

Malanchi, Ilaria

THI Laboratory, The Francis Crick Institute, London, UK

The tumour microenvironment or niche is the vital non-cancerous compartment of the tumour structure. The induction of suitable interaction at the metastatic site via the establishment of metastatic niche is key to the process, however, direct investigation of the early cellular changes induced by metastatic cells in the surrounding tissue in vivo is difficult to achieve. We developed a strategy whereby tissue infiltrating cancer cells label neighbouring cells in vivo and allows direct identification of metastatic niche cells within the whole tissue, to define changes in the local micro-metastatic niche. Using this strategy, we uncovered a remarkable local lung parenchymal regenerative response where lung alveolar epithelial cells in the metastatic niche show stem-cell features with multi-lineage differentiation potential. This highlights the radical local remodelling in the tissue accompanying cancer cell growth and the fact that acquisition of stem cell potential is a feature not only of the cancer cells pool, but also of the tissue microenvironment they instruct.

Keywords: Metastasis, Tumour microenvironment, Lung stem cell

THEME TSC (TISSUE STEM CELLS AND REGENERATION)
PLENARY II: STEM CELL NICHES
9:30 - 11:05 EDT
REBROADCAST WITH LIVE CHAT 21:30 - 23:05 EDT

OOGENESIS SPOTLIGHTED: HOW FAR ARE WE FROM MAKING (MATURE) HUMAN OOCYTES?

Chuva de Sousa Lopes, Susana

Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands

Human gametogenesis is a complex process that we are still far from understanding and even further from mimicking in the laboratory. Oogenesis starts with the specification of primordial germ cells and culminates with the production of mature (metaphase II) oocytes, ready to be fertilised and finally resume meiosis. I will discuss our ongoing efforts to characterise the different stages of human oogenesis at the single-cell (transcriptomics) level and how this framework together with bioengineering technologies, such as the use of microfluidics and biomaterials, is contributing to optimise protocols to develop and mature human oocytes from the (fetal and adult) ovary. Moreover, I will discuss our advances on in vitro gametogenesis towards oocytes, starting from pluripotent stem cells. Learning how to develop and mature oocytes in the laboratory may lead to more effective personalised-therapy for fertility preservation and contribute to the development of an in vitro mini-ovary organoid model to use in human reproductive toxicology and disease modelling.

Funding Source: European Research Council (ERC-CoG-2016-725722) Dutch Research Council (ZONMW-VICI-2018-91819642)

Keywords: human gametogenesis, human pluripotent stem cells, novel technologies

SKIN DEEP: STEM CELLS AT THE NEXUS OF THE NICHE, PHYSIOLOGY, AND THE EXTERNAL ENVIRONMENT

Hsu, Ya-Chieh

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

Modern society is overrun by stress, which affects people of all ages, genders, and occupations. Stress is thought to influence a wide variety of tissues and processes in the body, and is perhaps the single most common risk factor for any disease or change in the skin. Despite its profound effect, surprisingly little is known about the mechanisms by which stress impacts tissue biology, and whether stress-related tissue degeneration is associated with the effect of stress on somatic stem cells, their niches, or their progeny. To understand whether and how stress affects tissue changes, my lab adapted several approaches to model both transient acute stress and long lasting chronic stress and delineated their impacts on skin stem cells. Combining physiology, neurobiology, endocrinology, stem cell biology, and functional genetics, we have identified two distinct mechanisms by which stress affects different populations of stem cells in the skin. For melanocyte stem cells, stress-induced neuronal activity drives rapid loss of melanocyte stem cells directly, leading to premature hair graying. For hair follicle stem cells, elevated stress hormones from the adrenal glands under stress inhibits hair follicle regeneration and prolongs stem cell quiescence via the impact of stress hormones on the niche.

Keywords: Niche, Stress, Nerve-stem cell interaction

TISSUE STEM CELL-BASED ORGANOIDS TO MODEL HUMAN DISEASE

Clevers, Hans

Hubrecht Institute, Utrecht, Netherlands

Techniques for culturing functional human breast epithelium in three-dimensional (3D) matrices have been championed for more than 30 years by Mina Bissell. Additionally, around a decade ago, Sasai and colleagues pioneered pluripotent stem cell (PSC)–based technology to create organoids that mirror specific parts of the central nervous system (CNS). Lancaster and Knoblich modified this technology and provided particularly notable examples of "mini-brain" structures. Although PSCs can be used to model everything ranging from tissues to organismal development, adult stem cells (ASCs) can also be isolated to generate organoid models of the primary tissues in which they reside. Specific growth factor cocktails allow long-term expansion of ASC organoids by mimicking the organ stem cell niche, as first established for mouse and human intestine and airway epithelium. The organoid structures generated from PSCs and ASCs reflect crucial tissue features in terms of overall architecture, the collection of differentiated cell types, and tissue-specific function. Organoids thus represent a model system that can be compared to traditional genetically engineered mouse models (GEMMs), cell lines, and patient-derived xenografts (PDXs). As a definition, organoids are microscopic self-organizing, three-dimensional structures that are grown from stem cells in vitro. They recapitulate many structural and functional aspects of their in vivo counterpart organs. This

versatile technology has led to the development of many novel human cancer models. It is now possible to create indefinitely expanding organoids starting from tumor tissue of individuals suffering from a range of carcinomas. Alternatively, CRISPR-based gene modification allows the engineering of organoid models of cancer through the introduction of any combination of cancer gene alterations to normal organoids. When combined with immune cells and fibroblasts, tumor organoids become models for the cancer microenvironment enabling immune-oncology applications. Emerging evidence indicates that organoids can be used to accurately predict drug responses in a personalized treatment setting. I will illustrate the current state and future prospects of the rapidly evolving tumor organoid field through examples from my lab.

NICHE BIOLOGY: BEYOND GROWTH FACTORS

Morrison, Sean J.

Children's Research Institute, Howard Hughes Medical Institute and Children's Research Institute at the University of Texas Southwestern Medical Center, Dallas, TX, USA

Having identified the location and cellular composition of hematopoietic stem cell (HSC) niches in adult hematopoietic tissues, a key question is now whether these niches regulate the cells they maintain through mechanisms other than growth factor signaling. This is a major focus of my laboratory. Leptin Receptor+ (LepR+) stromal cells in adult bone marrow are a critical source of growth factors, including Stem Cell Factor (SCF), for the maintenance of HSCs and early restricted progenitors. LepR+ cells are heterogeneous, including skeletal stem cells, osteogenic, and adipogenic progenitors, though few markers have been available to distinguish these subsets or to compare their functions. Here we show that expression of an osteogenic growth factor, Osteolectin, distinguishes peri-arteriolar LepR+ cells poised to undergo osteogenesis from peri-sinusoidal LepR+ cells poised to undergo adipogenesis. Peri-arteriolar LepR+Osteolectin+ cells are rapidly dividing, short-lived, osteogenic progenitors that increase in number after fracture and are depleted during aging. Deletion of Scf from adult Osteolectin+ cells did not affect the maintenance of HSCs or most restricted progenitors but depleted common lymphoid progenitors (CLPs), impairing lymphopoiesis, bacterial clearance, and survival after acute bacterial infection. Peri-arteriolar Osteolectin+ cell maintenance required mechanical stimulation. Voluntary running increased, while hindlimb unloading decreased, the frequencies of peri-arteriolar Osteolectin+ cells and CLPs. Deletion of the mechanosensitive ion channel, Piezo1, from Osteolectin+ cells depleted Osteolectin+ cells and CLPs. A peri-arteriolar niche for osteogenesis and lymphopoiesis in the bone marrow is thus maintained by mechanical stimulation and depleted during aging. This provides a new mechanism by which mechanical stimulation promotes osteogenesis and lymphopoiesis and reveals that mechanical stimulation is sometimes required for the maintenance of mammalian niches.

Keywords: Hematopoietic Stem Cells, Mechanosensation, Osteolectin

ISSCR DR. SUSAN LIM OUTSTANDING YOUNG INVESTIGATOR AWARD SESSION 13:15 – 13:45 EDT

DECIPHERING MECHANISMS OF HUMAN BRAIN EXPANSION USING CEREBRAL ORGANOIDS

Lancaster, Madeline A.

Cell Biology, MRC Laboratory of Molecular Biology, Cambridge, UK

The human brain sets us apart as a species, with its size being one of its most striking features. Brain size is largely determined during development as vast numbers of neurons and supportive glia are generated. In an effort to better understand the events that determine the human brain's cellular makeup, and therefore its size, we use a human model system in a dish, called cerebral organoids. These 3D tissues are generated from pluripotent stem cells through neural differentiation and a supportive 3D microenvironment to generate organoids with the same tissue architecture as the early human fetal brain. Such organoids are allowing us to tackle questions previously impossible with more traditional approaches. Indeed, our recent findings provide insight into regulation of brain size and neuron number across ape species, identifying key stages of early neural stem cell expansion that set up a larger starting cell number to enable the production of increased numbers of neurons. We are also investigating the role of extrinsic regulators in determining numbers and types of neurons produced in the human cerebral cortex. Overall, our findings are pointing to key, human-specific aspects of brain development and function, that have important implications for neurological disease.

Keywords: Organoids, Brain, Evolution

THEME SESSION NT 2 (NEW TECHNOLOGIES) SINGLE-CELL OMICS 14:00 - 15:45 EDT REBROADCAST WITH LIVE CHAT 02:00 – 03:45 EDT

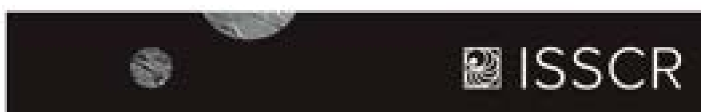
MOLECULAR ARCHITECTURE OF THE DEVELOPING HUMAN BRAIN

Linnarsson, Sten

Medical Biochemistry and Biophysics, Karolinska Insitutet, Stockholm, Sweden

The mammalian brain is arguably the most complex mechanism known. At a cellular and molecular level, it contains likely thousands distinct cell types underlying complex behaviour, sensation and emotion. The brain develops from a simple epithelium, the neural tube, unfolding through great cellular expansion, patterning by secreted morphogens, and wiring by precise and activity-dependent spatial cues. In order to understand the ontogeny of the brain, we have surveyed the adult and developing mouse and human brains, uncovering a previously unrecognized diversity of cell types, and a detailed spatiotemporal unfolding of distinct developmental cell states. In the adolescent mouse, we found hundreds of distinct cell types including novel spatially restricted astrocytes. In the developing mouse, we describe in molecular detail all the major lineages of the neural tube - neural crest leading to the meninges, radial glia leading to neurons and glioblasts leading to astrocytes and oligodendrocytes - as well as infiltrating immune cells. We used in situ RNA sequencing to map organizers during the critical patterning stage laying down the overall architecture of the brain. In the human adult brain, we have sampled 100 regions in three donors comprising over two million single cells, uncovering a tremendous diversity of neurons with surprising level of conservation to monkey and mouse. Like in the mouse, astrocytes showed spatially restricted subtypes, but unlike mouse, this diversity extended to oligodendrocyte precursor cells. Finally, we have mapped human first trimester brain development in unprecedented detail using both single-cell RNA-seq and spatial RNA single-molecule FISH. Altogether, these findings begin to reveal the true molecular and cellular complexity of the mammalian brain.

Keywords: single-cell RNA-seq, brain, development



MAPPING OF THE NICHE-SPECIFIC EXPRESSION USING PIC-SEQ ANALYSIS IN MOUSE EMBRYONIC DEVELOPMENT

Won, Kyoung Jae[1], Brickman, Jushua[2], Kim, Junil[1], Rothova, Michaela[2]

[1]*BRIC – Biotech Research and Innovation Centre, University of Copenhagen, Denmark,* [2]*DanStem, University of Copenhagen, Denmark*

Cells continuously interact with each other to provide signaling cues to nearby cells during development. Cell-cell contact is a fundamental way of communication between two interacting cells. However, our understanding about the role of cell contact is still very limited. To understand cell-contact dependent gene regulation and developmental potential, we used RNA sequencing from physically interacting cells (PIC-seq) as well as single cells (scRNAseq) from developing mouse embryo (at embryonic day E7.5, E8.5, and E9.5). Interestingly, we found cells change their expression when contacting with other type of cells. Among them are genes associated with developmental processes such as Lhx5, which is highly expressed in the clump of neural progenitor (NP) and definitive endoderm (DE) cells, but not in individual cell type. Using the niche specific gene expression, we predicted the identity of neighboring cells. Further analysis found the cells of origin for the niche specific gene expression. For instance, Lhx5 is expressed in NP neighboring with DE, while Gsc is expressed in DE interacting with NP. We developed a visualization tool to remap the neighboring structure of the mouse embryo in the 2 dimensional map. Our study using "relative" spatial information may tell how cell-contact gene induction influences mouse embryo development.

Keywords: single-cell RNA sequencing, physically interacting cells (PIC-seq), Niche-specific expression

CEPO UNCOVERS CELL IDENTITY THROUGH DIFFERENTIAL STABILITY

Kim, Hani Jieun[1], Wang, Kevin[2], Chen, Carissa[1], Lin, Yingxin[1], Tam, Patrick PL[3], Lin, Dave M.[4], Yang, Jean[1], Yang, Pengyi[1]

[1]*School of Mathematics and Statistics, University of Sydney, Camperdown, Australia,* [2]*School of Mathematics and Statistics, University of Sydney, Australia,* [3]*Faculty of Medicine and Health, Children's Medical Research Institute, Sydney, Australia,* [4]*Department of Biomedical Sciences, Cornell University, Ithaca, NY, USA*

Defining cell identity is fundamental to understand the cellular heterogeneity in populations. Whilst exploring cell identity has been enabled by rapid technological advances in genome-wide profiling of single cells, only a few methods have been designed to identify genes associated with cell identity. None of the current approaches, among which the most widely used is differential expression (DE), has been evaluated systematically for their attribute and fidelity for defining cell identity genes from scRNAseq data. Here, we present Cepo, a method to retrieve genes defining cell identity from scRNA-seq data. We propose a biologically motivated metric, differential stability (DS), to identify cell-type specific genes on the premise that stable gene expression is a key indicator of cell identity. We perform a comprehensive benchmark against several differential analysis methods to show that Cepo outperforms current methods in assigning cell identity and enhances several cell identification applications such as cell-type characterisation, spatial mapping of single cells, and lineage inference of single cells. Moreover, Cepo is computational fast and efficient, requiring only seconds to analyse datasets with tens of thousands of single cells. As a method for identifying cell identity genes, we foresee that Cepo will facilitate the mining of the growing resource of single-cell data and realise the potential of single-cell analytics technologies to pinpoint cell identities that are relevant to the cellular phenotype.

Funding Source: Funded by the Australian Research Council (ARC) Discovery Early Career Researcher Award (DE170100759) and a National Health and Medical Research Council Investigator Grant (1173469), an ARC Discovery Project Grant (DP170100654).

Keywords: cell identity, single-cell RNA sequencing, differential analysis method

DEVELOPMENT WITHOUT DIVISION IN ZEBRAFISH EMBRYOS

Kukreja, Kalki, Klein, Allon

Systems Biology, Harvard University, Cambridge, MA, USA

When embryonic cells develop into a full organism, they must achieve two goals: they must divide to generate all cells of the body, and they must also become specialized into different cell types. These two developmental clocks – division and differentiation – need to be coupled to correctly pattern the organism and to regulate cell division, which can be very dangerous if not controlled. In a limited number of cases, we understand how cell cycle and differentiation are coordinated in detail, but over most tissues and stages of development, we do not know if and how the two processes are coupled. To understand this coupling, we block one of the two clocks – cell cycle – in two complementary ways, and measure how differentiation is affected using zebrafish as our model system. We use single-cell RNA sequencing (scRNA-seq) to quantify changes in differentiation rate, and in fate choice. We find that in spite of the cell division block, embryos continue to differentiate and diversify in transcriptomic cell states. However, we observe widespread changes in gene expression and in proportions of different cell types. We observe retarded differentiation of blood cell types and fate bias towards specific neuronal states, while some cell types, like ionocytes, do not show any significant transcriptional changes. We find that the effect of cell cycle arrest is not localized to specific fate decision points or stages of differentiation and likely occurs as a result of indirect effects of the cell cycle, such as changes in cell size, morphogenesis, and cell numbers.

Keywords: cell cycle, blood, zebrafish

SINGLE-CELL INDIVIDUAL COMPLETE MTDNA SEQUENCING UNCOVERS HIDDEN MITOCHONDRIAL HETEROGENEITY IN HUMAN AND MOUSE OOCYTES

Li, Mo[1], Bi, Chongwei[1], Izpisua Belmonte, Juan Carlos[2]

[1]*Biological and Environmental Sciences and Engineering Division, King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia,* [2]*Gene Expression Laboratory, Salk Institute for Biological Studies, La Jolla, CA, USA*

The ontogeny and dynamics of mtDNA heteroplasmy remain unclear due to limitations of current mtDNA sequencing methods. We developed individual Mitochondrial Genome sequencing (iMiGseq)–of full-length mtDNA for ultra-sensitive variant detection, complete haplotyping, and unbiased evaluation of heteroplasmy levels, all at the individual mtDNA molecule level. iMiGseq uncovers unappreciated levels of heteroplasmic variants in single healthy human oocytes well below the current 1% detection limit, of which numerous variants are deleterious and associated with late-onset mitochondrial disease and cancer. Extreme mtDNA heterogeneity among oocytes of the same mouse female, and a strong selection against deleterious mutations in human oocytes are observed. iMiGseq could comprehensively characterize and haplotype single-nucleotide and structural variants of mtDNA and their genetic linkage in NARP/Leigh syndrome patient-derived cells. Therefore, iMiGseq could not only elucidate the mitochon-

drial etiology of diseases, but also help diagnose and prevent mitochondrial diseases with unprecedented precision.

Funding Source: The work was supported by a KAUST Competitive Research Grant (award number URF/1/3412-01-01) given to ML and JCIB.

Keywords: single-cell mitochondrial DNA sequencing, Nanopore sequencing, oocyte

MODELING THE IMPACT OF ALZHEIMER'S DISEASE GENETIC RISK ON MICROGLIA STATES AND FUNCTIONS

Therrien, Martine[1], Dolan, Micheal J.[1], Jereb, Saša[1], Kamath, Tushar[1], Lojek, Neal[1], Marsh, Samuel E.[2], Johnson, Matthew[1], Macosko, Evan[1], Eggan, Kevin[3], Stevens, Beth[1]

[1]*Stanley Center, Broad Institute of MIT and Harvard, Cambridge, MA, USA*, [2]*Kirby Center, Boston Children's Hospital, Boston, MA, USA*, [3]*Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA*

Microglia, the brain's resident immune cells, are highly dynamic and reactive to environment and genetic challenges. More than 40 genomic loci have been linked to late onset Alzheimer's disease (AD) and many risk genes are highly expressed in microglia. Our goal is to connect insights from genetic association studies to new ways of functionally modeling the cellular and molecular causes of disease to enable predictive tracking and targeting of detrimental immune cell states in patients in the early stages of disease. 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. To answer this question, we turned toward human iPSC-induced microglia (iMGL) and single cell transcriptomics. Single cell transcriptomics revealed the presence of multiple microglia states when iMGLs are stimulated with different brain-relevant challenges, including apoptotic neurons, synaptosomes, myelin and amyloid. Moreover, alignment of these data using Liger shows these states are similar to the ones observed in human and mouse in vivo, revealing several disease associated states, including disease-associated microglia (DAM). We also observed changes in microglia states depending on the challenge and genetic background. Together, our data identified key elements causing the formation of DAM and how AD risk genes affect disease-associated states and functions. This work will open the door to the identification of modulators of DAM and highlight new therapeutics avenues of AD.

Keywords: microglia, Alzheimer's disease, cell state

LINEAGE DYNAMICS DURING BRAIN ORGANOID FORMATION

Treutlein, Barbara

Department of Biosystems Science and Engineering, ETH Zürich, Basel, Switzerland

Diverse regions develop within cerebral organoids generated from human induced pluripotent stem cells (iPSCs), however it has been a challenge to understand the lineage dynamics associated with brain regionalization. Here we establish an inducible lineage recording system that couples reporter barcodes, inducible CRISPR/Cas9 scarring, and single-cell transcriptomics to analyze lineage relationships during cerebral organoid development. We infer fate-mapped whole organoid phylogenies over a scarring time course, and reconstruct progenitor-neuron lineage trees within microdissected cerebral organoid regions. We observe increased fate restriction over time, and find that iPSC clones used to initiate organoids tend to accumulate in distinct brain regions. We use lineage-coupled spatial transcriptomics to resolve lineage locations as well as confirm clonal enrichment in distinctly patterned brain regions. Using long term 4-D light sheet microscopy to temporally track nuclei in developing cerebral organoids, we link brain region clone enrichment to positions in the neuroectoderm, followed by local proliferation with limited migration during neuroepithelial formation. Our data sheds light on how lineages are established during brain organoid regionalization, and our techniques can be adapted in any iPSC-derived cell culture system to dissect lineage alterations during perturbation or in patient-specific models of disease.

Funding Source: ERC Starting Grant ORGANOMICS, EU Horizon 2020 grant BRAINTIME, SNF project grant

Keywords: brain organoids, single-cell genomics, light sheet microscopy

THEME SESSION TSC 2 (TISSUE STEM CELLS AND REGENERATION) TISSUE DEVELOPMENT AND MAINTENANCE 14:00 - 15:45 EDT REBROADCAST WITH LIVE CHAT 02:00 – 03:45 EDT

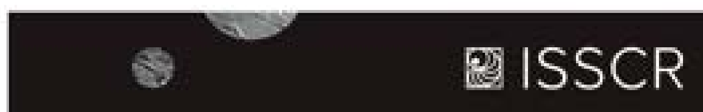
DISSECTING THE DAILY COMMUNICATION BETWEEN TISSUES TO MAINTAIN A COHERENT ORGANISMAL PHYSIOLOGY. HOW ITS LOSS CONTRIBUTES TO AGING AND PATHOLOGY

Aznar Benitah, Salvador

Aging and Metabolism, Institute for Research in Biomedicine (IRB Barcelona), Spain

Our body's circadian clock allows cells to know the time of the day and to function according to it. This incredible mechanism ensures that all tissues function in a synchronized manner, which is essential for remaining healthy. Importantly, our clock progressively fails and is reprogrammed to cope with daily stress and damage as we age, significantly contributing to neural, heart, and muscle degeneration, obesity, arthritis, loss of vision, infections, and cancer. Within the brain, a region known as the suprachiasmatic nuclei detects changes in light and communicates this information to all tissues in our body, which then communicate between each other to perform their daily functions in a concerted manner. How does this communication network happen? Why it is lost during aging? How does the misalignment of clocks of different tissues contribute to age-related pathologies? We are mapping all systemic nodes that govern clock communication between the central clock in the brain and tissues, and between peripheral tissues. We have generated different models in which we can restore the clock in any tissue of choice, or combinations of thereof. I will present data obtained from these models regarding: i) Molecular signatures (metabolome, transcriptome, epigenome) that enable communication between the clocks in brain and peripheral tissues; ii) how this communication enables a proper daily tissue function, and how it is perturbed during aging, and iii) identify global signaling nodes whose modulation might promote healthier aging and how to restore functions lost during aging. Our lifestyle continuously perturbs our circadian rhythms (irregular sleep-wake rhythms, jet lag, high-fat/poor diet choices, etc.) and occurs within an increasingly aged population. We hope to obtain an atlas of the connections that ensure a coherent daily physiology, and of the critical clock nodes that fail during aging and that can be targeted to promote a healthier aging.

Keywords: Tissue communication, clocks, aging



UNIQUE REGULATORY MODULES UNDERLIE SKELETAL MUSCLE STEM CELL DIVERSITY AND FUNCTION

Tajbakhsh, Shahragim, Benavente-Diaz, Maria, Comai, Glenda, Grimaldi, Alexandre

Developmental and Stem Cell Biology, Institut Pasteur, Paris, France

Mouse muscle stem cells (MuSCs) are functionally heterogeneous at the anatomical level, including proliferative capacity and regenerative potential, yet a comprehensive understanding of the mechanisms underlying these differences is lacking. We have proposed that this diversity might explain, in part, why only a subset of muscle groups succumb to a variety of myopathies. Here, we used a combination of clonal analysis, live imaging and spatiotemporal scRNA-seq analysis and identified critical regulators of cranial and limb MuSC diversity. Unexpectedly, embryonic and adult extraocular muscle stem cells expressed distinct extracellular matrix components, growth factors, signalling molecules, and transmembrane proteins that are typically associated with mesenchymal cells. These unique features are regulated by specific sets of transcription factors that constitute a coregulating module throughout development and postnatal growth. Interestingly, these molecular signatures are actively maintained even after several days in culture in a niche-independent context. Further, functional studies show that specific transcription factors and signaling regulatory modules confer the unique proliferative and differentiation properties that distinguish extraocular muscle stem cells from those in the limb. These findings provide mechanistic insights into how high-performing MuSCs, such as those in the extraocular, actively regulate commitment and maintenance of progenitors with concomitant remodelling of their local environment. They also raise the intriguing possibility that other tissues, like skin and blood that have wide anatomical distribution, could also operate with distinct regulatory properties.

Keywords: muscle stem cell heterogeneity, genetic regulatory networks, spatiotemporal transcriptomics

A BIOMECHANICAL SWITCH REGULATES THE TRANSITION TOWARDS HOMEOSTASIS IN MOUSE ESOPHAGEAL EPITHELIUM

Alcolea, Maria P.[1], McGinn, Jamie[1], Hallou, Adrien[1], Krizic, Kata[2], Ulyanchenko, Svetlana[2], Iglesias-Bartolome, Ramiro[3], England, Frances J.[1], Verstreken, Christophe[1], Chalut, Kevin J.[1], Jensen, Kim B.[2], Simons, Benjamin D.[4]

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Epithelial cells are highly dynamic and can rapidly adapt their behavior in response to tissue perturbations and increasing tissue demands. However, the processes that finely control these responses and, particularly, the mechanisms that ensure the correct switch to and from normal tissue homeostasis are largely unknown. Here we explore changes in cell behavior happening at the interface between postnatal development and homeostasis in the epithelium of the mouse esophagus, as a physiological model exemplifying a rapid but controlled tissue growth transition. Single cell RNA sequencing and histological analysis of the mouse esophagus reveal significant mechanical changes in the epithelium upon tissue maturation. Organ stretching experiments further indicate that tissue strain caused by the differential growth of the mouse esophagus relative to the entire body promotes the emergence of a defined committed population in the progenitor compartment as homeostasis is established. Our results point to a simple mechanism whereby the mechanical changes experienced at the whole tissue level are integrated with those "sensed" at the cellular level to control epithelial cell behavior and tissue maintenance.

Funding Source: This work was mainly supported by funding from the Wellcome Trust and The Royal Society (105942/Z/14/Z; 098357/Z/12/Z) and Cancer Research UK.

Keywords: Epithelial cell fate transitions, Mouse esophagus, Tissue mechanics

SOCIALIZING WITH THE NEIGHBORS: LYMPHATIC NICHE SYNCHRONIZES STEM CELL FATE DECISION AND TISSUE REGENERATION

Gur-Cohen, Shiri[1], Yang, Hanseul[2], Baksh, Sanjeethan C.[2], Miao, Yuxuan[2], Levorse, John[2], Kataru, Raghu P.[3], Dela Cruz-Racelis, June[2], Mehrara, Babak J.[3], Fuchs, Elaine[1]

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As the natural units of tissue repair and homeostasis, adult stem cells (SCs) have a unique capacity for long-term self-renewal and the execution of differentiation programs for one or more tissues. Elucidating how SC usage is controlled and maintained is a fundamental prerequisite to understanding why tissues lose their capacity to repair wounds and become tumor-prone with age. It is increasingly evident that SCs are never alone in the tissue, and that in order to preserve their stemness identity yet maintain their fate lineage commitment, SCs are intimately associated with their local environment ('niche'). Knowledge of how the environment changes to restrict SC identity selections and of whether the architectural design of the niche can be tailored to suit the needs of its resident SCs is still scant. Taking an interdisciplinary approach to address this challenge and by using the hair follicle (HF) as a model, we uncovered the dermal lymphatic vessels as a newly identified dynamic SC niche environment and demonstrated that SCs play a role in organizing and diversifying their niches. By employing a new clearing methodology with high-resolution microscopy to visualize large tissue segments in the context of preserved skin morphology, we identified the lymphatic capillaries to be highly associated with the resting HF-SCs. Notably, while the resting SCs intimately interact with lymphatic capillaries, we documented a disrupted and discontinuous lymphatic capillary structure as the SCs become activated, suggesting a dynamic reciprocal association between the SCs and the lymphatic niche. We revealed a preceding transcriptional switch in the transition from SC quiescence to an active state, as reflected by a significant downregulation of Angptl7 and elevation of Angptl4 and Ntn4. At the genetic level, we have functionally linked the SC-driven secretome switch to lymphatic remodeling dynamics and demonstrated that the switch governs the synchronized regenerative process. In sum, our results identify the lymphatic capillaries as a hitherto under-appreciated SC niche element and illuminate the way that lymphatic fitness, driven by SC cues, integrates and synchronizes the regenerative process. These findings may provide major implications for advancing tissue regeneration and wound-repair.

Keywords: Hair Follicle Stem Cells, Stem Cell Niche, Tissue Regeneration

UNDERSTANDING A MECHANISM UNDERLYING BONE REPAIR BY COMBINATORIAL ANALYSIS OF LINEAGE TRACING AND SINGLE-CELL RNA SEQUENCING

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In our super-ageing society, the number of bone fractures is increasing and nonunion after fragility fracture is difficult to treat by traditional methods. To repair irreversible bone loss, a regenerative strategy is required; however, the behaviour of the skeletal progenitors and the mechanism underlying the bone repair process have not been well elucidated. We investigated the mechanism of bone repair by combinatorial analyses of lineage tracing and single-cell RNA-sequencing (scRNA-seq) in a mouse calvarial defect model. We labelled Sox9-positive cells and their descendants with tdTomato by injecting tamoxifen into 8-week-old Sox9-CreERT2;R26RtdTomato mice before and after making 1-mm bone defects. Histological analysis revealed that the tomato-positive cells emerged de novo at bone defect sites 3 days after surgery; the tomato-positive cells had also migrated from the suture of the calvaria — a known skeletal progenitor pool — on day 10. Immunohistochemistry revealed co-expression of tomato and either RUNX2 or SP7, master regulators of osteoblasts, providing a potential clue to the contribution of the Sox9-positive progenitors to bone repair. Next, we performed scRNA-seq analysis with 2,977 cells isolated from the bone defect site on day 10. Clustering analysis identified 19 cell clusters with distinct signatures among the heterogeneous cell-types at the bone defect site which include skeletal progenitors and bone-forming osteoblasts. Pseudo-time analysis with tomato-positive cells revealed bifurcated differentiation pathways derived from the skeletal progenitors to either osteoblasts or adipogenic cells. The ligand-receptor analysis predicted signalling pathways enriched explicitly during the trajectory of cell differentiation. We tested the in vivo effects of the enriched pathways on the bone repair by manipulating the pathways' activities with molecules, including FDA-approved drugs and drugs undergoing clinical trials, in the mouse calvarial defect model. MicroCT analysis suggested that one molecule had a positive impact on the bone repair process. In conclusion, we identified Sox9-positive skeletal progenitors in calvaria, which had bidirectional behaviour during bone repair. The identified drug might be repositioned for use in bone repair.

Keywords: bone repair, single-cell RNA-sequencing, skeletal progenitor

TISSUE AND CELL-SCALE MECHANICS DRIVE HAIR FOLLICLE MORPHOGENESIS

Ylivinkka, Irene, Hashmi, Ali, Miroshnikova, Yekaterina A., Villeneuve, Clementine, Bertillot, Fabien, Biggs, Leah C., Wickström, Sara A.

Helsinki Institute of Life Sciences, Stem Cells and Metabolism Research Program, Wihuri Research Institute, University of Helsinki, Finland

How epithelia are patterned and folded to give rise to complex organs and their correct tissue architecture and geometry during morphogenesis remains as open question in biology. During mouse embryogenesis a single layer of epidermal stem cells differentiate into two separate compartments: the stratified epidermis and hair follicle. Specification of placodes, which mark the position of future hair follicles, occur at embryonic day E14 and the morphogenesis continues throughout the development. We hypothesized that the compartmentalization of the placode from the epidermis and its specification are controlled by tissue and cell-scale mechanical forces. Using in vivo embryo imaging in combination with mathematical modelling we observe emergence of elevated tissue pressures and stresses at early placodes. Moreover, atomic force microscopy reveal that the underlying basement membrane is softer in the placodes when compared to the surrounding epidermis, suggesting that the combination of high tissue pressure and local basement membrane compliance could drive placode invagination. To ask if this invagination required force generation by the epidermis, we analyzed the placodes of an epidermal specific non-muscle myosin IIA knock-out mice. These mice exhibited inverted placode architecture but normal basement membrane structure. These findings collectively indicate that contractility of the epidermal cells to deform the epithelium acts in collaboration with basement membrane remodeling and folding to drive hair follicle morphogenesis.

Funding Source: Helsinki Institute of Life Science, Sigrid Juselius Foundation

Keywords: tissue morphogenesis, basement membrane, mechanotransduction

BUILDING THE HUMAN LUNG: LESSONS FROM ORGANOIDS

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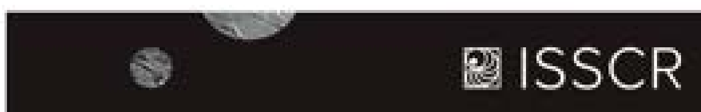
The developmental cues which regulate lung branching, epithelial and mesenchymal differentiation and maturation have been investigated extensively in the mouse lung. How many of the morphogenetic events and signals are conserved in human lung embryonic development? Can we develop improved models of in vitro human lung development that will facilitate drug screening and disease modelling? And gain insights into lung regeneration? To address these questions, we have developed organoid-based culture systems in which we can grow human embryonic distal tip cells isolated from 6-20 week gestation human lungs. We can self-renew these epithelial tip progenitor cells as karyotypically stable organoids and subsequently differentiate to mature lung lineages. Our recently-developed "Organoid Easytag" system for gene "knock-in" and our adaptation of CRISPRi and CRISPRa techniques allows the latest genetic toolsets to be employed in the organoids. Most recently, we have determined the timing of a key developmental transition in the human lung – the switch from airway to alveolar cell production – and isolated organoids from the alveolar stage of lung development. These have allowed us to functionally define key pathways and transcription factors which regulate the airway – alveolar fate switch, including factors associated with congenital lung disease.

Funding Source: Medical Research Council, Wellcome Trust

Keywords: differentiation, single cell, CRISPR-Cas9



THEME SESSION NT 3 (NEW TECHNOLOGIES) CRISPR BASED TECHNOLOGIES 7:30 - 9:15 EDT REBROADCAST WITH LIVE CHAT 16:30 - 18:15 AND 19:30 - 21:15 EDT

USING STEM CELLS TO EXPLORE THE GENETICS UNDERLYING BRAIN DISEASE

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The complex genetic mechanisms underlying psychiatric and neurodegenerative disease remain unclear. We employ a functional genomics approach that integrates stem cell models and genome engineering, resolving the combinatorial impact of patient-specific variants across cell types, donor genetic backgrounds, and environmental contexts. Individually small risk effects combine to yield much larger impacts in aggregate, but the interactions between the myriad variants remain undetermined. First, we evaluated the impact of patient-specific NRXN1+/- deletions in hiPSC-neurons, observing greater than two-fold reduction of half of the wildtype NRXN1α isoforms and detecting dozens of novel isoforms expressed from the mutant allele; reduced neuronal activity in patient hiPSC-neurons was ameliorated by overexpression of individual control isoforms in a genotype-dependent manner, whereas individual mutant isoforms decreased neuronal activity levels in control hiPSC-neurons. Second, we integrated CRISPR-mediated gene editing, activation and repression technologies to study putative causal common variants and their associated target genes, alone and in combination. This allowed us to uncover an unexpected synergistic effect between risk genes that converges on synaptic function and links the rare and common variant genes implicated in psychiatric disease risk, one which may represent a generalizable phenomenon occurring more widely in complex genetic disorders. We demonstrate a systematic and scalable strategy to interpret and evaluate the additive impact of a growing number of disease-associated variants and genes within and across pathways, neural cell types and treatments. We seek to decode highly complex genetic insights into medically actionable information, better connecting the expanding list of genetic loci associated with human disease to pathophysiology. Our goal is to improve diagnostics, predict clinical trajectories, and identify pre-symptomatic points of therapeutic intervention.

Funding Source: This work was partially supported by National Institute of Health (NIH) grants R56MH101454 R01MH106056, R01MH109897 and R01MH121074.

Keywords: neurogenomics, CRISPR, hiPSCs

THE IPSC NEURODEGENERATIVE DISEASE INITIATIVE (INDI)

Ward, Michael[1], Skarnes, William C.[2], Cookson, Mark R.[3], Ramos, Daniel[3], Singleton, Andrew B.[3]

[1]*NINDS, National Institutes of Health (NIH), Bethesda, MD, USA,* [2]*Cellular Engineering, The Jackson Research Laboratory, Farmington, CT, USA,* [3]*NIA, National Institutes of Health (NIH), Bethesda, MD, USA*

Over the past few decades, breakthroughs in genomics, CRISPR/Cas9, and iPSCs have provided an unprecedented opportunity to understand the basic biology of Alzheimer's disease and related dementias (ADRD). However, practical application of these advances by the ADRD research community has, to a large degree, been hampered by a lack of foundational cellular disease models and datasets. Here we describe a large-scale iPSC genome engineering and phenotyping effort for ADRD, the iPSC Neurodegenerative Disease Initiative (iNDI). iNDI is a two-phase collaborative project between the NIH, non-profits, and industry to generate a large series of disease-relevant iPSC lines and related foundational datasets for the ADRD research community. In Phase 1, iNDI will create >600 genetically-engineered isogenic iPSC lines, including those harboring ADRD mutations, gene knockouts, and endogenous tags. All lines will be deeply characterized through a panel of genomic and phenotypic quality control assays, accompanied by the release and sharing of all associated datasets. Following their validation, lines will be distributed to the research community on a rolling basis through JAX laboratories. To date, over 130 mutant lines across 70 ADRD genes have been successfully created from the KOLF2.1 iPSC parental line, and are currently undergoing QC. In Phase 2, through the use of high-throughput robotic platforms, iNDI will differentiate mutation-harboring iPSC lines into CNS-relevant cell types such as neurons and microglia. We will use a series of phenotypic assays, including transcriptomics, proteomics, imaging, and functional genomics to characterize the effect of ADRD mutations on downstream molecular pathways. We anticipate that these resulting tools and datasets will enable discovery of fundamental disease mechanisms, thereby unlocking new therapeutic targets and reducing the burden of these diseases on affected patients, their families, and society.

Keywords: iPSCs, Neurodegenerative Diseases, Multi-omics

A CRISPR PRIME EDITING PIGGYBAC TRANSPOSON ALLOWS FOR ENRICHMENT OF GENE EDITED CELLS IN HUMAN PLURIPOTENT STEM CELLS

Eggenschwiler, Reto[1], Gschwendtberger, Thomas[2], Petri, Susanne[2], Cantz, Tobias[1]

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CRISPR prime-editors are emergent tools for genome editing and offer a versatile alternative approach to HDR-based genome engineering or DNA base-editors. However, sufficient expression levels and availability of optimized transfection protocols may hamper editing efficiencies, especially in hard-to-transfect cells like human induced pluripotent stem cells (hiPSC). Here, we show that piggyBac prime-editing (PB-PE) allows for sustained expression of prime-editor and can edit more than 50% of hiPSC cells even when non-optimized transfection conditions are applied. We demonstrate proof-of-concept for PB-PE in a lentiviral traffic light reporter, which allows for estimation of gene correction and defective editing resulting in indels, based on expression of two different fluorophores. We also show that improper design of pegRNA cannot simply be overcome by extended expression, but PB-PE allows for estimation of effectiveness of selected pegRNAs after few days of cultivation time. Finally, we implemented PB-PE for efficient editing of an amyotrophic lateral sclerosis-associated mutation in the SOD1-gene of patient-derived hiPSC. Progress of genome editing can be monitored by Sanger-sequencing, whereas PB-PE vectors can be removed after editing and excised cells can be enriched by fialuridine selection. Together, we present an efficient prime-editing system which can be robustly used in a variety of cell lines.

Keywords: CRISPR prime editing, piggyBac, hiPSC

GENOME-WIDE FUNCTIONAL SCREENING OF HUMAN GENETIC DISORDERS IN PLURIPOTENT STEM CELLS AND THEIR NEURAL DERIVATIVES

Yilmaz, Atilgan, Braverman-Gross, Carmel, Peretz, Mordecai, Bialer-Tsypin, Anna, Benvenisty, Nissim

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Early developmental phenotypes of many human genetic disorders are yet to be characterized. To study the involvement of disease-causing mutations in early human development, we have performed genome-wide loss-of-function screens in haploid human pluripotent stem cells (hPSCs) and their differentiated derivatives, utilizing CRISPR/Cas9 technology with over 180,000 sgRNAs. We have carried out screens at different embryonic stages from blastocyst-stage cells to neural progenitors and into neuronal differentiation and analyzed the genes causing human disorders for their potential growth and differentiation phenotypes at these early developmental windows. At the stage of pluripotency, we uncovered that nearly one fifth of all genes known to cause autosomal recessive disorders with growth retardation phenotypes regulates normal growth of hPSCs, suggesting early embryogenesis phenotypes for these disorders. Similarly, our screens on early neuroectoderm specification revealed that a significant portion of genes causing a range of neurological conditions such as microcephaly, autism, hypomyelination and gliosis exhibit differentiation phenotypes at this early stage of neural fate. More recently, we utilized our screening platform to identify genes regulating the differentiation into more advanced stages of neuronal differentiation, showing that genes causing pattern-specific neurological conditions such as lissencephaly and colpocephaly have differentiation phenotypes during these cell fate transitions. Overall, our work reveals stage-specific early embryogenesis phenotypes for large fractions of human disorders and suggests that at least some aspects of these disorders can be modelled by using cells at these earlier stages of development.

Keywords: Modeling human genetic disorders, CRISPR screens, Neural differentiation

A PHENOTYPE-AGNOSTIC FUNCTIONAL SCREENING PLATFORM OPTIMIZED FOR HUMAN PLURIPOTENT STEM CELL-DERIVED LINEAGES

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Deconvolution of transcriptional responses to barcoded gene expression perturbations through single cell RNA sequencing (scRNA-seq) has emerged as a powerful tool for large scale assessment of gene function. Nevertheless, efficient and robust execution of this strategy in human pluripotent stem cell (hPSC)-derived lineages remains challenging. We previously developed an OPTimized inducible KnockDown (OPTiKD) platform to dissect stage- and lineage-specific gene function during hPSC differentiation. Here we expand this approach to enable pooled functional screenings through scRNA-seq, a strategy we name OPTiKD-seq. The method relies on the generation of isogenic genome-edited pools of hPSCs, each expressing a single barcoded and tetracycline-inducible short hairpin RNA (shRNA) from a wide library. Barcodes are included in the 3' end of a highly expressed transgene, to allow efficient recovery through scRNA-seq approaches that rely on 3' mRNA detection. Besides an shRNA-specific identifier, each barcode includes a random sequence – which we call Unique Clonal Identifier (UCI) – that marks cells arising from an individual genome-editing event. The matching of single cell transcriptomes to shRNA barcodes and UCIs allows for the deconvolution of cell-autonomous loss-of-function perturbations, while controlling for clonal variability. We exemplify this approach in human induced pluripotent stem cells (hiPSCs) and hiPSC-derived cardiomyocytes. Commercially available microfluidics-based scRNA-seq (10X Genomics) allows to assign over 80% of cells to individual perturbations in both cell types. Adaptation of an in house scRNA-seq protocol based on combinatorial indexing (sci-RNA-seq) reaches a comparable efficacy. Thus, OPTiKD-seq has wide applicability across technologies that differ greatly in their sensitivity, scalability, cost, and input material (including live cells and fixed nuclei). We identify lineage-specific roles of epigenetic regulators (KMT2D and CHD7) and transcription factors (GATA4, NKX2-5 and SMAD2) implicated in the pathogenesis of congenital heart disease. Overall, OPTiKD-seq empowers phenotype-agnostic pooled functional screenings in hPSC-derived cell types, accelerating the pursuit to functionally annotate human genome function in development and disease.

Funding Source: This work was supported by an Innovation Pilot Award from the Institute for Stem Cell and Regenerative Medicine at the University of Washington, and by a gift from the Tietze Family Foundation.

Keywords: single cell RNA sequencing, short hairpin RNA, human pluripotent stem cells

TINC - A METHOD TO DISSECT REGULATORY COMPLEXES AT SINGLE-LOCUS RESOLUTION - REVEALS AN EXTENSIVE PROTEIN COMPLEX AT THE NANOG PROMOTER

Knaupp, Anja S.[1], Mohenska, Monika[1], Larcombe, Michael R.[1], Ford, Ethan[2], Lim, Sue Mei[1], Wong, Kayla[1], Chen, Joseph[1], Firas, Jaber[1], Huang, Cheng[3], Liu, Xiaodong[1], Nguyen, Trung[2], Sun, Yu B.[1], Holmes, Melissa L.[1], Tripathi, Pratibha[1], Pflueger, Jahnvi[4], Rossello, Fernando J.[1], Schröder, Jan[1], Davidson, Kathryn C.[1], Nefzger, Christian M.[1], Das, Partha P.[1], Haigh, Jody J.[5], Lister, Ryan[2], Schittenhelm, Ralf B.[3], Polo, Jose M.[1]

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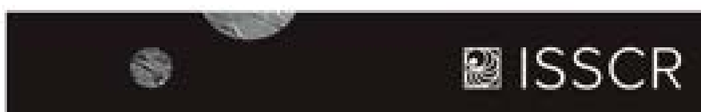
Cellular identity is ultimately dictated by the interaction of transcription factors with regulatory elements (REs) to control gene expression. Advances in epigenome profiling techniques have significantly increased our understanding of cell-specific utilization of REs. However, it remains difficult to dissect the majority of factors that interact with these REs due to the lack of appropriate techniques. Therefore, we developed TINC: TALE-mediated isolation of nuclear chromatin. Using this new method, we interrogated the protein complex formed at the Nanog promoter in embryonic stem cells (ESCs) and identified many known and previously unknown interactors, including RCOR2. Further interrogation of the role of RCOR2 in ESCs revealed its involvement in the repression of lineage genes and the fine-tuning of pluripotency genes. Consequently, using the Nanog promoter as a paradigm, we demonstrated the power of TINC to provide insight into the molecular makeup of specific transcriptional complexes at individual REs as well as into cellular identity control in general.

Keywords: Single locus isolation, Transcriptional complex, Nanog



CRISPR-BASED FUNCTIONAL GENOMICS UNCOVER REGULATORS OF DISEASE-ASSOCIATED STATES OF GLIA AND NEURONAL PATHWAYS CONTRIBUTING TO NEURODEGENERATIVE DISEASE

Kampmann, Martin

Institute for Neurodegenerative Diseases, University of California, San Francisco, CA, USA

Human genes associated with brain-related diseases are being discovered at an accelerating pace. A major challenge is the identification of the mechanisms through which these genes act, and of potential therapeutic strategies. To elucidate such mechanisms in human cells, we established a CRISPR-based platform for genome-wide screens in human iPSC-derived neurons, glia, and multi-lineage assembloids. Our approach relies on CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa), in which a catalytically dead version of the bacterial Cas9 protein recruits transcriptional repressors or activators, respectively, to endogenous genes to control their expression, as directed by a small guide RNA (sgRNA). Complex libraries of sgRNAs enable us to conduct genome-wide or focused loss-of-function and gain-of-function screens. I will present unpublished results from two applications: A screen in human iPSC-derived microglia and astrocytes to uncover regulators of disease-associated cell states, and a screen in human iPSC-derived neurons with familial mutations linked to frontotemporal dementia to identify neuronal pathways controlling tau aggregation. Hits from these screens provide mechanistic insights and reveal potential therapeutic targets.

Keywords: tau, microglia, Alzheimer's Disease

THEME SESSION TSC 3 (TISSUE STEM CELLS AND REGENERATION) WOUND HEALING, STRESS AND AGING 7:30 - 9:15 EDT REBROADCAST WITH LIVE CHAT 16:30 - 18:15 AND 19:30 - 21:15 EDT

EMERGENCY MYELOPOIESIS PATHWAYS

Emmanuelle Passegué

Columbia Stem Cell Initiative, Department of Genetics and Development, Columbia University, New York, USA

In the context of myeloid malignancies or during aging, activation of the emergency myelopoiesis pathways drive the expansion of malignant hematopoietic stem and progenitor cells (HSPCs) and the overproduction of myeloid cells. At steady-state, blood production reflects the differential production by rare quiescent hematopoietic stem cells (HSC) of a small number of myeloid-biased multipotent progenitors (MPP2/3) and a large number of lymphoid-biased multipotent progenitors (MPP4), which both give rise to granulocyte/macrophage progenitors (GMP) and contribute to limited myeloid output. In contrast, during regeneration, activated HSCs overproduce myeloid-biased MPP2/3, lymphoid-biased MPP4 are reprogrammed towards myelopoiesis, and GMP expand in the bone marrow (BM) cavity as GMP clusters (cGMP), driving burst production of myeloid cells. This remodeling of the HSPC compartment reflects the engagement of emergency myelopoiesis pathways, which are transiently activated during regeneration but are co-opted and constantly triggered in inflammatory conditions like aging or during the development of myeloid malignancies. However, the regulatory mechanisms controlling emergency myelopoiesis remain poorly understood, and the relationships between acute triggering during regeneration and chronic activation in malignancy and aging are still largely unexplored. I will present results identifying how HSPCs adapt to these demands and adjust both self-renewal and differentiation properties to tailor myeloid cell production in regenerative and disease conditions.

Funding Source: R35 HL135763

Keywords: Hematopoietic stem cells, regeneration, lineage commitment

AGING OF MOUSE AND HUMAN SKELETAL STEM CELLS UNDERLIES LINEAGE SKEWING THAT ALTERS BONE MARROW NICHE DYNAMICS

Ambrosi, Thomas H.[1], Marecic, Owen[1], Sinha, Rahul[2], Steininger, Holly[1], Weissman, Irving[2], Longaker, Michael T.[1], Chan, Charles[1]

[1]*Surgery - Stem Cell Institute, Stanford University, CA, USA,* [2]*Stem Cell Institute, Stanford University, CA, USA*

The skeleton is maintained by a constellation of diverse, closely interacting cell types that coordinate distinct homeostatic and regenerative requirements. Aging and disease disrupt the delicate balance between the opposing anabolic and catabolic actions of skeletal and hematopoietic cell types, respectively. Adult stem cells hold great promise for clinical therapies. We recently described bona fide mouse and human skeletal stem cells (SSCs) and their defined downstream progenitor populations. Here we show, through detailed functional as well as single-cell genomic studies, that intrinsic aging of mouse and human SSCs skews lineage output and alters niche signaling thereby generating an aged bone marrow niche characterized by a decline in osteochondrogenic capacity and increased expression of pro-inflammatory signaling. Purified SSCs from 24-month-old mice and geriatric patients showed fibrogenic skewing compared to their young counterparts during in vitro differentiation assays or when transplanted under the renal capsule of immuno-deficient mice in vivo. Heterochronic parabiosis and hematopoietic reconstitution of aged hosts with young hematopoietic stem cells (HSCs) did not improve skeletal parameters. Reversely, transplantation of young HSCs into aged mice or co-culture with aged versus young SSCs favored myelopoiesis, indicative of a seed (HSC) and soil (SSC) mechanism. Trajectory inference analysis of SmartSeq2 single cell RNA-sequencing data from mouse and human SSCs suggested a loss of stem cell diversity characterized by a pro-fibrotic differentiation shift concurrent with elevated pro-hematopoietic signaling. Combinatorial activation of aged SSCs with simultaneous blockade of inflammatory signaling by specific cytokines in a mouse bi-cortical fracture regeneration model reinstated youthful SSC potency and healing as assessed by mechanical strength testing, flow cytometric analysis, and 10X single cell RNA-sequencing. These findings provide mechanistic insight into the dysregulation of inter-stem cell communication underlying stem cell aging and offer new therapeutic vantage points for rejuvenating the aged skeletal system by targeting the bone marrow niche.

Keywords: Skeletal Aging, Bone Marrow Niche, Skeletal Stem Cell Biology

NICHE MECHANICS CONTROLS STEM CELL POTENTIAL THROUGH REGULATING CHROMATIN ARCHITECTURE

Miroshnikova, Yekaterina A.[1], Koester, Janis[2], Wickstrom, Sara[1]

[1]*HiLife, University of Helsinki, Finland,* [2]*SW, Max Planck Institute for Biology of Ageing, Cologne, Germany*

Tissue homeostasis and regeneration require activation and subsequent lineage commitment of tissue-resident stem cells (SCs). These state changes are controlled by epigenetic barriers. Our studies have implicated mechanical forces as regulators of chromatin architecture and transcription. To study physiological consequences of force-mediated fate regulation through chromatin remodeling we focused on the aged skin as a paradigm, where we detect large scale changes in tissue mechanics. Aged

skin displayed wide-spread alterations in extracellular matrix and basement membrane composition and mechanics, resulting in niche stiffening and compressive forces on skin hair follicle stem cells (HFSCs). Analyses of genome-wide chromatin accessibility revealed that aged HFSCs displayed widespread reduction of chromatin accessibility, specifically at key SC self-renewal and differentiation genes that were characterized by bivalent promoters carrying both activating and repressive chromatin marks. Consistently, aged HFSCs showed reduced self-renewing capacity and attenuated ability to activate expression of these bivalent genes upon regeneration. These functional defects were niche-dependent as transplantation of aged HFSCs into young recipients or into ex vivo niches restored SC functions and transcription of poised genes. Mechanistically, altered niche mechanics led to transcriptional repression of HFSCs, leading to loss of bivalent promoters. Tuning tissue mechanics both in vivo and in vitro recapitulated age-related SC changes, implicating niche mechanics as a central regulator of genome organization and function leading to age-dependent SC exhaustion.

Funding Source: Human Frontier Science Program fellowship LT000861/2018 to YAM

Keywords: hair follicle stem cells, aging, chromatin mechanotransduction

DEFINING THE TRANSCRIPTIONAL SIGNATURE OF ESOPHAGEAL-TO-SKIN LINEAGE CONVERSION

Bejar, Maria T.[1], Jimenez-Gomez, Paula[1], Moutsopoulos, Ilias[1], Colom, Bartomeu[2], Calero-Nieto, Fernando[1], Gottgens, Berthold[1], Mohorianu, Irina[1], Alcolea, Maria P.[1]

[1]*Wellcome-MRC Cambridge Stem Cell Institute, University of Cambridge, UK,* [2]*Wellcome Sanger Institute, Cambridge, UK*

The ability of epithelial cells to rewire their cell fate program beyond their physiological repertoire has become a new paradigm in stem cell biology. This plasticity leaves behind the concept of strict stem cell hierarchies, opening up new exciting questions about its limits and underlying regulation. Here we developed a heterotypic 3D culture system to study the mechanisms modulating changes in the identity of adult esophageal epithelial cells. We demonstrate that, when exposed to the foreign stroma of adult skin, esophageal cells transition towards hair follicle identity and architecture. Heterotypic transplantation experiments recapitulated this cell fate conversion process in vivo. Single-cell RNA sequencing and histological analysis, capturing the temporality of this process, reveal that most esophageal cells switching towards skin identity remain in an intermediate state marked by a transient regenerative profile and a particularly strong hypoxic signature. Inhibition of HIF1a establishes the central role of this pathway in regulating epithelial cell plasticity, driving cells away from their transition state in favor of cell fate conversion.

Funding Source: M.T.B received funding from the European Union's Horizon 2020 under the Marie Sklodowska-Curie grant agreement No 794664. Work supported by the Wellcome Trust and The Royal Society (105942/Z/14/Z to M.P.A.) and core grant WT-MRC SCI.

Keywords: Adult epithelial plasticity, Esophageal 3D organ culture, Cell fate transition

SOFT BIOMECHANICAL PROPERTIES OF THE LIMBUS SUSTAIN YAP ACTIVITY TO PREVENT SMAD2/3 MEDIATED CELL DIFFERENTIATION IN THE MOUSE AND HUMAN CORNEAL EPITHELIUM

Bhattacharya, Swarnabh[1], Pisano, Sabrina[2], Mukherjee, Abhishek[1], Altshuler, Anna[1], Nasser, Waseem[1], Amitai-Lange, Aya[1], Mimouni, Michael[3], Socea, Sergiu[3], Hasson, Peleg[1], Feral, Chloe[2], Wolfenson, Haguy[1], Shalom-Feuerstein, Ruby[1]

[1]*Genetics and Developmental Biology, Technion - Israel Institute of Technology, Haifa, Israel,* [2]*Epithelial Homeostasis and Tumorigenesis, University of Nice - Sophia-Antipolis, Nice, France,* [3]*Opthalmology, Rambam Health Care Campus, Haifa, Israel*

The corneal epithelium is the outermost layer covering the eye. Its transparency, high accessibility, and compartmentalization for stem cells (SCs) and differentiated cells make cornea an excellent SC research model. We recently established an in-vivo microscopy approach to identify corneal (limbal) SC using K15-GFP transgene and discovered that depletion of the entire SC pool is restored by committed cells which de-differentiate into bona-fide SCs. Contrarily, niche destruction resulted in irreversible SC loss and corneal opacification. However, the mechanism of SC differentiation and reprogramming of committed cells remained unclear. Here we report that the post-natal formation of SC and differentiation compartments in the murine cornea was hallmarked by zonated changes in matrix rigidity. Enhanced matrix stiffening of the differentiation compartment (central cornea) was coupled by switching from nuclear to cytoplasmic localization of Yes-associated protein (YAP). Simultaneously, the low rigidity SC niche (limbus) retained nuclear YAP and undifferentiated state. In line, ectopic matrix stiffening resulted in disrupted LSC function and corneal opacification. Inhibition of YAP activity or forced Rho activation that mediates actomyosin contractility repressed LSC function in vivo, delayed wound healing response and also perturbed injury-induced dedifferentiation. In agreement, human LSCs that were grown on stiffer matrices generated stronger contractile forces and induced cytoplasmic YAP localization through activation of LATS1/2, facilitating SMAD2/3-mediated cell differentiation. Altogether, we propose biomechanical forces play a vital role in the limbal niche formation during post-natal development, regulating LSC fate, cell plasticity and regeneration.

Funding Source: RSF has received funding from the Israel Science Foundation (1308/19 and 2830/20), NIH-exploratory R21 (800040), European Union's Horizon 2020 research & innovation program (828931).

Keywords: Epithelial stem cells, Mechanobiology of stem cells, Limbal stem cells

EXPLORING THE HUMAN LACRIMAL GLAND USING ORGANOIDS AND SINGLE-CELL SEQUENCING

Bannier-Hélaouët, Marie[1], Post, Yorick[1], Korving, Jeroen[1], Trani Bustos, Marc[1], Gehart, Helmuth[1], Begthel, Harry[1], Bar-Ephraim, Yotam[1], van der Vaart, Jelte[1], Imhof, Saskia M.[2], Kalmann, Rachel[2], Clevers, Hans[1]

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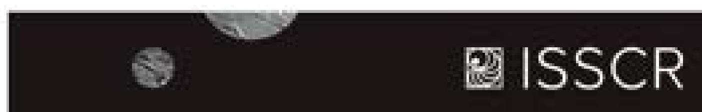
The lacrimal gland is essential for lubrication and protection of the eye. Disruption of lacrimal fluid production, composition or release results in dry eye, causing discomfort and damage to the ocular surface. Here, we describe the establishment of long-term 3D organoid culture conditions for mouse and human lacrimal gland. Organoids can be expanded over multiple months and recapitulate morphological and transcriptional features of lacrimal ducts. CRISPR/Cas9-mediated genome editing reveals the master regulator for eye development Pax6 to be required for differentia-



tion of adult lacrimal gland cells. We address cellular heterogeneity of the lacrimal gland by providing a single-cell atlas of human lacrimal gland tissue and organoids. Finally, human lacrimal gland organoids phenocopy the process of tear secretion in response to neurotransmitters and can engraft and produce mature tear products upon orthotopic transplantation in mouse. Together, this study provides an experimental platform to study (patho-)physiology of the lacrimal gland.

Keywords: lacrimal gland, organoids, single-cell sequencing

MAPPING CELL FATE CONTROL MECHANISMS DURING STATE TRANSITIONS

Jensen, Kim B.

Biotech Research & Innovation Centre and Novo Nordisk Center for Stem Cell Biology, University of Copenhagen, Denmark

The intestine is essential for digestion and absorption of nutrients. Moreover, the epithelium, which lines the luminal surface, constitutes a barrier that protects our body from gut microbiota. Adult stem cells located at the bottom of crypts are responsible for the life-long replenishment of the epithelium by giving rise to differentiated offspring. Using a combination of mouse models and tissue biopsies from humans, we recently outlined the relationship between fetal progenitors in the developing epithelium and adult stem cells. Here we observed that fetal cells are not organised in a strict cellular hierarchy as has been observed for adult epithelium and appear to be inherently more plastic. Moreover, during injury of the adult epithelium, which is associated with cellular de-differentiation, the adult epithelial cells transition into a fetal-like state. It will consequently be important to address the difference between stem cells in the adult and fetal state and identify mechanisms that control the transitions between these two states. We believe that understanding the mechanisms that direct cellular identity and plasticity will enable us to fuel tissue regeneration in diseases such as ulcerative disorders.

Keywords: Intestinal stem cells, organoids, Tissue regeneration

THEME NT (NEW TECHNOLOGIES) PLENARY III: EMERGING TECHNOLOGIES / ENABLING TECHNOLOGIES 9:30 - 11:00 EDT REBROADCAST WITH LIVE CHAT 21:30 - 23:00 EDT

USING THE ALLEN CELL COLLECTION TO VISUALIZE AND MAP CELL STATES FROM PLURIPOTENCY THROUGH DIFFERENCIATION

Gunawardane, Ruwanthi

Allen Institute for Cell Science, Allen Institute, Seattle, WA, USA

The Allen Institute for Cell Science is creating a dynamic visual model of cell organization from hiPSCs to differentiated cells with a collection of fluorescent-tagged clonal hiPSC lines and an associated suite of image-based computational tools for quantitation and analyses. Our approach utilizes CRISPR/Cas9 to fluorescently tag proteins that localize to the major organelles of the cell. Live cell imaging, image analysis, modeling and visualization, and open distribution to the scientific community define our endeavor. To date we have generated fluorescently tagged hiPSC clonal lines for ~50 major cellular structures and performed key genetic, cell biological, and stem cell validation. We will demonstrate the utility of these cell lines for generating image-based integrated models of cell organization and dynamics. We will present imaging data demonstrating the epithelial organization of the undifferentiated hiPS cells, dynamics of major organelles during cell division and early mesoderm differentiation, and the organization of the sarcomere in hiPSC-derived cardiomyocytes. We will describe the use of these high-resolution, 3D live images for quantitative analysis and development of integrative cell models to map cell states. These include approaches to study natural variation in subcellular organization of hiPSCs and conjoining gene expression signatures with cellular organization in cardiomyocytes. We are also starting to integrate structural landmarks in the nucleus with chromatin architecture in collaboration with the 4DN to better understand the organization of the genome. With these tools and approaches we are starting to develop a generalizable framework for understanding principles of cell organization that can be tested in various cell types and states in normal, pathological, and regenerative contexts. Our cell lines, plasmids, 3D images, various analysis and visualization tools, integrated cell models, and biological findings are available to the research community (www.allencell.org).

Keywords: iPSCS, Imaging, gene editing

BASE EDITING AND PRIME EDITING: GENOME EDITING WITHOUT DOUBLE-STRAND BREAKS

Liu, David

Chemical Biology and Therapeutic Sciences, Harvard University, Broad Institute and HHMI, Cambridge, MA, USA

In this lecture I describe the development of precision genome editing technologies that do not require double-strand DNA breaks or donor DNA templates. Through a combination of protein engineering and protein evolution, we developed two classes of base editors (CBE and ABE), proteins that enable all four types of transition mutations (C to T, T to C, A to G, and G to A) to be efficiently and cleanly installed or corrected at target positions in genomic DNA without making double-strand DNA breaks. We also engineered a novel double-strand DNA deaminase discovered by Joseph Mougous's lab into a mitochondrial base editor, enabling the first precision edits in the mitochondrial DNA of living cells. Base editing has been used by laboratories around the world in a wide range of organisms and cell types. By integrating base editors with in vivo delivery strategies, we have addressed animal models of human genetic diseases such as progeria, with strong phenotypic rescue. I will also describe prime editing, a versatile and precise genome editing method that directly writes new genetic information into a specified DNA site using a catalytically impaired Cas9 fused to an engineered reverse transcriptase, programmed with a prime editing guide RNA (pegRNA) that both specifies the target site and encodes the desired edit. We used prime editing to perform >175 edits in human cells including targeted insertions, deletions, and all 12 types of point mutations without requiring double-strand breaks or donor DNA templates. Prime editing offers efficiency and product purity advantages over HDR, complementary strengths and weaknesses compared to base editing, and lower off-target editing than Cas9 nuclease at known Cas9 off-target sites. Prime editing further expands the scope and capabilities of genome editing.

Keywords: Genome Editing, Base Editing, Prime Editing

ENGINEERING EPITHELIAL ORGANOIDS-ON-A-CHIP

Lutolf, Matthias P.

Institute of Bioengineering, EFPL, Lausanne, Switzerland

Organoids form through poorly understood morphogenetic processes in which initially homogeneous ensembles of stem cells spontaneously self-organize in suspension or within permissive three-dimensional extracellular matrices. Yet, the absence of virtually any predefined patterning influences such as morphogen gradients or mechanical cues results in an extensive heterogeneity. Moreover, the current mismatch in shape, size and lifespan between native organs and their in vitro counterparts hinders

their even wider applicability. In this talk I will discuss some of our ongoing efforts in developing organoids-on-a-chip that are assembled within novel hydrogel devices by guiding cell-intrinsic self-patterning through engineered stem cell microenvironments.

Keywords: Organoids, Tissue Engineering, Guided Self-Organization

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

Schroeder, Timm

Dept Biosystems Science & Engineering (D-BSSE), Swiss Federal Institute of Technology Zürich (ETHZ), Basel, Switzerland

Surprisingly many long-standing questions in stem cell research remain disputed. One major reason is the fact that we usually analyze only populations of cells - rather than individual cells – and at very few time points of an experiment – rather than continuously. We therefore develop imaging approaches and software to image, segment, and track cells long-term, and to quantify e.g. divisional history, position, interaction, and protein expression or activity of all individual cells over many days and generations. Live-cell imaging is complemented by novel large-volume multi-color 3D imaging with up to single-molecule sensitivity. Dedicated software, machine learning and computational modeling enable data acquisition, curation, and analysis. Custom-made microfluidics and other hardware devices improve single-cell observation, dynamic manipulation, molecular analysis, and the high-dimensional snapshot 'omics' quantification of individual cells with known history, kinship and dynamics. The resulting continuous single-cell data is used for analyzing the dynamics, interplay and functions of signaling pathway and transcription factor networks in controlling the fate decisions of hematopoietic, pluripotent, and neural stem cells.

Keywords: imaging, single-cell, dynamics

ISSCR TOBIAS AWARD SESSION 13:15 - 13:45 EDT

TURNING THE CLOCK BACK FOR THERAPY OF THE MAJOR HEMOGLOBIN DISORDERS

Orkin, Stuart H.

Harvard Medical School and Howard Hughes Medical Institute, Boston, MA, USA

Globin genes are expressed in both a cell-specific and stage-specific manner. The basis of red cell specification during hematopoiesis and erythroid-specific expression has been explored largely through studies of GATA1, the master transcription factor for the lineage. However, the mechanism(s) of stage-specific expression of globin genes remained elusive until just over a decade ago. The critical switch from fetal-to-adult globin within the human β globin gene cluster has attracted the most attention due to classical studies demonstrating that elevated fetal hemoglobin (HbF, $\alpha2\gamma2$) greatly lessens the severity of β-thalassemia and sickle cell disease (SCD). In this talk I will review how the fetal-to-adult switch is controlled through the repressor protein BCL11A. Proceeding from genome-wide association studies to genome editing, the details of the switch are coming into focus. The translation of these basic discoveries to recent clinical trials has centered on down-regulation of expression of BCL11A via gene therapy and CRISPR/Cas9 editing of an erythroid specific enhancer in the BCL11A gene, culminating in reports of disease-altering treatment of patients with SCD and β-thalassemia. Current challenges and prospects for the future will be discussed.

Keywords: hemoglobin, gene editing, BCL11A

THEME SESSION NT 4 (NEW TECHNOLOGIES) BIOENGINEERING 14:00 - 15:45 EDT REBROADCAST WITH LIVE CHAT 02:00 – 03:45 EDT

IMPACT PLATFORM FOR VASCULARIZED MICROPHYSIOLOGICAL SYSTEMS

Jeon, Noo Li

Mechanical Engineering, Seoul National University, Korea

Recent advances in microfluidic organ-on-a-chip technology have enabled the growth of 3D microphysiological systems (MPS). Although soft lithography based polydimethylsiloxane (PDMS) based microfluidic devices have been widely used, demands for more accessible and standardized devices are required for wider applications. This presentation will introduce a novel injection-molded plastic array 3D culture (IMPACT) platform, a microfluidic system designed for robust patterning of 3D cellular hydrogels. The flexibility of the IMPACT platform enabled custom designs for diverse applications such as angiogenesis, vasculogenesis, vascularized organoids and cancer spheroids. We propose IMPACT as the next generation of 3D microfluidic co-culture platform compatible with biological, clinical, and pharmaceutical investigations requiring robust high-content assays.

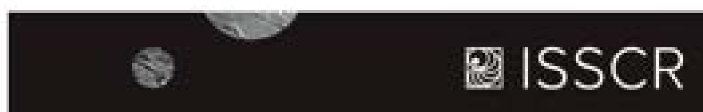
Keywords: organ on a chip, vascularization, micro physiological system

CYBORG ORGANOIDS: MEASURING HUMAN ISLET-WIDE CELL PHYSIOLOGY WITH SOFT IMPLANTED NANOELECTRONICS

Alvarez, Juan R.[1], Li, Qiang[2], Nan, Kewang[2], Kenty, Jennifer[1], Liu, Jia[2], Melton, Douglas[1]

[1]*Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA,* [2]*School of Engineering and Applied Sciences, Harvard University, Cambridge, MA, USA*

Nanoelectronics implanted during organ formation enable organ-wide electrophysiology studies. We created pancreatic islets with integrated soft electronics to investigate how alpha and beta cells communicate to generate whole-islet functions. The "cyborg islets" are assembled by embedding soft, stretchable multielectrode arrays during organogenesis of islets differentiated from human pluripotent stem cells. This enables non-invasive, chronic electrophysiology recordings with single-cell and millisecond resolution over substantial time windows. We show the utility of this approach to trace the spatiotemporal coordination within and between alpha and beta cell populations as they gain functional maturity. Nonuniform activities with clear time latency support islet-wide propagation of local field potentials, revealing a role for alpha cells in synchronizing islet-wide electrophysiological activities to generate coordinated stimulus-coupled hormone responses. Applying STARmap (spatially-resolved transcript amplicon readout mapping) to cyborg organoids with barcoded sensors, we paint a molecular picture of alpha and beta cells within their intact 3D environment, effectively integrating a cell's electrical activity to its gene expression. Finally, we show that functional maturation is marked by increases in the amplitude of membrane depolarization and by synchronization of bursting phases without changes in burst duration or frequency. The approach and results offer a general framework for understanding how human alpha and beta secretory activities evolve and become coordinat-



ed during maturation, and how these properties are lost during diabetes onset and progression.

Funding Source: J.R.A.-D. is an HHMI Fellow of the Life Sciences Research Foundation. D.A.M. is an HHMI investigator. This work was supported by grants from the JDRF, Helmsley Charitable Trust, JPB Foundation, and NSF.

Keywords: Organoids, Diabetes, Nanoelectronics

MACHINE-GUIDED CELL-FATE ENGINEERING

Appleton, Evan M.[1], Tao, Jenhan[2], Ng, Alex[1], Khoshakglagh, Parastoo[1], Church, George M.[1]

[1]*Genetics, Harvard Medical School, Boston, MA, USA,* [2]*Computational Biology, Generate Biomedicines, Boston, MA, USA*

Stem cells are the progenitor cells of all differentiating multi-cellular organisms. In principle, it is possible to differentiate these cells into any other type of cell, which can then be used for many different possible therapeutic or diagnostic applications. The creation of induced pluripotent stem cells (iPSCs) has enabled scientists to explore the derivation of many types of cells. While there are many general approaches for cell-fate engineering, one of the fastest and most efficient approaches is transcription factor (TF) over-expression. Over-expression of specific combinations of TFs is often a reliable method to differentiate stem cells, but since there are at least 1732 transcription factors in the human genome, selecting the right combination to differentiate iPSCs directly into other cell-types is a difficult task. Here were describe a machine-learning (ML) pipeline, called CellCartographer, for using chromatin accessibility next-generation sequencing (NGS) data to produce a multiplex TF pooled-screen for converting stem cells into other cell types. We then describe a barcoded bulk RNA-seq method for refining the set of TFs using iterative NGS experiments. We validate this method by differentiating stem cells into six medically-relevant cell types with the human TFome originating from all germ layers: cytotoxic T-cells, regulatory T-cells, B-cells, microglia, type II astrocytes, and hepatocytes. We demonstrate iterative improvement in differentiation efficiency and functionally characterize the cell lines to validate fast, robust, and functionally accurate differentiation of stem cells into cell types useful for downstream therapeutic and diagnostic pipelines.

Funding Source: This work was funded by the Intelligence Advanced Research Projects Activity (IARPA), via the Army Research Office (ARO) under Federal Award No. W911NF-17-2-0089 and the EGL Charitable Foundation.

Keywords: Machine Learning, Cell-Fate Engineering, Epigenetics

BMP2 SURROGATE USING BISPECIFIC NANOBODIES FOR CARTILAGE REGENERATION

Takematsu, Eri, Zhao, Liming, Wang, Sicong, Wang, Yuting, Arouge, Elizabeth, Longaker, Michael

Surgery, Stanford University, Palo Alto, CA, USA

The most common type of musculoskeletal disorder is osteoarthritis (OA), which afflicts 15% of the adult population with a lifetime risk of 40%. Damaged articular cartilage has no ability to regenerate and extensive cartilage damage often necessitates invasive joint replacements. Stem cell therapy to regenerate damaged articular cartilage would be a highly attractive alternative to invasive joint replacement surgery. Recently, our group identified an effective combination of BMP2 and VEGF antagonism (soluble VEGF receptor) facilitated efficient regeneration of articular cartilage at OA joints after microfracture. These proteins target endogenous skeletal stem cells (SSCs) at the injury site and directed them towards cartilage differentiation. This BMP2-VEGFR protein therapy is very effective, however the efficiency of the recombinant proteins is still low, requiring high dosage. Our team is developing BMP2 surrogate made of two nanobodies (NBs) which have strong affinity to BMP2 receptors, bringing the two receptors to the optimal signaling configuration. NB1 and NB2 have a high affinity to BMPR1a and BMPR2 respectively, thus forcing the receptors to be in proximity, enabling stronger signaling than recombinant BMP2. To generate the BMP2 surrogate, we first synthesized the extracellular domain of BMPR1a and BMPR2 using E. coli with pET 26 vector. Proteins are purified with nickel ion chromatography using His-tag, and expression confirmed by western blot and Coomassie blue. Size chromatography was used to isolate the monomer form of BMPR1a and BMPR2 for nanobody selection. A yeast nanobody library was used to screen the candidate NB1 and NB2. After 6-8 selection rounds, we had a several candidate NBs. These candidate NB1 and NB2 were conjugated by peptide linker, varying the distance between BMPR1a and BMPR2 for the optimal BMP2 signaling configuration. This approach is very unique and has potential to apply to any other ligand for optimizing the efficiency of the protein therapy. In this presentation, the details of fabrication process and functional activities of the BMP2 surrogate will be discussed. Our BMP2 surrogate will serve as an alternative for the conventional BMP2 with better functional activities, ultimately reducing the dosage of treating protein for cartilage regeneration.

Keywords: Nanobody, BMP2 surrogate, Cartilage regeneration

A MICROFLUIDIC ORGANOID PLATFORM FOR STUDYING HUMAN HEART DEVELOPMENT AND FUNCTION

Sachs, David[1], Allen, Nicole[1], Chang, Serena[1], Ebrahim, Tasneem[2], Mayourian, Joshua[1], Nelson, Zachary[1], Van Neste, Camille[1], Dubois, Nicole[2], Turnbull, Irene C.[1], Costa, Kevin D.[1]

[1]*Cardiovascular Research Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA,* [2]*Department of Cell, Developmental, and Regenerative Biology, Icahn School of Medicine at Mount Sinai, New York, NY, USA*

Animal models of embryological development have contributed fundamentally to our understanding of cardiac regeneration and congenital heart disease. However, species-specific effects limit translation of such studies to humans, and although breakthroughs in stem cell biology now provide virtually unlimited supplies of human cells of any type, existing 2D cell culture and 3D tissue engineering techniques do not replicate important anatomical features and developmental milestones essential to cardiac morphogenesis. To address this need, a system was created that aims to reproduce key structural, biophysical and multi-lineage elements of the niche environment to enable investigation of human heart development in vitro. A microfluidic cardiovascular chip (μCVchip) was designed with eight independent micro-circulatory systems recapitulating size constraints and flow patterns of the early embryonic heart tube. Custom robotics were developed to deposit cells at controlled rates while the chip is being incubated and monitored with a microscope. Comprehensive testing of various hydrogel components led to a combination of laminin/entactin, fibronectin, and gelatin as an effective cell encapsulation material. We have demonstrated in situ microfluidic differentiation of healthy iPSCs into multiple cardiac cell types and beating 3D microfluidic cardiac organoids after hydrogel encapsulation in the μCVchip, with 200nm fluorescent beads used to monitor fluid flow patterns and hydrogel organization. Cardiac organoid contraction generated pulsatile flow in the perfusable μCVchip, exposing other cell types to cyclic shear stress. Organoid cell types have been characterized by immunofluorescence and qPCR, and single cell RNA sequencing efforts are ongoing. Another robotic system for characterizing organoid function controls and monitors the μCVchips while in an incubator, and includes a fluorescent microscope for time lapse imaging of organoid morphology and

flow patterns, a tilting platform for generating transient pressure impulses, and a custom cardiac micro-pacing system with platinum electrodes. This µCVchip platform has been selected to fly to the International Space Station in 2022 to study the effects of extended microgravity on cardiovascular organoid development and function.

Funding Source: This work was support by NIH (R21 EB023573) and NSF-CASIS (1929028)

Keywords: Microfluidic, Cardiac, Organoid

NANOPATTERNED SCAFFOLDS AUGMENT SURVIVAL OF HUMAN IPSC-DERIVED ENDOTHELIAL CELLS IN THE MURINE ISCHEMIC LIMB

Huang, Ngan F.[1], Alcazar, Cynthia[2], Hu, Caroline[2], Paukshto, Michael[3], Yang, Guang[1], Zaitseva, Tatiana[3]

[1]*Cardiothoracic Surgery, Stanford University, Stanford, CA, USA*, [2]*Research and Development, Veterans Affairs Palo Alto Health Care System, Palo Alto, CA, USA*, [3]*Research and Development, Fibralign Corporation, Union City, CA, USA*

Human induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) are a promising cell type for treatment of peripheral arterial disease (PAD), but poor post-transplantation survival is a major limitation. This study aims to quantitatively assess the role of topographical cues from nanopatterned collagen scaffolds on iPSC-EC organization and survival, for improved survival post-transplantation. In this work, we examined the effect of nanofibrillar collagen dimensions and nanopatterning on iPSC-EC spatial organization and survival in a murine model of PAD. Parallel-aligned nanofibrillar collagen scaffolds of low (100 nm) or high (200 nm) nanofibril diameters were prepared by tuning the ionic strength of monomeric collagen I and then inducing fibrillogenesis in the presence of shear. Human iPSC-ECs were seeded onto the aligned nanofibrillar scaffolds for quantification of cellular organization based on immunofluorescence staining of F-actin. Signaling pathways mediating the process were elucidated using RNA Sequencing. The regenerative potential of iPSC-EC-seeded scaffolds was further examined in a mouse hind limb ischemia model. Our results showed that parallel-aligned scaffolds with 100 nm or 200 nm fibrils reorganized the endothelial cytoskeleton along the direction of nanofibrils, whereas cells on randomly oriented scaffolds depicted a random orientation. The iPSC-ECs-seeded scaffolds were implanted acutely to the site of murine hindlimb ischemia, and bioluminescence imaging demonstrated markedly higher cell survival in the ischemic limb when seeded on aligned nanofibrillar scaffolds with 200 nm fibril diameter, compared to on randomly oriented scaffold or when delivered in saline. To assess underlying mechanisms, RNA Sequencing revealed that aligned scaffolds promoted the positive regulation of signaling pathways related to cell-substrate adhesion, cytoskeletal adhesion, and focal adhesion assembly, compared to randomly oriented scaffolds. In conclusion, aligned collagen nanofibrillar scaffolds with 200 nm fibrils promote iPSC-EC survival and alignment along the fibril direction. This study provides new insight into the role of biophysical cues in the survival of iPSC-ECs and has important implications in cell therapy for treatment of PAD.

Funding Source: National Institutes of Health

Keywords: angiogenesis, peripheral arterial disease, endothelial cells

IN VITRO VASCULARIZATION OF HUMAN KIDNEY AND CARDIAC TISSUES

Lewis, Jennifer A.

Bioengineering, Wyss Institute, Harvard University, Cambridge, MA, USA

Recent protocols in developmental biology are unlocking the potential for stem cells to undergo differentiation and self-assembly to form "mini-organs", known as organoids. To bridge the gap from organoid building blocks (OBBs) to therapeutic functional tissues, integrative approaches that combine bottom-up organoid assembly with top-down bioprinting are needed. While it is difficult, if not impossible, to imagine how either organoids or bioprinting alone would fully replicate the complex multiscale features required for organ-specific function – their combination may provide an enabling foundation for de novo tissue manufacturing. My talk will begin by describing our recent efforts to generate microvascularized organoids in vitro that exhibit enhanced maturation and function. Next, I will describe the generation of 3D vascularized organ-specific tissues by assembling OBBs into living matrices that support embedded printing of macro-vessels by a process known as sacrificial writing in functional tissue (SWIFT). Though broadly applicable, I will highlight our recent work on kidney and cardiac tissue engineering.

Funding Source: NIDDK (Re)Building a Kidney Consortium NCATS Tissue Chips 2.0 (4UH3TR002155-03) NSF CELL-MET ERC-1647837 ONR NSSEFF (N000141612823) Wyss Institute Organ Engineering Initiative

Keywords: organoids, vasculature, biomanufacturing

THEME SESSION TSC 4 (TISSUE STEM CELLS AND REGENERATION) HEMATOPOIETIC STEM CELLS 14:00 - 15:45 EDT REBROADCAST WITH LIVE CHAT 02:00 – 03:45 EDT

REGULATION OF HEMATOPIETIC AND LEUKEMIC STEM CELLS

Trumpp, Andreas

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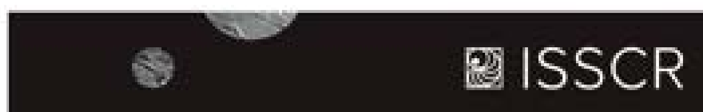
Hematopoietic stem cells (HSCs) are characterized by their self-renewal potential associated to dormancy. Entry and exit from dormancy are mediated by MYC activity, but the niche mediated signals controlling HSC dormancy remains poorly understood. We have recently identified Netrin-1 produced by arteriolar endothelial and periarteriolar stromal cells as a ligand activating the Neogenin-1 (Neo1) receptor. Neo1 is specifically expressed on dormant HSCs and Netrin-1::Neo1 interaction leads to EGR1 expression and promotion of a dormant state. Ageing associated bone marrow remodelling leads to the decline of Netrin-1 expression in niches and a compensatory but reversible upregulation of Neogenin-1 on HSCs. In summary, niche produced Netrin-1 preserves HSC quiescence and self-renewal via Neogenin-1 function and its decline in the bone marrow during ageing leads to the gradual decrease of Neo1 mediated HSC self-renewal. In addition, quiescent and active states of HSCs are controlled by differential Alternative Polyadenylation (APA). Upon transition of HSCs from quiescence to proliferation as well as during differentiation there is an overall shortening of their 3'-UTRs. Specifically, APA regulates the rewiring of the metabolic network in HSCs upon exit from quiescence for example by Glutaminase (Gls) isoform switching, which is required for the proper stress response of quiescent HSCs. Our data establish APA as a critical layer of regulation orchestrating HSC self-renewal, state and commitment.

Keywords: Hematopoietic Stem Cell, Leukemic Stem Cell, Netrin1-Neo1



DICHOTOMOUS REGULATION OF LYSOSOMES BY MYC AND TFEB CONTROLS HEMATOPOIETIC STEM CELL FATE

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Human long-term hematopoietic stem cells (LT-HSC), at the apex of the hematopoietic hierarchy, must meet enormous daily demand (~1011 cells) while also sustaining life-long maintenance of the stem cell pool. This hierarchical organization is widely thought to protect LT-HSC from exhaustion by their maintenance in a quiescent state, activating only in response to microenvironment signals to generate highly proliferative but more short-lived populations including short-term HSC and committed progenitors. Upon cues to exit quiescence, HSC must respond and adapt their metabolism and nutrient uptake to meet increased bioenergetic demands for cell growth and differentiation. Simultaneously, the events underlying cellular and metabolic activation must also be suppressed within a subset of LT-HSC to enable re-entry to quiescence and maintaining the LT-HSC pool through self-renewal. However, the demand-adapted regulatory circuits of these early steps of hematopoiesis are largely unknown. Sensing signals or nutrient uptake depends on proteins that are embedded within the plasma membrane. These proteins internalize through endocytosis and can be degraded in the lysosomes or rerouted back to the cell surface and reused. However, little is known about the regulation and role of the endolysosomal system in the stem cell context. Here, we describe the unexpected finding that lysosomes, whose activity is intricately balanced by TFEB and MYC, are instrumental for regulating the stemness and differentiation properties of human LT-HSC. We found that TFEB induces a constitutive lysosomal flux in unperturbed LT-HSC that actively maintains quiescence, preserves self-renewal and governs lineage commitment. These effects are mediated by endolysosomal degradation of membrane receptors, such as the transferrin receptor 1, pointing to a role for TFEB in coordinating how LT-HSC sense environmental changes and initiate the earliest steps of their fate and lineage commitment decisions. These transitions are regulated by a TFEB/MYC dichotomy where MYC is a driver of LT-HSC anabolism and activation and counteracts TFEB function by serving as a negative transcriptional regulator of lysosomes. Collectively, our study identifies lysosomes as a central regulatory hub for proper and coordinated stem cell fate determination.

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Keywords: Lysosomes, long-term HSC, Stemness

ADULT HEMATOPOIETIC STEM CELL CLONAL CONTRIBUTION IS DETERMINED BY MACROPHAGE SENSING OF CALRETICULIN 3 ON HEMATOPOIETIC STEM CELLS DURING DEVELOPMENT

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During development, local signals and cell-cell interactions induce the emergence and expansion of a set of hematopoietic stem cell and progenitor cells (HSPCs) responsible for life-long hematopoiesis. Using spinning disk confocal microscopy, we followed runx1+23:mCherry+ HSPCs through early zebrafish development and identified intimate interactions with mpeg1:GFP+ macrophages in the fetal niche. By tracking individual cells over time, we found that 30% of HSPCs in the niche experienced sustained physical contact with macrophages and were either fully engulfed or had a fragment of cytoplasm removed. Using the transgenic cell cycle reporter FUCCI, we found HSPCs in G2M phase were more likely to interact with macrophages (60% of FUCCI+ vs 17% of FUCCI- HSPCs) and frequently completed mitosis shortly afterwards. To evaluate how these interactions may affect HSC clonality, we depleted embryonic macrophages in a brainbow color barcoding system and raised fish to adulthood. Transient macrophage depletion in embryos with either the irf8 morpholino or clodronate liposome injection significantly reduced the average number of HSC clones in adulthood compared to sibling controls (14 vs 24.6 clones ($p = 0.0002$)). To molecularly characterize macrophage interactions, we performed few-cell proteomics. This identified 166 peptides enriched in the macrophages which had recently taken material from HSPCs, including three isoforms of calreticulin (calr, calr3a, and calr3b). Though normally an ER-bound chaperone protein, calr also acts as a 'come-eat-me' signal for nearby phagocytes when displayed on the cell surface. Single-cell RNA-seq also revealed expression of lrp1ab, the canonical surface calr receptor, specifically in recently-engaged macrophages. Morpholino knockdown of calr3a or calr3b reduces macrophage-HSPC interactions by up to 2-fold ($p = 0.0008$), and HSPCs overexpressing a non-ER bound form of calr3a are 4-fold more likely to engage macrophages ($p < 0.0001$). Morpholino knockdown of calr3a in brainbow embryos also reduced hematopoietic clonality (15.6 vs 19.7 clones ($p < 0.0001$)). Together our data support a model in which newly-formed HSCs display surface calr3a for macrophages, leading to either engulfment and apoptosis, or grooming and cell division, thereby shaping lifelong HSC clonality.

Keywords: Hemtapoiesis, Clonality, Live Imaging

PRENATAL INFLAMMATION PERTURBS FETAL HEMATOPOIESIS AND DRIVE PERSISTENT CHANGES TO POSTNATAL IMMUNITY

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During development, transient fetal hematopoietic stem cells (HSCs) are responsible for the production of "unconventional" innate-like immune cells that persist into adulthood and contrib-

ute to adult immunity. Dysregulation of fetal-derived immune cells contributes to pathogenesis in a variety of immune tolerance disorders, but the cellular and molecular drivers of pathogenesis are unknown. We have previously identified a transient developmentally-restricted HSC (drHSC) that specifically gives rise to innate-like lymphocytes during the perinatal period. Under homeostatic conditions, the drHSC disappears postnatally, but its innate-like lymphocyte progeny persist into adulthood. Our discovery of a transient cell-of-origin for a specialized component of adult immunity underscores a "critical window" of immune development, during which phenotype of the adult immune system can be shaped by extrinsic inputs in early life. Here we tested, for the first time, how fetal HSCs respond to prenatal inflammation during development, and how this shapes hematopoiesis and immunity postnatally. We used a maternal immune activation (MIA) model, in which an immune response to viral infection during pregnancy is mimicked by injection of the viral mimetic poly(I:C) at mid-gestation. Prenatal inflammation disrupted the entrance of fetal HSCs into quiescence, causing disproportionate expansion of drHSCs, and a resultant shift in multipotent progenitor output. Single-cell sequencing and lineage tracing of cytokine-induced response of fetal hematopoietic stem and progenitors also revealed the sensitivity of specific populations to prenatal inflammation. Postnatally, we observed sustained expansion and inappropriate persistence of the drHSC population into adulthood. These fundamental changes to the postnatal HSC compartment resulted in parallel expansion and hyperactivation of innate-like lymphocytes, thereby altering immune landscape and function in offspring. Our work validates the existence of a critical window of hematopoietic and immune development by revealing how early perturbation of distinct fetal HSCs can drive long-term changes to immunity and disease susceptibility in offspring.

Funding Source: NIH/NHBLI award K01HL130753 to AEB, the Pew Biomedical Scholars award to AEB, and the Hellman Fellows Award to AEB.NIH/NIDDK R01DK100917 to ECF; and Max Planck Society and the ERC-Stg-2017 (VitASTEM) Research to NC-W.

Keywords: hematopoiesis, development, inflammation

MITOCHONDRIAL DYNAMICS REGULATE INTERFERON SIGNALING AND AGE-RELATED CHANGES IN HSPC

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Blood cells are predominantly short-lived and hematopoietic stem cells (HSCs) are required throughout life to replenish multilineage and more committed precursors, ultimately giving rise to mature blood cells. Despite major advances in our understanding of the biology of the hematopoietic system, numerous critical issues remain to be addressed. Some of those are the mechanisms underlying the aging of hematopoietic stem and progenitor cells (HSPCs). With age, HSPCs expand and become more myeloid biased, leading to immune impairment and increased risk of clonal hematopoiesis and myeloid malignancies. This work is based on the finding that Mitofusin 2 (MFN2), a protein involved in mitochondrial fusion, is required for the maintenance of HSCs with extensive lymphoid potential and for overall HSC quiescence. Here we show that deletion of Mfn2 causes an 'aged' HSPC phenotype in young mice, with relative expansion of the stem and progenitor cell compartment in both medullary and extra-medullary compartments, with exacerbated loss of lymphoid potential during aging. RNAseq combined with single cell-RNAseq experiments revealed striking up-regulation of Interferon stimulated genes in HSCs as well as whole bone marrow (BM) and spleen cells from Mfn2-/- knock-out (KO) mice compared to wild-type (WT). Detectable IFNa was furthermore found in the serum of Mfn2-/- mice. Moreover, we found that Mfn2 does not functionally interact with Mitochondrial activator of viral signaling (Mavs), a central mediator of innate immunity signaling associated with mitochondria. Instead, double deletion of Mfn2 and Stat1 or the type I interferon receptor fully rescued the effect of Mfn2 deletion on HSPC cycling. Finally, aged Mfn2-deleted BM has decreased hematopoietic regenerative function compared to WT with a full rescue deleting Stat1 together with Mfn2. Our data indicate that MFN2 is required to dampen tonic IFN signaling and production and may play a role in the development of age-related hematopoietic malignancies. They raise the question where and how MFN2 interferes with IFNa induction in the hematopoietic system. Taken together, these findings suggest that manipulating mitochondrial dynamics might constitute an approach to alleviate some aspects of aging, myeloproliferative diseases as well as genetic interferonopathies.

Funding Source: Cancer Research Institute Irving Post-doctoral Fellowship 2020

Keywords: Hematopoietic stem cells, Immune system, Aging

OVERLAPPING DEFINITIVE PROGENITOR WAVES DIVIDE AND CONQUER TO BUILD A LAYERED HEMATOPOIETIC SYSTEM

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Adult innate immune cells are part of a layered hematopoietic system constructed from definitive hematopoietic stem and progenitor cells (HSPC) with diverse origins during development. One source of HSPC are fetal hematopoietic stem cells (HSC) that provide long-term reconstitution throughout life. However, the extent to which HSC produce mature cells in utero is only recently being uncovered. This is in part due to the added complexity of an overlapping wave of definitive progenitors that derive from yolk sac erythro-myeloid progenitors (EMP). HSC and EMP are generated from spatiotemporally distinct hemogenic endothelia, yet they both migrate to the fetal liver niche where they co-habitate and are presumed to reach their full potential. Delineation of the respective HSC and EMP pathways towards developmental immune cell differentiation has been confounded by challenges in ontogeny-specific cell labeling. In this study, in vivo inducible pulse chase labeling revealed that HSC contribute little to fetal myelopoiesis and that EMP are the predominant source of mature myeloid cells until birth. This is similar to what has been reported for the erythroid branch of hematopoiesis thereby establishing a developmentally-restricted privilege for erythro-myeloid differentiation from EMP compared to HSC. Tracing the origins of mature cells to the progenitor level by immunophenotyping and single cell RNA sequencing uncovered a dichotomy in the allocation of fetal liver EMP and HSC to myeloid progenitor subsets, both in timing and lineage bias. This has exposed an uncoupling between developmental granulopoiesis and monopoiesis from EMP and HSC pathways, and provides a framework for future studies of HSC-dependent and -independent hematopoiesis.

Keywords: erythro-myeloid progenitors, fetal liver, developmental hematopoiesis

STEM CELL CLONALITY AND THE NICHE

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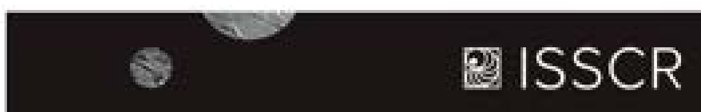
Hematopoietic stem cell transplantation involves the homing of stem cells to the marrow, an active process of engraftment, and the self-renewal of the blood stem cells. We have been using the



zebrafish as a model to study the molecular biology of this process. Blood stem cells are born in the dorsal aorta of the developing embryo. By imaging RUNX1 GFP+ cells arriving in the next site of hematopoiesis (the caudal hematopoietic territory), engraftment can be visualized. The endothelial cells of the niche cuddle the hematopoietic stem cells, and the stem cells divide in the niche. We have used RNA tomography to find transcripts that are restricted in expression to the niche endothelial cells. After sorting these endothelial cells, we used ATAC seq to reveal regions of chromatin that are open. The regions function in enhancer assays to drive expression of GFP in the niche. By computing the binding sites in these regions, and examining RNA seq, we found transcription factors that may participate in niche endothelial development. Overexpression of three of these transcription factors is sufficient to reprogram embryonic cells to express the markers of the niche. New niches form in ectopic locations and HSPCs arrive in the new niche, akin to extramedullary hematopoiesis, are cuddled by endothelial cells and divide. Using a brainbow color barcoding system, we previously demonstrated that zebrafish produce 20-30 HSCs from the developing aorta. HSCs traffic to the embryonic niche where they exit circulation and divide. To evaluate a possible role of macrophages in attenuating HSC clone number, we depleted macrophages in our brainbow barcoding system. Unique color barcodes were induced in individual HSCs at 24 hpf, just prior to stem cell emergence, and clodronate loaded liposomes injected into circulation. On average, animals injected with clodronate liposomes had only 14 HSC color clones, compared to 24.6 HSC clones in sibling controls ($p = 0.0002$). Our studies show that there is a transcriptional code for niche endothelial cells and that macrophages are required for establishing clonality.

Keywords: clonality, niche, hematopoiesis

THURSDAY, JUNE 24

THEME SESSION MDD 1(MODELING DEVELOPMENT AND DISEASE) AGING
7:30 - 9:15 EDT
REBROADCAST WITH LIVE CHAT 16:30 - 18:15 AND 19:30 - 21:15 EDT

STEM CELL BASED HETEROGENEITY OF INTERFERON SIGNALING IN THE HEMATOPOIETIC SYSTEM

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Inflammation or infection has a great impact on an organism. In order to protect the hematopoietic system from exhaustion during pathogenic insult, heterogeneity in metabolic activity, gene expression patterns, differentiation capacity, and responsiveness to cytokines such as interferons (IFN) have been revealed. However, how this diversity in the system is generated and maintained remains poorly understood. Here, we show that intra-cell type heterogeneity in expression of IFN-stimulated genes (ISGs) is already established at the level of the hematopoietic stem cell (HSC). Transplantation experiments using different reporter mouse models for ISG expression indicated stably inheritance of the ISG expression to the downstream progeny. Single cell transplantation experiments of wild type HSCs supported these findings, proposing a stable inheritance of IFN signaling heterogeneity from HSCs to mature blood cells. To investigate the origin of Interferon signaling heterogeneity we have analyzed ISG expression in stem cells during development. From a first streak of primitive hematopoiesis early in embryonal development to the expansion of definitive HSCs in the fetal liver and the homing in the bone marrow around birth, HSCs pass through a number of embryonal organs. Fetal liver HSCs at E13.5 already showed ISG expression heterogeneity, comparable to the adult stem cells. However, at earlier time points during development the degree of ISG expression was depending on the time point and organ the cells were isolated from. In both wild type and reporter mice the highest degree of ISG expression was found in the placenta. These data suggest an important role for the placenta during establishment of the heterogeneity. Furthermore, transplantation experiments confirmed inheritance of the stable ISG expression established during development. Thus, our data suggest that heterogeneity of IFN signaling in the hematopoietic system, stably inherited from stem cells to progeny, is already established early during hematopoietic development. Uncovering the origin and function of the heterogeneity will have far-reaching implications for HSC biology in health, disease, and upon infection.

Keywords: Hematopoietic stem cells, Inflammation, Interferon

IDENTIFICATION OF AN AGE-RELATED PARKINSON'S DISEASE RISK FACTOR WHICH REGULATES SULFUR METABOLISM

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Aging is the main risk factor for Parkinson's disease (PD). Despite the research into the mechanisms leading to cell death in PD, the fundamental age-related factors causing the development of this disease remain unclear. To better understand age-related pathogenesis, we reprogrammed human fibroblasts into dopaminergic neurons (iDA) for modeling. With transcriptome analysis, we identified 240 aging-related genes. By comparing healthy and PD donor-derived iDA we identified 52 sporadic PD-related genes and 61 genetic PD-related genes. The aging and disease comparisons only share one gene in common, TSTD1, which is upregulated. This gene codes for a protein that catalyzes the metabolism of glutathione. To validate TSTD1 expression in human brains using immunochemistry, we acquired postmortem tissues of substantia nigra from donors aged from 0-83 years. We observed an age-dependent up-regulation of TSTD1 in healthy donors. In PD donors, TSTD1 expression level were several folds higher than healthy donors, which suggests that up-regulation of TSTD1 is age and disease-associated in the human brain. Forced expression of TSTD1 in dopaminergic (DA) neurons led to a depletion of cellular glutathione. By analyzing the amino acid metabolome, we found that levels of cysteine, the precursor amino acid for glutathione synthesis, decreased upon TSTD1 overexpression. This is strong evidence pointing to a shift in cellular thiol metabolism equilibrium caused by TSTD1. Cysteine metabolism has a prominent impact on cellular H2S levels. In iDA derived from aged and PD donor fibroblasts, we found a lower cellular H2S level and mitochondrial membrane potential (MMP). The same phenomena were also observed on iPSC-derived DA neurons with TSTD1 overexpression, suggesting that H2S metabolism and MMP are also altered by TSTD1. This phenotype is further validated with a shRNA-based loss of function experiment in an iDA model that successfully rescued the H2S level and MMP decrease in the PD iDA groups. Our results suggest that TSTD1 is an age-related PD risk factor. The protein depletes glutathione, which alters sulfur metabolism and mitochondrial function in neurons. Additional characterization of TSTD1 in animal models should further clarify the mechanisms of age-dependent neural degeneration due to chronic glutathione depletion.

Funding Source: JPB Foundation

Keywords: reprogramming, aging, Parkinson's disease

CPEB4 REGULATES MOUSE MUSCLE STEM CELL FUNCTION DURING AGING BY MODULATING MITOCHONDRIAL PROTEOMIC LANDSCAPE AND ACTIVITY

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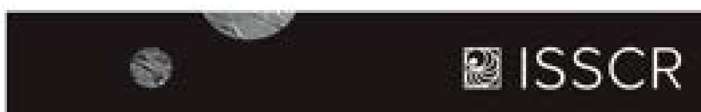
Age-associated impairments in stem cell function correlate with the decline in somatic tissue regeneration capacity after diseases or tissue injury. The mitochondria activity is essential for stem cell homeostasis and function. It is thus crucial to understand how mitochondria activity affects stem cell function during aging. Using skeletal muscle stem cells, or satellite cells (SCs), we first illustrated an initial blueprint of the proteomics landscape of SCs during aging. The senescence-associated proteins are significantly upregulated in aged SCs. In contrast, the transcription and translation-related proteins are downregulated, suggesting a change in the maintenance of basal stem cell cellular activity during aging. Moreover, this initial blueprint indicates that a subset of mitochondrial proteins is downregulated in aged SCs. Functional analysis suggests that these proteins are enriched in fatty acid degradation and the oxidative phosphorylation pathways. The Seahorse mitochondria activity assay revealed that mitochondrial respiration is dramatically decreased, indicating that the energy production process is impaired in aged SCs. Intriguingly, we identified a translation regulator, termed Cytoplasmic Polyadenylation Elements Binding protein 4 (CPEB4), functions to regulate mitochondrial proteomic landscape and activity in SCs. Further analysis demonstrated that CPEB4 regulates SC function in myogenic lin-



eage progression and muscle regeneration. Importantly, CPEB4 targets the Idh1 transcripts and regulates its protein expression in SCs. We found that Idh1 is strongly expressed during SC activation and is required for SC activation and proliferation. Idh1 overexpression in CPEB4 knockout SCs or aged SCs partially rescued the SC function. Altogether, our study suggests that CPEB4 is a crucial regulator for mitochondria activity modulation during aging and sheds light on its therapeutic potential for the treatment of age-associated muscle regeneration impairment.

Funding Source: Funding source: Hong Kong Research Grant Council (GRF16102319, GRF16102420, C6018-19G, C6027-19G, AoE/M-604/16, T13-605/18W), Lee Hysan Foundation (LHF17SC01), Croucher Innovation Award (CIA14SC04) from Croucher Foundation.

Keywords: Stem cell aging, Mitochondria proteome and activity, CPEB4

HYALURONIDASE-1-MEDIATED GLYCOCALYX IMPAIRMENT UNDERLIES ENDOTHELIAL ABNORMALITIES IN POLYPOIDAL CHOROIDAL VASCULOPATHY

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Polypoidal choroidal vasculopathy (PCV) is characterized by polyp-like aneurysmal dilations of blood vessels and variable blood flow in the choroid of the eye. Our understanding of the pathogenesis of PCV has been hampered by limitations of current animal models. Here, we cultivated human blood outgrowth endothelial cells (BOECs) and exposed them to heterogeneous flow generated by an orbital rotator. Single-cell profiling revealed that PCV patient-derived BOECs preferentially adopted an angiogenic-migration cell state, while healthy control BOECs undertook a proinflammatory cell state in response to heterogeneous flow. Gene set enrichment showed that PCV BOECs had downregulated cellular response to stress but upregulated processes governing extracellular matrix organization. Functionally, PCV BOECs demonstrated greater migratory capacity and reduced barrier permeability. We uncovered that hyaluronidase-1 was significantly enriched in PCV BOECs. Inhibition of hyaluronidase-1 decreased degradation of hyaluronic acid, a major component of glycocalyx which interfaces between flow stresses and vascular endothelium. Mechanosensitivity of PCV BOECs was hence restored by hyaluronidase-1 inhibition, leading to normalization of the level of Krüppel-like factor 2, a flow-responsive transcription factor, which in turn modulated PCV BOEC migration. This was corroborated by higher amounts of hyaluronidase-1 in PCV plasma samples than in vitreous humor samples. Therefore, choroidal vessels may be susceptible to hyaluronidase-1-mediated degradation of glycocalyx lining the luminal surface of endothelial cells. Our findings present hyaluronidase-1 as a novel therapeutic modality in preserving glycocalyx integrity and endothelial stability in one of the most common causes of age-related vision impairment.

Funding Source: Academic Research Fund (MOE2018-T2-1-042) from the Ministry of Education, Singapore, and the SERI-IMCB Program in Retinal Angiogenic Diseases grant (SPF2014/002) from A*STAR Singapore.

Keywords: Endothelial dysfunction, Polypoidal choroidal vasculopathy, Hyaluronidase-1

INHIBITION OF LONGEVITY REGULATOR PAPP-A MODULATES TISSUE HOMEOSTASIS VIA RESTRAINT OF MESENCHYMAL STROMAL CELLS

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Pregnancy-associated plasma protein-A (PAPP-A) is a secreted metalloprotease that increases insulin-like growth factor (IGF) availability by cleaving IGF-binding proteins. Reduced IGF signaling extends longevity in multiple species, and consistent with this, PAPP-A deletion extends lifespan and healthspan; however, the mechanism remains unclear. To clarify PAPP-A's role, we developed a PAPP-A neutralizing antibody and treated adult mice with it. Transcriptomic profiling across tissues showed that anti-PAPP-A reduced IGF signaling and extracellular matrix (ECM) gene expression system wide. The greatest reduction in IGF signaling occurred in the bone marrow, where we found reduced bone, marrow adiposity, and myelopoiesis. These diverse effects led us to search for unifying mechanisms. We identified mesenchymal stromal cells (MSCs) as the source of PAPP-A in bone marrow and primary responders to PAPP-A inhibition. Mice treated with anti-PAPP-A had reduced IGF signaling in MSCs and dramatically decreased MSC number. As MSCs are (1) a major source of ECM and the progenitors of ECM-producing fibroblasts, (2) the originating source of adult bone, (3) regulators of marrow adiposity, and (4) an essential component of the hematopoietic niche, our data suggest that PAPP-A modulates bone marrow homeostasis by potentiating the number and activity of MSCs. We found that MSC-like cells are the major source of PAPP-A in other tissues also, suggesting that reduced MSC-like cell activity drives the system-wide reduction in ECM gene expression due to PAPP-A inhibition. Dysregulated ECM production is associated with aging and drives age-related diseases, and thus, this may be a mechanism by which PAPP-A deficiency enhances longevity.

Funding Source: Calico Life Sciences

Keywords: Aging, Mesenchymal stromal cell, Hematopoiesis

PERSISTENT NF-KB ACTIVATION IN MUSCLE STEM CELLS INDUCES PROLIFERATION-INDEPENDENT TELOMERE SHORTENING

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During the repeated cycles of damage and repair in many muscle disorders, including Duchenne muscular dystrophy (DMD), the muscle stem cell (MuSC) pool becomes less efficient at responding to and repairing damage. The underlying mechanism of such stem cell dysfunction is not fully known. Here, we demonstrate that the distinct early telomere shortening of diseased MuSCs in both mice and young DMD patients is associated with aberrant NF-κB activation. Mechanistically, we discovered that prolonged NF-κB activation in MuSCs in chronic injuries leads to shortened telomeres, Ku80 dysregulation and results in severe skeletal muscle defects. Our studies provide evidence of a previously unrecognized role for NF-κB in regulating stem cell-specific telomere length, independently of cell replication, and could be a congruent mechanism that is applicable to additional tissues and/or diseased characterized by systemic chronic inflammation.

Keywords: Muscle stem cells, muscular dystrophy, telomere biology

MECHANISMS OF NEURAL STEM CELL AGING

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Aging is accompanied by a decline in the regenerative potential of most tissues. The mammalian brain contains regenerative neurogenic niches composed of neural stem cells (NSCs), neural progenitors, and other cells, including microglia, and endothelial cells. Neurogenic niches become less functional with increasing age. This deterioration could underlie cognitive and sensory restriction with age, although the exact age at which it occurs is still debated in humans. How the neurogenic niche changes during aging, and whether new cell types arise in older individuals, is not known. Our lab has embarked on a global characterization of the neurogenic niche during aging. This work provides a global understanding of the old neurogenic niche and suggests possible cause for NSC decline during aging. Results from these studies could open new avenues to counter age-related decline in the neurogenic niche and brain aging.

Keywords: aging, neural stem cells, neurogenesis

THEME SESSION CI 1 (CELLULAR IDENTITY) METABOLISM AND CELL IDENTITY 7:30 - 9:15 EDT REBROADCAST WITH LIVE CHAT 16:30 - 18:15 AND 19:30 - 21:15 EDT

HIRA AS A PHENOTYPE INHERITANCE BIOMARKER IN A MOUSE MODEL OF TRANSGENERATIONAL EPIGENETIC INHERITANCE

Watson, Erica D.[1], Blake, Georgina[1], Zhao, Xiaohui[1], Yung, Hong Wa[1], Burton, Graham J.[1], Ferguson-Smith, Anne C.[2], Hamilton, Russell S.[2]

[1]*Physiology, Development, and Neuroscience, University of Cambridge, UK,* [2]*Genetics, University of Cambridge, UK*

Transgenerational epigenetic inheritance (TEI) is when an environmental stressor induces a phenotype that is inherited for several generations, even in the absence of the original stressor. While the mechanism behind TEI is unclear, particularly in mammals, it occurs independent of changes to the DNA base sequence and likely involves the inheritance of an epigenetic factor via the germline. We previously showed that disruption of folate metabolism in mice by the Mtrr^gt hypomorphic mutation results in TEI of congenital malformations (e.g., neural tube, heart and placenta defects) at midgestation. Either maternal grandparent can initiate this phenomenon, which persists for at least four wildtype generations. Folate metabolism is important for transmitting methyl groups for all methylation reactions in the cell, and thus is directly linked to epigenetic regulation. We used a genome-wide approach to reveal genetic stability in the Mtrr^gt model, which improves confidence in exploring an epigenetic mechanism. Furthermore, we showed epigenome-wide differential DNA methylation in the germline of Mtrr+/gt maternal grandfathers. While epigenetic reprogramming occurs, wildtype grandprogeny and great grandprogeny exhibit transcriptional memory of germline methylation defects. One region encompasses the Hira gene, which is misexpressed in embryos at least until the F3 generation in a manner that distinguishes Hira transcript expression as a biomarker of phenotypic inheritance.

Keywords: Epigenetic inheritance, Folate metabolism, Congenital malformations

METABOLIC REPROGRAMMING DURING EARLY EMBRYOGENESIS REGULATES 2-HG/A-KG HOMEOSTASIS TO PROMOTE ERASURE OF HISTONE METHYLATION

Zhao, Jing[1], Yao, Ke[2], Yu, Hua[3], Zhang, Ling[1], Xu, Yuyan[1], Xie, Wei[3], Hu, Zeping[2], Zhang, Jin[1]

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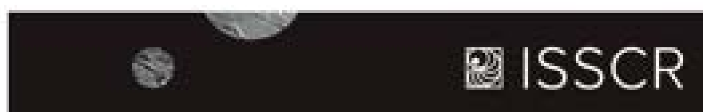
During early mammalian embryogenesis, cell growth and proliferation are dynamically changed and tightly linked to the underlying genetic and metabolic regulation. However, our understanding of metabolic reprogramming and its impact on epigenetic regulation in early embryo development remains elusive. Here, we profiled metabolomes of embryos from the 2-cell and blastocyst stages, and their in vitro counterpart 2-cell like cells and ES cells, and reconstructed their metabolic landscape through the transition from totipotency to pluripotency. Our integrated metabolomics and genomics analysis showed that 2-cell embryos favor methionine, polyamine and phosphatidylinositol metabolism and stay in a more reductive state, whereas blastocyst embryos and ES cells mainly use the mitochondrial TCA cycle and are in a more oxidative state. Moreover, we identify a reciprocal relationship between α-ketoglutarate (α-KG) and its competitive inhibitor L-2-hydroxyglutarate (L-2-HG), namely, higher L-2-HG in the 2-cell embryos inherited from oocytes and 1-cell zygotes, and higher a-KG in the blastocyst. Supplementing 2-HG or knocking down L2hgdh, a gene encoding the 2-HG consuming enzyme L-2-hydroxyglutarate dehydrogenase impeded erasure of global histone methylation markers. Together, our data demonstrate dynamic and interconnected metabolic, genetic and epigenetic network remodeling during murine early embryo development.

Keywords: embryogenesis, metabolic reprogramming, L-2-hydroxyglutarate



SENESCENCE SUPPRESSION TO IMPROVE MATURATION OF STEM CELL-DERIVED CARDIOMYOCYTES

Garbern, Jessica, Elwell, Hannah, Mancheno Juncosa, Estel, van den Berg, Daphne, Escalante, Gabriela O., Sokol, Morgan K., Aoyama, Junya, Lee, Richard T.

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

Inadequate maturation of induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) may be a major barrier to clinical translation, as delivery of immature cardiomyocytes increases arrhythmogenic risk. Our group recently identified that inhibition of the mechanistic target of rapamycin (mTOR) pathway improves maturation of iPSC-CMs in two-dimensional (2D) culture via induction of p53-mediated cellular quiescence and suppression of cellular senescence. Cellular quiescence is a resting state triggered by nutrient deprivation characterized by the ability to re-enter the cell cycle in response to appropriate stimuli. In contrast, cellular senescence is a state of irreversible cell cycle arrest associated with an aging or diseased phenotype, characterized by DNA damage, elevated reactive oxygen species levels, and a senescence-associated secretory phenotype (SASP) that can lead to a pro-inflammatory state in nearby cells. Because SASP can have detrimental effects on nearby cells due to a paracrine effect, we tested whether removal of senescent cells with a senolytic can enhance the maturation of surviving cells. The senolytic, quercetin, is a flavonol found in foods such as apples that can induce apoptosis in senescent tumor cells. We treated three-dimensional (3D) iPSC-CM spheroids with 200 μM quercetin for 2-5 days starting 2 days after onset of beating. We found that treatment with quercetin for 5 days increased expression of p53 and also decreased levels of reactive oxygen species, suggestive of a quiescent state. In addition, quercetin treatment increased gene expression of PPARGC1a, a key regulator of mitochondrial biogenesis, and also increased protein expression of Kir2.1, the ion channel largely responsible for maintaining the cardiomyocyte resting membrane potential. These results suggest that quercetin may improve maturation of surviving iPSC-CMs. Removal of senescent cells using senolytics may facilitate maturation of iPSC-CMs in culture prior to transplantation.

Funding Source: K08 HL150335-01A1 (to J.C.G.), R01HL151684-01 (to R.T.L.)

Keywords: cardiomyocyte, maturation, senescence

LIPID DROPLET AVAILABILITY INFLUENCES NEURAL STEM/PROGENITOR CELL PROLIFERATION AND DIFFERENTIATION INTO NEURONS

Knobloch, Marlen[1], Ramosaj, Mergim[1], Madsen, Sofia[1], Scandella, Valentina[1], Sudria-Lopez, Daniel[1], Maillard, Vanille[1], Yuizumi, Naoya[2], Telley, Ludovic[3]

[1]*Department of Biomedical Sciences, University of Lausanne, Switzerland,* [2]*Graduate School of Pharmaceutical Sciences, University of Tokyo, Japan,* [3]*Department of Fundamental Neurosciences, University of Lausanne, Switzerland*

Neural stem/progenitor cells (NSPCs) generate new neurons throughout adulthood, however, the underlying regulatory processes are still not fully understood. Lipid metabolism plays an important role in NSPC regulation: Build-up of lipids is crucial for NSPC proliferation, whereas break-down of lipids has been shown to regulate NSPC quiescence. Despite their central role for cellular lipid metabolism, the role of lipid droplets (LDs), the lipid storing organelles, in NSPCs remains underexplored. We show here that LDs are highly abundant in adult mouse NSPCs, and that LD accumulation is significantly altered upon fate changes such as quiescence and differentiation. Artificially increasing the number of LDs in NSPCs augments the number of neuronal progenies. Further, NSPC proliferation can be influenced by the number of LDs, inhibition of LD breakdown, and the asymmetric inheritance of LDs during mitosis. Together, these data suggest an instructive role for LDs in driving NSPC behaviour.

Funding Source: Swiss National Science Foundation (#31003A_175570)

Keywords: lipid metabolism, neural stem cells, lipid droplets

REGULATION OF HEMATOPOIESIS BY MITOCHONDRIAL DYNAMICS

Yao, Yan[1], de Almeida, Mariana Justino[2], Luchsinger, Larry L[1], Snoeck, Hans-Willem[1]

[1]*Medicine, Columbia University Medical Center, New York, NY, USA,* [2]*Department of Microbiology and Immunology, Columbia University Medical Center, New York, NY, USA*

Despite their established therapeutic potential, maintaining and expanding hematopoietic stem cells (HSCs) in vitro remains a challenge. It is therefore crucial to understand mechanisms that balance HSC self-renewal, maintenance and differentiation. HSCs generate ATP predominantly through glycolysis and have low mitochondrial respiration activity. We previously showed however that HSCs have higher mitochondrial mass and highly fused mitochondrial network compared to more mature populations3. These findings raise the question what the role of the abundant mitochondria might be in HSCs. To further uncover the roles of mitochondria in HSCs, I disrupted mitochondrial dynamics by conditional knockout of the two mediators of mitochondrial outer membrane fusion, Mitofusin (Mfn) 1 and 2, in hematopoietic system. My preliminary observations show that the HSC pool was expanded in double knockout (DKO) mice embryos, while their reconstitution capacity was abolished. One allele of Mfn1 (1Mfn1) largely restored DKO HSCs functions, whereas one allele of Mfn2 (1Mfn2) only restored myeloid reconstitution. Specific deletion of Mitofusins in lymphoid-lineage also caused lymphopenia. Both Mfns furthermore play differential roles in erythroid, B, T and platelet, but not in NK cell development. Genome-wide expression analysis of purified HSCs showed deletion of Mitofusins leads to downregulation of transcripts associated with HSCs, early progenitors and the lymphoid lineage and reciprocal upregulation of transcripts expressed in the myeloid lineage, suggesting epigenetic regulation of fate decisions by mitochondrial dynamics at the level of the HSCs. 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.

Keywords: Hematopoiesis, Mitochondria, Mitofusin

RIBOSOMAL RNA BIOGENESIS REGULATES MOUSE 2C-LIKE STATE AND 2-CELL/4-CELL EMBRYO DEVELOPMENT BY 3D STRUCTURE REORGANIZATION OF PERI-NUCLEOLAR HETEROCHROMATIN

Yu, Hua, Pan, Hongru, Sun, Zhen, Tan, Tianyu

Center for Stem Cell and Regenerative Medicine, Department of Basic Medical Sciences, Zhejiang University, Hangzhou, China

Nucleolus is the organelle for ribosome biogenesis and for sensing various types of stress. Its role in regulating stem cell fate is unclear. Here, we present multiple lines of evidences that nucleolar stress induced by interfering rRNA biogenesis can drive two-cell stage embryo-like (2C-like) transcriptional program and induce an expanded 2C-like cell population in mouse embryonic stem (mES) cells. Mechanistically, the liquid-liquid phase separation (LLPS) mediated by rRNA and nucleolar proteins maintains the formation of peri-nucleolar heterochromatin (PNH). Upon

rRNA biogenesis defect, the normal LLPS of nucleolus is disrupted, causing dissociation of NCL/TRIM28 complex from PNH and changes of epigenetic states and reorganization of the 3D structure of PNH, which leads to Dux, a 2C program transcription factor gene, to be released from the PNH region and activation of 2C-like program. Embryos with rRNA biogenesis defect are incompatible to develop from 2-cell (2C) to 4-cell embryos, with delayed repression of 2C/ERV genes and a transcriptome skewed toward earlier cleavage embryo signatures. Our results highlight that nucleolar LLPS-mediated 3D chromatin structure reshaping of PNH compartment regulates the fate transition of mES cells to 2C-like cells, and that rRNA biogenesis is a critical regulator during the 2-cell-to-4-cell transition of murine pre-implantation embryo development.

Keywords: Stem Cell Fate Transition, Nucleolar Phase Separation, 3D Chromatin Structure

THEME MDD (MODELING DEVELOPMENT AND DISEASE)
PLENARY IV: SELF-ORGANIZATION OF DEVELOPMENTAL PROCESSES
9:00 - 11:15 EDT
REBROADCAST WITH LIVE CHAT 21:00 - 23:15 EDT

METABOLIC CONTROL OF MOUSE EMBRYONIC PATTERNING AND TIMING

Aulehla, Alexander

Developmental Biology Unit, EMBL, Heidelberg, Germany

How environmental cues are integrated into developmental programs and impact the phenotypic outcome is a fundamental, unresolved question that we aim to address. To this end, we study how cellular metabolic activity, which responds dynamically to external environmental conditions, impacts cellular signaling. Specifically, we investigate the role of glycolytic flux in mouse embryo mesoderm development. We generated a novel mouse model to genetically increase glycolytic flux by conditional overexpression of PFKFB3 and found it results in altered mesoderm segmentation, Wnt-signaling down regulation and importantly, a glucose-dose dependent slowing down of segmentation rate and segmentation clock oscillations. I will also present our recent experiments that aim to connect these intriguing findings and will discuss how changes in glycolytic flux and metabolite levels impact developmental programs and embryonic timing.

Keywords: metabolism/glycolysis, mouse embryonic patterning, mesoderm segmentation, clock oscillations

THREE DIMENSIONAL MODELS OF PANCREAS ORGANOGENESIS: FROM SELF-ORGANIZATION TO UNDERSTANDING DIABETES

Grapin-Botton, Anne[1,2], Gonçalves, Carla A.[1], Larsen, Michael[1], Jung, Sascha[2], Stratmann, Johannes[3], Nakamura, Akiko[1], Leuschner, Marit[3], Hersemann, Lena[3], Keshara, Rashmiparvathi[3], Amit, Ido[4], Jørgensen, Anne[5], Kim, Yung Hae[3], del Sol, Antonio[2,6,7]

[1]The Novo Nordisk Foundation Center for Stem Cell Biology, Copenhagen, Denmark, [2]CIC bioGUNE-BRTA (Basque Research and Technology Alliance), Derio, Spain, [3]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany, [4]The Weizmann institute, Rehovot, Israel, [5]Department of Growth and Reproduction, Copenhagen University Hospital (Righshospitalet), Copenhagen, Denmark, [6]Luxembourg Centre for Systems Biomedicine (LCSB), University of Luxembourg, Belvaux, Luxembourg, [7]IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

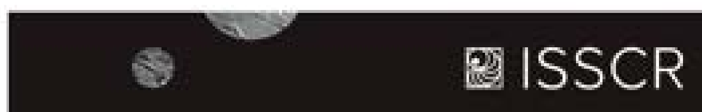
To understand pancreas development, as a complement to in vivo investigations, we designed simplified in vitro systems that can be monitored and manipulated better than the whole embryo. We established 3D (three-dimensional) culture conditions that enable the efficient expansion, differentiation and morphogenesis of pancreatic progenitors isolated from mouse embryos, human fetuses or produced from human pluripotent stem cells (hPSCs). The human system enables robust expansion, cryopreservation, differentiation and morphogenesis of human pancreatic progenitors. Using single-cell sequencing, we compared the transcriptional profile of cells grown in vitro in 2D, and 3D from hPSC-derived, to those isolated from fetal pancreas. Our experiment show a good molecular stability of pancreas progenitors over time, retention of the endocrine differentiation capacity and a molecular signature closer to the in vivo counterparts when grown in 3D. We used this system to perform a screen for molecules controlling human pancreas progenitor expansion. The systems developed enable to address the mechanisms of pancreas development, a process normally hidden from us in the womb. Moreover, the method enables to address the function of genes controlling architectural events and morphogenesis operating in 3D that would be difficult to address in 2D culture. Our ongoing investigations notably address the mechanisms of complex lumen formation in the pancreas. The human organoids also open a way to address whether some genes identified in genome-wide association studies (GWAS) control the development of beta cells thereby predisposing to diabetes later in life. We initiated this process by studying the effect of GLIS3 in human development and started to clarify the mechanisms by which it promotes diabetes and pancreatic cysts.

BLASTOID: MODELING MAMMALIANS BLASTOCYST DEVELOPMENT AND IMPLANTATION

Rivron, Nicolas

Institute of Molecular Biotechnology, Austrian Academy of Science, Vienna, Austria

The blastocyst is the early mammalian conceptus, from which all embryonic and extra-embryonic tissues develop. It is made up of a spherical layer called trophectoderm that surrounds a fluid-filled cavity sheltering the embryonic and primitive endoderm cells. Stem cell lines can be derived from mouse and human blastocysts. Our lab showed that such stem cells self-organize in vitro into structures that morphologically and transcriptionally resemble mouse and human blastocysts (blastoids). Blastoids form analogs of the three founding lineages (trophoblast, epiblast, and primitive endoderm) and model aspects of implantation in utero and in vitro. Blastoids, like blastocysts, form due to inductive molecules secreted by the embryonic cells and driving trophec-



toderm development. We identified a set of inductive molecules that promote trophoblast proliferation and self-renewal while tuning trophoblast epithelial morphogenesis. Altogether, these inductions are paramount to generate a trophectoderm state capable of implanting in utero. Thus, at this stage, the nascent embryo invests in its own future by fueling the development of trophoblasts that mediate implantation and placentation. Overall, mouse and human blastoids are powerful models that can be reproducibly generated in large numbers and finely tuned to mimic aspects of blastocyst development and implantation. Molecular insights into these processes are crucial for improving IVF procedures, developing contraceptives, and managing early pregnancy therapeutically.

Funding Source: ERC-CoG, HFSP early investigator.

Keywords: Blastoid, Blastocyst, Implantation

PURSUING ORGANOID MEDICINE DURING GLOBAL PANDEMIC

Takebe, Takanori

Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Organoids are multicellular structures that can be derived from adult organs or pluripotent stem cells. Early versions of organoids range from simple epithelial structures to complex, disorganized tissues with large cellular diversity. The current challenge is to engineer cellular complexity into organoids in a controlled manner that results in organized assembly and acquisition of tissue function. These efforts have relied on studies of organogenesis during embryonic development and have resulted in development of organoids with multilayer tissue complexity and higher order functions. Our group and others have established in vitro self-organizing principles to introduce, for example, vascular, mesenchymal, neuronal and immunity components into organoids from pluripotent stem cells. The application of our unique multicellular organoid model enabled the inflammatory liver disease model system in human that includes viral hepatitis, steatohepatitis and drug induced liver injury. Now, we extended our organoid model for studying SARS-CoV-2 associated thrombotic complications. Organoid based mechanistic investigation highlights potentially therapeutic pathways to alleviate vicious cycle of endothelial cell damage and thrombosis, that can be transformed into potential cure for severe COVID-19-assocaited fatal coagulopathy. Here, I will summarize how the next generation of organoids can be designed by utilizing an engineering-based narrative design, and discuss promise and impact of organoid medicine approach towards addressing unmet clinical challenges.

Funding Source: NIH DP2, AMED, T-CiRA

Keywords: Organoids, iPSC, COVID-19

GENERATION OF MOUSE PANCREATIC ISLET ORGANOIDS USING RESIDENT PROCR PROGENITORS

Zeng, Yi A., Wang, Daisong, Wang, Jingqiang, Bai, Lanyue

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

It has generally proven challenging to produce functional β cells in vitro. Our recent study uncovers a novel Procr cell population in adult mouse pancreatic islets. The cells do not express differentiation markers and feature epithelial-to-mesenchymal transition (EMT) characteristics. By genetic lineage tracing, Procr islet cells undergo clonal expansion and generate all four endocrine cell types during adult homeostasis. Sorted Procr cells, representing ~1% of islet cells, can robustly form islet-like organoids when cultured at clonal density. Exponential expansion can be maintained over long time periods by serial passaging, while differentiation can be induced at any time point in culture. b cells dominate in differentiated islet organoids, while ±, ´ and PP cells occur at lower frequencies. The organoids are glucose-responsive and insulin-secreting. Upon transplantation in diabetic mice, the organoids reverse disease. These findings demonstrate that the adult pancreatic islet contains a population of Procr progenitors. We will also describe the physiological relevance of Procr progenitors during postnatal islet development and homeostasis.

Keywords: pancreatic islet, adult stem cells, Procr

REBUILDING KIDNEY TISSUE FROM PLURIPOTENT STEM CELLS: THE CHALLENGES OF SCALING UP AND DOWN

Little, Melissa H.

Cell Biology, Murdoch Children's Research Institute, Melbourne, Australia

The development of protocols for the differentiation of human pluripotent cells to complex multicellular organoids provides novel opportunities for stem cell medicine. We have developed a protocol for the generation of multicellular human kidney organoids from human pluripotent stem cells that capitalises on the ability for cells to self-organise to form nephrons in vitro. As such, kidney organoids represent a remarkably complex and developmentally congruent tissue type when compared to developing human kidney. Critically, the protocols for generation of such tissue are robust and transferable. However, remaining challenges include the minimisation of off target cell types, improvements in tissue scale and hence nephron number, appropriate tissue structure and ultimately successful maturation and renal function. Using changes in culture format, we have developed approaches for the 'scale up' of kidney tissue sheets using bioprinting, resulting in a substantial increase in nephron number generated via an automated manufacturing process. Transplantation of human pluripotent stem cell-derived kidney organoids has revealed evidence for vascularisation, filtration and maturation in vivo. Conversely, we are also developing approaches for the 'scale out' of kidney organoid production such that we can generate large numbers of miniaturised kidney organoids with a very low coefficient of variation for use in high content compound screening. These modifications continue to support appropriate morphogenesis, illustrating the power of self-organisation. They will also improve our ability to apply human kidney organoids to disease modelling, drug screening, tissue engineering and regenerative medicine.

Keywords: Kidney development, Pluripotent stem cell, Regenerative medicine

ISSCR ACHIEVEMENT AWARD SESSION 13:15 - 13:45 EDT

FROM EMBRYOS TO STEM CELLS TO STEM CELL-BASED EMBRYO MODELS- WHY UNDERSTANDING EARLY DEVELOPMENT MATTERS

Rossant, Janet

Program in Developmental and Stem Cell Biology and the Department of Molecular Genetics, The Hospital for Sick Children, University of Toronto, and The Gairdner Foundation, Toronto, ON, Canada

My first published experiments in the 1970s dealt with the events of cell commitment to the epiblast, primitive endoderm and trophectoderm in the mouse blastocyst. Lacking in situ lineage tracers and any clue as to molecular lineage determinants, I made some correct predictions (trophectoderm is committed by position prior to the ICM) and some that were wrong (primitive endoderm

is also specified by position on the surface of the ICM). Happily, it was my lab that later disproved the positional theory for epiblast/primitive endoderm formation and showed the importance of stochastic localized FGF signaling. With increased understanding of the signaling pathways and downstream transcriptional networks involved in blastocyst formation came the possibility to derive stem cells that retained the lineage restriction of the three cell types in the blastocyst. Pluripotent embryonic stem cells represent the epiblast but cannot normally make the other blastocyst lineages. We derived trophoblast stem (TS) cells and XEN cells from the primitive endoderm. These cell lines have provided key insights into the placental and yolk sac tissues critical to embryo and fetal development. And today ES, TS and XEN cells are part of the toolkit for generating stem cell-derived embryo models, first in the mouse and more recently in human. The events of lineage formation in the early embryo are more relevant than ever and the blastocyst remains my passion.

Keywords: trophoblast, pluripotent stem cells, embryo models

THEME SESSION CI 2 (CELLULAR IDENTITY) CELL STATE TRANSITIONS IN DEVELOPMENT AND CANCER 14:00 - 15:45 EDT REBROADCAST WITH LIVE CHAT 02:00 – 03:45 EDT

INTEGRATING LINEAGE-TRACING WITH SINGLE CELL GENOMICS ACROSS EXPERIMENTAL DESIGNS

Klein, Allon

Harvard Medical School, USA

Single cell assays have recently been integrated with lineage tracing, a set of methods that identify cells of common ancestry to establish bona fide dynamic relationships between cell states. These integrated methods identify early changes in cell state that predict future cell behaviors over days or weeks of differentiation, and thus provide a powerful addition to the arsenal of stem cell biology. They are not yet simple to deploy and interpret, in part because lineage data can be noisy and sparse. I will report on a robust computational approach, named CoSpar, that integrates single-cell genomics with lineage tracing to learn long-term cell dynamics. CoSpar is robust to severe down-sampling and dispersion of lineage data, which enables simpler, lower-cost experimental designs and requires less calibration. In datasets representing hematopoiesis, reprogramming, and directed differentiation, CoSpar identifies fate biases not previously detected, predicting transcription factors and receptors implicated in fate choice.

Keywords: Lineage-tracing; Dynamic inference; Single cell genomics

SINGLE CELL CHROMATIN ACCESSIBILITY PROFILING OF MOUSE HEART DEVELOPMENT IDENTIFIES REGULATORY UNDERPINNING OF CARDIAC OUTFLOW TRACT ANOMALIES

Ranade, Sanjeev, Ye, Lin, Nishino, Tomohiro, Alexanian, Michael, Wallace, Langley Grace, Krup, Alexis, Pelonero, Angelo, Huang, Yu, Srivastava, Deepak

GICD, The Gladstone Institutes, San Francisco, CA, USA

Congenital Heart Disease (CHD) is the most common form of birth defects and arises due to aberrant gene regulatory networks during heart development. Genetic analyses of CHD patients have identified an enrichment for variants in transcription factors (TFs) and chromatin modifying genes, highlighting the sensitivity of transcriptional network dosage. However, mechanistic insight into cardiac TF function in vivo is limited by technical challenges, thus the cell type specific regulatory logic of heart development remains poorly understood. Single Cell Assay for Transposase Accessible Chromatin (scATAC-seq) has emerged as a transformative method for elucidating regulatory networks in heterogeneous developing tissues in vivo. Here, we perform scATAC-seq on >65,000 single cells from four early stages of cardiogenesis in mice and identify cell type specific, temporally dynamic regulatory elements that drive progenitor cell differentiation to mature cardiac structures. Integration of scRNA-seq data predicted targets of putative enhancers, which we validate using CRISPR-based perturbations in vitro. We further uncover dysregulated epigenomic and transcriptomic states of cells from mice deficient for TBX1, a TF responsible for CHD occurring in DiGeorge Syndrome, a complex disorder caused by microdeletions on Chromosome 22q11.2. Loss of TBX1 altered chromatin accessibility most prominently in two progenitor cell populations, the anterior second heart field and cardiopharyngeal mesoderm, while differentiated cardiomyocytes were unaffected. scRNA-seq within these populations revealed aberrant expression of secreted growth factors and Semaphorin genes, such as Sema3c, a guidance molecule necessary for appropriate migration of neural crest cells during outflow tract septation. Moreover, scATAC-seq identified TBX1 dependent enhancers enriched in progenitor cells for Sema3c, which we validate in vitro, providing a mechanistic link to the transcriptional dysregulation. These results suggest that TBX1 directly impacts the progenitor cell state by disrupting expression of genes required to properly pattern the outflow tract and, more broadly, identify regulatory networks for progenitor cell fate specification into mature cell types in heart development.

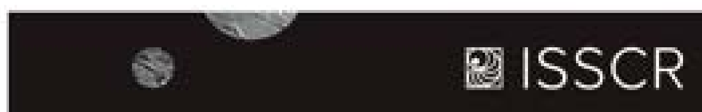
Keywords: epigenomic regulation of heart development, Integrated single cell ATAC-seq and RNA-seq, Congenital Heart Disease

DEVELOPMENTAL CHROMATIN PROGRAMS DETERMINE ONCOGENIC COMPETENCE IN MELANOMA

Baggiolini, Arianna[1], Callahan, Scott J.[1], Montal, Emily[2], Weiss, Joshua[2], Trieu, Tuan[3], Tagore, Mohita[2], Tischfield, Sam E.[2], Walsh, Ryan M.[1], Suresh, Shruthy[2], Fan, Yujie[1], Campbell, Nathaniel[2], Perlee, Sarah C.[2], Saurat, Nathalie[1], Hunter, Miranda[2], Simon-Vermot, Theresa[2], Huang, Ting-Hsiang[2], Ma, Yilun[2], Hollmann, Travis[4], Tickoo, Satish K.[4], Taylor, Barry[5], Khurana, Ekta[3], Koche, Richard P.[6], White, Richard[2], Studer, Lorenz[1]

[1]*Developmental Biology, Memorial Sloan Kettering Cancer Center, New York, NY, USA*, [2]*Cancer Biology & Genetics, Memorial Sloan Kettering Cancer Center, New York, NY, USA*, [3]*Computational Biomedicine, Cornell University, New York, NY, USA*, [4]*Pathology, Memorial Sloan Kettering Cancer Center, New York, NY, USA*, [5]*Memorial Sloan Kettering Cancer Center, New York, NY, USA*, [6]*Center for Epigenetics Research, Memorial Sloan Kettering Cancer Center, New York, NY, USA*

Oncogenes cause malignant transformation only in certain cellular contexts, a phenomenon called oncogenic competence. Cellular lineage plays a crucial role in determining the transcriptional response to oncogenic mutations. However, the mechanisms linking developmental lineage programs to oncogenic competence remain poorly understood. Here, using a combination of a human pluripotent stem cell (hPSC)-based cancer model along with zebrafish transgenesis, we demonstrate that the transforming ability of BRAFV600E depends upon the intrinsic transcriptional program present in the cell of origin. To understand which cells along the melanocytic differentiation trajectory are most sensitive to an oncogenic insult and more permissive to give rise to melanoma, we engineered zebrafish to express BRAFV600E in either neural crest (NC), melanoblasts, or melanocytes by using stage-specif-



ic promoters in a p53-/- background. Moreover, we used gene targeting in hPSCs to introduce oncogenic BRAFV600E and to inactivate the tumor suppressors RB1, TP53 and P16 (referred to hereafter as 3xKO cells). The 3xKO cells were differentiated into NC cells, melanoblasts and mature melanocytes and BRAFV600E was induced by doxycycline. These cells were then subcutaneously injected into NSG mice and assessed for tumor formation. In both systems, we showed that melanocytes are refractory to melanoma formation, while their progenitors, NC cells and melanoblasts, are highly responsive to the same tumorigenic insults. Molecular profiling reveals that NC cells and melanoblasts have distinct expression of chromatin modifying enzymes, and we learned that the chromatin factor ATAD2 is required for response to BRAFV600E and tumor initiation. Expression of ATAD2 in melanocytes allows for malignant transformation. In particular, we showed that ATAD2 forms a complex with SOX10, allowing for expression of downstream oncogenic programs and for the activation of a developmental signature typical of the NC. These data suggest that oncogenic competence is mediated by the regulation of developmental chromatin factors, which then allow for proper response to those oncogenes.

Keywords: Melanoma competent states, hPSC-derived melanoma model, ATAD2, a melanoma competence factor

INTEGRATIVE MOLECULAR ROADMAP FOR REPROGRAMMING MOUSE FIBROBLASTS INTO INDUCED MYOGENIC STEM AND PROGENITOR CELLS

Kim, Inseon, von Meyenn, Ferdinand, Bar-Nur, Ori

Department of Health Sciences and Technology, ETH Zürich, Switzerland

Direct lineage reprogramming provides a unique system to study cell fate transitions and unearth molecular mechanisms that safeguard cellular identity. We previously reported on direct conversion of mouse fibroblasts into induced myogenic progenitor cells (iMPCs) by transient MyoD overexpression in concert with small molecules treatment. Here we employed integrative multi-omic assays to delineate the molecular landscape of fibroblast reprogramming into iMPCs in comparison to transdifferentiation into myogenic cells solely by MyoD overexpression. Utilizing bulk RNA-sequencing and mass spectrometry we uncovered molecular regulators and pathways that endow a myogenic stem cell identity only in the presence of small molecule treatment. Furthermore, we demonstrate that iMPC reprogramming is a step-wise process, commencing with the appearance of myofibers and committed myogenic progenitors prior to the formation of satellite-like progenitors that express a suite of stem cell markers including Pax7, Myf5, Sox8, and Dmrt2. To assess the equivalency of iMPCs to satellite cell-derived myoblasts, we employed a fluorescent Pax7-GFP reporter to purify Pax7+ cell from the heterogeneous iMPC cultures and molecularly compare them to Pax7+ myoblasts. We demonstrate that Pax7+ iMPCs share molecular attributes with myoblasts, however in addition express unique genes, proteins and pathways that are indicative of a more activated satellite cell-like state in vitro. We further establish that iMPC formation and maintenance is dependent on the Notch pathway, as small molecule inhibition of Notch abrogates iMPC formation and derails stable iMPC cultures via depletion of Pax7 cells. Lastly, using single cell RNA-sequencing we determine the transcriptional trajectory present in the heterogeneous iMPC cultures and demonstrate that a highly proliferative satellite cell-like population differentiates into committed myoblasts and myocytes that further give rise to myofibers, thus capturing a dynamic myogenic program in vitro. Collectively, this study charts a molecular blueprint for reprogramming fibroblasts into muscle stem and progenitor cells and further establishes the fidelity of stable iMPC cultures in capturing skeletal muscle regeneration in vitro for disease modeling and basic research applications.

Keywords: Reprogramming, skeletal muscle, Omics

ILLUMINATING POST-TRANSCRIPTIONAL REGULATION OF PLURIPOTENT CELL STATE TRANSITION AND CELL FATE AT SINGLE CELL RESOLUTION

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Post-transcriptional regulation orchestrates diverse cellular mechanisms that regulate pluripotent stem cell biology and pattern early mammalian development. The fibroblast growth factor-extracellular signal-regulated kinase (FGF-ERK) signaling pathway drives pluripotent stem cells toward trilineage specification and lineage commitment. By analysis of both FGF-ERK signal transduction and FGF-receptor endocytosis at single-cell resolution, we have shown that post-transcriptional regulation of Profilin-2, an actin/dynamin-binding protein, enables FGF signaling, ERK activation, and early stem cell differentiation by controlling endocytosis. Remarkably, these findings define a previously unknown axis of post-transcriptional control, endocytosis, and signal transduction important for stem cell biology (including proliferation and cell cycle status) and early differentiation. Our investigations have furthermore sought to determine how post-transcriptional regulation of PFN2 controls signaling and cell fate during trilineage specification and the gastrulation-stage embryo, and we subsequently reveal that coordinate control of this axis by multiple post-transcriptional regulators fine-tunes lineage commitment.

Funding Source: This work was supported by NIH/Eunice Kennedy Shriver National Institute of Child Health and Human Development Grant F32HD088051 and NIH/National Institute of General Medical Sciences Grants R01 GM125089 and R01 GM122439.

Keywords: cell fate, FGF-ERK signaling, post-transcriptional

YAP:NODAL SIGNALING AXIS REGULATES THE CELL FATE PATTERNING IN HUMAN GASTRULOIDS

Stronati, Eleonora[1], Giraldez, Servando[2], Huang, Ling[2], Abraham, Elizabeth[1], Estaras, Conchi[1]

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A fundamental question in developmental biology is how unpatterned cells of the early embryo gain their identity necessary to generate the mature organism. This process takes place during gastrulation that culminates in the specification of the three germinal layers and the establishment of the antero-posterior axis (AP). The gastrulation is mediated by the coordinated crosstalk between morphogens, including BMP, Activin/NODAL and WNT signaling pathways, and the activity of transcription factors. The transcriptional effector of the Hippo-pathway, YAP, emerged as a regulator in several biological processes, ranging from cell proliferation to tumorigenesis. However, the role of YAP in the cell fate patterning decision occurring during gastrulation and its interplay with morphogenetic signals is not fully understood. Here we identified YAP as a key regulator of cell fate specification repressing NODAL signaling. By using human ESCs 2D-micropatterned gastruloids we showed that YAP is essential for the ectodermal lineage acquisition and the spatial self-organization of the three germinal layers. Our data demonstrate that NODAL inhibition, mediated by YAP, is crucial to exit the pluripotency and acquire the ectodermal fate. In the WT hESCs, a gradient of NODAL:SMAD2.3

from the edge to center of the gastruloid controls the self-organization of the germ layers. In the absence of YAP, a hyperactive NODAL signaling and SMADs nuclear retention impede the ectoderm layer specification and expand the meso and endodermal layers. Moreover, our single-nuclei ATAC-seq analysis reveals that YAP controls the chromatin accessibility of key genes in the NODAL pathway, including NODAL gene itself, repressing their transcription. Accordingly, we show that the NODAL inhibition is sufficient to restore the correct patterning, indicating that YAP repression of NODAL signaling is essential for proper gastrulation. Our study defines a novel YAP:NODAL signaling axis necessary to regulate the spatial differentiation of the epiblast cells during gastrulation and potentially, in the establishement of the AP axis in the embryo. Finally, our study provides new mechanistic insights in human developmental defects caused by the altered NODAL signaling.

Keywords: micropatterned gastruloid, YAP, ectoderm

QUANTITATIVE LANDSCAPES OF CELL FATE DECISIONS

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Fate decisions in developing tissues involve cells transitioning between a set of discrete cell states, each defined by a distinct gene expression profile. Geometric models, often referred to as Waddington landscapes, in which developmental paths are given by the gradient and cell states by the minima of the model, are an appealing way to describe differentiation dynamics and developmental decisions. To construct and validate accurate dynamical landscapes, quantitative methods based on experimental data are necessary. To this end we took advantage of the differentiation of neural and mesodermal cells from pluripotent mouse embryonic stem cells exposed to different combinations and durations of signalling factors. We developed a principled statistical approach using flow cytometry data to quantify differentiating cell states. Then, using a framework based on Catastrophe Theory and approximate Bayesian computation, we constructed the corresponding dynamical landscape. The result was a quantitative model that accurately predicted the proportions of neural and mesodermal cells differentiating in response to specific signalling regimes. Analysis of the geometry of the landscape revealed two distinct ways in which cells make a binary choice between one of two fates. We discuss the biological relevance of these mechanisms and suggest that they represent general archetypal designs for developmental decisions. Taken together, the approach we describe is broadly applicable for the quantitative analysis of differentiation dynamics and for determining the logic of developmental cell fate decisions.

Keywords: Cell decision making, quantitative models, dynamical systems

THEME SESSION MDD 2 (MODELING DEVELOPMENT AND DISEASE) COMPARATIVE EARLY DEVELOPMENT 14:00 - 15:45 EDT REBROADCAST WITH LIVE CHAT 02:00 – 03:45 EDT

DECIPHERING THE MECHANISMS OF HOW PRIMATES ARE FORMED

Wang, Hongmei

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A fine-tuned development of the embryo is a prerequisite for a successful pregnancy. Abnormal embryonic development may lead to adverse pregnancy outcomes, such as miscarriage and congenital anomalies. By combining in vitro culture models for mouse, monkey, and human embryos, construction of stem cell-based embryoid, and a well-established platform for the extra-embryonic placenta research (including various trophoblast cell fusion/invasion/migration models, trophoblast stem cells, live-cell imaging, tissue clearing, high-throughput proteomics, single-cell RNA-seq, etc), we spend all our efforts in trying to decipher the hidden secrets of how primate embryos are developed and what are the roles of the extraembryonic tissues in supporting the embryos at different stages of pregnancy.

Funding Source: Strategic Priority Research Program of the Chinese Academy of Sciences

Keywords: primate, embryogenesis, placenta

SYNTHETIC EMBRYOLOGY: REPROGRAMMING EPIBLAST STEM CELLS INTO PRE-IMPLANTATION BLASTOCYST CELL-LIKE CELLS

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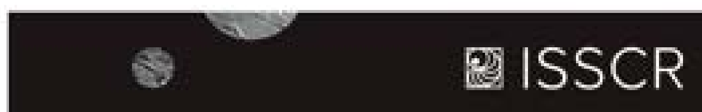
Recently, a new wave of synthetic embryo systems (SESs) have been established from cultured cells for efficient and ethical embryonic development research. We recently reported our epiblast stem cell (EPISC) reprogramming SES that generates numerous blastocyst (BC)-like hemispheres (BCLH) with pluripotent and extraembryonic cell features detected by microscopy. Here, we further explored the system over key time points with single-cell RNA sequencing (scRNA-seq) analysis. We found broad induction of the 2C-like reporter MERVL and RNA velocities diverging three major cell populations with gene expression profiles resembling those of pluripotent epiblast, primitive endoderm, and trophectoderm. Enrichment of those three induced BC-like cell fates involved key gene regulatory networks, zygotic genome activation-related genes and specific RNA splicing, and specific cells closely resembled in silico models. This analysis confirms the induction of extraembryonic cell populations during EPISC reprogramming. We anticipate that our unique BCLH SES and rich dataset may uncover new facets of cell potency, improve developmental biology, and advance biomedicine.

Funding Source: RIKEN Organoid Project

Keywords: reprogramming, synthetic embyrology, early embryo



3D BIOMIMETIC IMPLANTATION NICHE REVEALS THE FIRST INTERACTIONS OF THE EMBRYO AND THE MATERNAL BLOOD VESSELS

Bedzhov, Ivan

Embryonic Self-Organization Research Group, Max Planck Institute for Molecular Biomedicine, Muenster, Germany

The process of implantation and the cellular interactions at the embryo-maternal interface are intrinsically difficult to analyse, as the implanting embryo is concealed by the uterine tissues. Therefore, the mechanisms mediating the interconnection of the embryo and the mother are poorly understood. Here, we established a 3D biomimetic culture environment that harbours the key features of the murine implantation niche. This culture system enabled direct analysis of trophoblast invasion and revealed the first embryonic interactions with the maternal vasculature. We found that implantation is mediated by the collective migration of penetrating strands of trophoblast giant cells, which acquire the expression of vascular receptors, ligands and adhesion molecules, assembling a network for communication with the maternal blood vessels. In particular, Pdgf signalling cues promote the establishment of the heterologous contacts. Together, the biomimetic platform and our findings thereof elucidate the hidden dynamics of the early interactions at the implantation site.

Funding Source: German Research Foundation (DFG)

Keywords: 3D embryo culture, implantation, biomimetic platform

A GENOME-WIDE CRISPR-CAS9 KNOCKOUT SCREEN IDENTIFIES ESSENTIAL AND GROWTH-RESTRICTING GENES IN HUMAN TROPHOBLAST STEM CELLS

Dong, Chen, Fu, Shuhua, Chew, Brian, Zhang, Bo, Theunissen, Thorold W.

Developmental Biology, Washington University School of Medicine, St. Louis, MO, USA

The recent development of culture conditions that support the derivation and maintenance of bona fide human trophoblast stem cells (hTSCs) significantly enhances our ability to study human placental biology and pathologies, but so far few studies have investigated the molecular regulators of hTSC identity. To comprehensively identify potential regulators of the hTSC state, we utilized a genome-wide CRISPR-Cas9 knockout screen to define genes essential for or restricting the survival and self-renewal of hTSCs. The screen produced 2139 essential genes (EGs) and 619 growth-restricting genes (GRGs), and includes both well-established trophoblast regulators such as GATA2, TFAP2C, and EGFR, as well as many genes without previously reported association with the trophoblast lineage. Our analyses indicate that these genes are biologically and functionally relevant and instrumental for defining human trophoblast identity. We also systematically uncovered pathways that play important roles in regulating hTSCs, such as ferroptosis, NF-κB, mTOR, and TNF signaling pathways. In addition, we referenced our data to those of similar genetic screens performed in cancer cell lines and primed human pluripotent stem cells (hPSCs), as well as gene expression data of early human embryos and the human maternal-fetal interface, to identify potential hTSC-specific and -enriched regulators. Among those are TEAD1, a gene previously reported to be dispensable for mouse placentation. We validated the essentiality of TEAD1 and assayed its genome-wide DNA binding sites using Cleavage Under Targets and Tagmentation (CUT&Tag) in hTSCs, which revealed that TEAD1 cooperates with other transcription factors such as TFPA2C to target downstream trophoblast regulators, including GATA3 and KRT7. Intersection with ATAC-seq and RNA-seq data suggests that TEAD1 plays a major role in the specification, maintenance, and maturation of the human trophoblast lineage. Overall, our study presents the first CRISPR/Cas9 knockout screen in a human extraembryonic lineage, systematically reports potential regulators of hTSCs, and provides a valuable resource for future research into human placental development and disease.

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: Human trophoblast stem cell, CRISPR screen, Essential gene

TRANSGENIC PIG MODEL REVEALS CONSERVED LGR5 EXPRESSION IN HAIR FOLLICLE STEM CELLS IN POSTNATAL SKIN, BUT DIVERGENT EXPRESSION IN FETAL DEVELOPMENT ACROSS SPECIES

Polkoff, Kathryn[1], Gupta, Nithin K.[1], Green, Adrian[2], Marquez-Napoles, Yanet[1], Chung, Jaewook[1], Gleason, Katherine[1], Piedrahita, Jorge[1]

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LGR5+ hair follicle stem cells in mice are key to hair follicle growth and homeostasis, can contribute to all lines of the pilosebaceous unit, and are known to migrate from the hair follicle niche to contribute to re-epithelialization during cutaneous wound healing. At present, due to a lack of validated antibodies, cellular detection methods of LGR5 are limited to RNA FISH or fluorescent reporter systems. As a result, the study of LGR5+ hair follicle stem cells (HFSC) has been mostly limited to the mouse, which has many physiological differences from the human. Porcine skin, however, shares many characteristics with the human, including thick epidermis and dermis, asynchronous hair follicle cycling, and sparse hair coats. In this research, we use CRISPR-Cas9 mediated gene editing in combination with somatic cell nuclear transfer to develop a transgenic pig model expressing H2BGFP under the control of the endogenous LGR5 promoter. Our results show that in pigs, like the mouse, LGR5 is expressed throughout the lower bulge in all stages of the hair cycle, and fluorescent in situ hybridization confirms an analogous pattern in the human. Furthermore, in anagen, as in mice, the LGR5+ cells in the pig give rise to a transient amplifying population in the hair matrix which ultimately becomes the hair shaft and inner root sheath. Comparison of RNAseq transcriptomes of human, mouse and pig LGR5+ epidermal cells reveal many conserved pathways, but also major differences in upregulated genes across species. Finally, analysis of embryonic hair follicle development shows that in pigs, LGR5 is expressed at an earlier stage and more broadly than reported in mice: at least as early as the hair germ stage and consistently from the bulge to the bulbar base throughout morphogenesis. Overall, these findings point to a conserved role for LGR5 in postnatal HFSC homeostasis, but also highlight potential differences across species between stem cell dynamics in embryonic development and in postnatal gene regulatory networks. Understanding the differences between species could be key in translating regenerative pathways discovered in the mouse toward therapies the human.

Funding Source: This work was supported by NIH F31AR077423 (K.P.) and NIH R21OD019738 and R01OD023138 (J.P.).

Keywords: Hair Follicle Stem Cells, Porcine, Skin

LANDMARKS OF HUMAN EMBRYONIC DEVELOPMENT INSCRIBED IN SOMATIC MUTATIONS

Bizzotto, Sara[1], Dou, Yanmei[2], Ganz, Javier[1], Doan, Ryan N.[1], Kwon, Minseok[2], Bohrson, Craig L.[2], Kim, Sonia N.[1], Bae, Taejeong[3], Abyzov, Alexej[3], Park, Peter J.[2], Walsh, Christopher A.[1]

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The human body is made of cellular clones that are ultimately traceable to the first post-zygotic cell division. Although cell lineage information is fundamental to understanding organismal development, very little direct information is available about humans, as DNA editing strategies that mark individual cells to follow their progenies in vivo are not applicable. We performed high-depth (>250X on average) whole-genome sequencing (WGS) of multiple tissues from three individuals to identify hundreds of somatic single nucleotide variants (sSNVs) and estimate their average whole-body mosaic fractions, defined as percentage of cells carrying the sSNV. Using variants as "endogenous barcodes" in single cell WGS, we reconstructed early embryonic cell divisions, and revealed asymmetric contributions of early progenitors to extraembryonic tissues and to different organs. Using a maximum-likelihood approach to identify first cell generation variants in WGS data from 74 individuals, we found overall asymmetric contributions of the first cell generation clones to the human body with strong inter-individual variability, from a 50:50 symmetry in few individuals to a 20:80 asymmetry and potentially higher. Ultra-deep (~25,000X) targeted sequencing of clonal sSNVs across 94 biopsies from 17 different organs and >1,000 cortical single cells revealed asymmetries in the clonal contributions to embryonic germ-layers, suggested the onset of gastrulation at ~170 epiblast cells and ~50-100 founders for the forebrain. Finally, we performed single nuclei (sn)RNA-seq and snATAC-seq of ~100,000 cortical single cells and identified sSNV lineage markers in a subset of them to couple lineage information with cell type classification. Although limited by the per-cell coverage sparsity, snATAC-seq revealed better suited to identify lineage markers as reads were more uniformly distributed across the genome compared with snRNA-seq reads. By these means, we pointed to the potential of newer methods for combining analysis of DNA and RNA at high-throughput to systematically analyze the formation of distinct cell types at scale in humans. Thus, with our study we show that mosaic mutations identified in post-mortem tissue provide a permanent record of human embryonic development at remarkably high-resolution.

Keywords: Human embryonic development, Lineage tracing, Somatic mosaic mutations

RECONSTITUTION OF OVARIAN FOLLICLES USING MOUSE PLURIPOTENT STEM CELLS

Hayashi, Katsuhiko, Yoshino, Takashi

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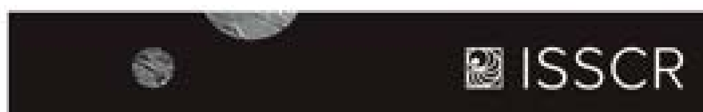
In mammalian species, oocytes are grown in the ovarian follicles for a long period of time in order to store a characteristically large cytoplasm containing maternal proteins and RNAs that are essential for conferring totipotency to the zygote. In mice, the interaction of oocytes with surrounding somatic cells commences at embryonic day (E) 10, when the primordial germ cells (PGCs) migrate into the genital ridges. Somatic cells in the genital ridge provide signal(s) for the proliferation of PGCs, while proliferating themselves to form a pair of gonads at the dorsal wall of the coelom. Upon sex determination, female gonadal somatic cells start to differentiate into granulosa cells and interstitial cells, which eventually form ovarian follicle structures. Formation of ovarian follicle structures is intimately involved in feminization of the individuals with production of oocytes as well as estrogens. Therefore, reconstitution in vitro of the entire process of follicular development is a key step toward a better understanding of sex differentiation. Moreover, reconstitution of follicle structures will contribute to a robust production of oocytes in culture. In mice, we recently developed a culture system that produces functional oocytes from pluripotent stem cell-derived PGC-like cells (PGCLCs) by reaggregation with female gonadal somatic cells isolated from E12.5 mouse embryos. However, the scarcity of embryonic gonadal somatic cells has been an inevitable obstacle, since it is technically and ethically challenging to prepare embryonic tissues at a certain developmental stage. In this study, we tried to induce female gonadal somatic cells from pluripotent stem cells that are capable of forming functional follicle structures.

Keywords: Ovary development, Oocytes, Pluripotent stem cells



FRIDAY, JUNE 25

THEME SESSION CI 3 (CELLULAR IDENTITY) EPIGENETIC REGULATION OF CELL IDENTITY
7:30 - 9:15 EDT
REBROADCAST WITH LIVE CHAT 16:30 - 18:15 AND 19:30 - 21:15 EDT

DISTAL AND PROXIMAL CIS-REGULATORY ELEMENTS SENSE X-CHROMOSOMAL DOSAGE AND DEVELOPMENTAL STATE AT THE XIST LOCUS

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Max Planck Institute for Molecular Genetics, Berlin, Germany

Developmental genes such as Xist, the master regulator of X-chromosome inactivation, are controlled by complex cis-regulatory landscapes, which decode multiple signals to establish specific spatio-temporal expression patterns. In mice, Xist is expressed nearly ubiquitously in females, but not at the naive pluripotent state, were the transition from imprinted to random X inactivation occurs. The Xist locus must thus integrate information on developmental stage and on X-chromosomal dosage to ensure female-specific upregulation at the correct developmental time. Stage-specific expression is thought to be mainly controlled by active repression at the naive pluripotent state through pluripotency factors. We have performed a high-throughput functional dissection of the Xist locus to identify cis-regulatory elements and transcription factors that control the Xist expression pattern. We found that distinct transcription factors actively drive Xist expression during pre-implantation development and upon exit from the naive pluripotent state. They are sensed by the locus through dedicated distal enhancer elements, which are associated with cis-acting long non-coding RNAs. Female-specificity by contrast is ensured through a promoter-proximal element, which controls responsiveness to distal enhancers in a switch-like, X-dosage sensitive manner. Developmental cues and X-dosage are thus decoded by distinct regulatory regions, which cooperate to actively drive Xist upregulation only in females at the correct developmental time. Our study is the first step to disentangle how multiple, functionally distinct regulatory elements interact to generate complex expression patterns in mammals.

Keywords: X-chromosome inactivation, enhancers, CRISPR screens

DNA SEQUENCE LOGIC AT ENDODERMAL ENHANCERS DETERMINES CELL FATE ALLOCATION THROUGH REGULATION OF FOXA PIONEER FACTOR RECRUITMENT

Geusz, Ryan[1], Wang, Allen[2], Lam, Dieter K.[2], Vinckier, Nicholas K.[2], Alysandratos, Konstantinos-Dionysios[3], Roberts, David A.[4], Wang, Jinzhao[2], Kefalopoulou, Samy[2], Qiu, Yunjiang[5], Chiou, Joshua[2], Gaulton, Kyle J.[2], Ren, Bing[5], Kotton, Darrel N.[3], Sander, Maike[6]

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FOXA pioneer transcription factors (TFs) displace nucleosomes and prime chromatin across enhancers of endodermal organs in multipotent precursors before lineage induction. We examined patterns and mechanisms of FOXA target site engagement using human pluripotent stem cell (hPSC) models of endodermal organ development. Unexpectedly, we found that only a subset of pancreatic, hepatic, and lung alveolar enhancers are FOXA-primed, whereas the majority are unprimed and engage FOXA only upon lineage induction. Analysis of underlying DNA sequence revealed stronger FOXA motifs at primed than unprimed enhancers and enrichment for lineage-specific TF motifs at unprimed enhancers. We showed that FOXA recruitment to unprimed but not primed enhancers is dependent on lineage-specific TFs, suggesting that regulatory DNA sequence governs temporal FOXA recruitment. To validate these findings, we used genome editing to strengthen FOXA motifs at an unprimed enhancer near the pancreatic beta cell lineage-determining TF NKX6.1. As predicted, optimizing FOXA motifs led to earlier FOXA recruitment independent of pancreatic lineage-specific TFs. The motif changes also forward-shifted and increased NKX6.1 expression. As a result, progenitors underwent a fate conversion from a pre-alpha to a pre-beta cell identity. Together, our findings identify regulatory sequence logic as the underlying mechanism for temporal differences in FOXA recruitment to endodermal organ enhancers. Based on our demonstration that small changes to regulatory sequence can alter cell fate, we propose that binding site (sub)optimization and "directed pioneering" could be used to manipulate cell identity across various in vitro differentiation systems.

Funding Source: T32 GM008666 (R.J.G.), R01 DK068471 and R01 DK078803 (M.S.), U54 DK107977 (B.R.), the I.M. Rosenzweig Junior Investigator Award (K.D.A.), R01 HL095993, R01HL128172, U01TR001810, and N01 75N92020C00005 (D.N.K).

Keywords: Chromatin, Pioneer factor, Endoderm

DENSE CHROMATIN AND TRANSCRIPTIONAL PROFILING ALONG HEMATOPOIETIC DEVELOPMENT DELINEATES THE REGULATORY LANDSCAPE OF LINEAGE COMMITMENT AND DIFFERENTIATION

Georgolopoulos, Grigorios[1], Psatha, Nikoleta[2], Iwata, Mineo[2], Nishida, Andrew[2], Som, Tannishtha[2], Yiangou, Minas[3], Stamatoyannopoulos, John A.[2], Vierstra, Jeff[2]

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Cell fate establishment and differentiation programs are orchestrated by the binding of lineage specific transcription factors (TFs) to their cognate cis- regulatory elements. While numerous efforts have characterized key lineage transcriptional regulators, their temporal interactions with chromatin and how this imparts gene expression and fate choice remains largely unexplored. Here, we perform dense time-course profiling of chromatin accessibility (DNase I-seq) and gene expression (total RNAseq) along ex vivo erythroid differentiation from human Hematopoietic Stem and Progenitor Cells (HSPCs) to map >11,000 developmentally regulated DNase I hypersensitive sites (DHSs) and >5,000 transcripts. By correlating DHSs to genes in cis we capture ~23,000 potential enhancer-promoter connections. Modelling the temporal profiles of TF expression and DHS accessibility allowed us to map individual TFs to their target DHS genome-wide. This uncovered the regulatory landscape of erythropoiesis which is organized in discrete regulatory modules which are associated with distinct lineage potential states. Functional progenitor assays revealed rapid lineage restriction events during erythropoiesis, concomitant with the modules identified. By directly comparing transcriptional dynam-

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ics (total and single-cell) between erythropoiesis and the closely related megakaryocytic lineage we identify a shared regulatory program responsible for the exit from HSPC stage. Integrating lineage potential assays during megakaryopoiesis with single-cell trajectories we find the megakaryocytic lineage to rapidly commit upon HSPC exit while erythropoiesis undergoes a transient yet distinct myeloid permissive state prior to terminal commitment. Collectively, we present high-resolution temporal maps of the developmentally regulated cis- (chromatin) and trans- (TFs) elements during erythropoiesis. Through integrative analysis of TF and DHS dynamics in combination with functional assays and single-cell trajectories we demonstrate that discrete regulatory states instruct rapid transitions in lineage potential.

Keywords: erythropoiesis, lineage commitment, regulatory dynamics

GENETICALLY DIVERSE MOUSE EMBRYONIC STEM CELLS ENABLE INFERENCE OF GENETIC REGULATORY STRUCTURE

Kuffler, Lauren[1,2], Skelly, Daniel A.[2], Czechanski, Anne[2], Churchill, Gary A.[2], Munger, Steven C.[2], Baker, Christopher L.[2], Reinholdt, Laura G.[2], Carter, Gregory W.[2]

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Mouse embryonic stem cells can display highly variable molecular phenotypes, depending on their genetic background. These phenotypes include genome-wide differences in chromatin openness and local regulatory activity, with varying and sometimes unpredictable effects on gene expression. Molecular genetic studies have demonstrated genetic-epigenetic interactions between genetic variants, epigenetic state, and downstream gene expression, but the vast majority of these studies focus on just a few loci, and limited genetic diversity. Our goal is to take advantage of genomic data from a genetically diverse population to analyze these regulatory mechanisms on a whole-genome scale, and to use this information to infer and define the regulatory boundaries around all expressed genes. This could help us understand how these regulatory limits relate to the maintenance of pluripotency, and broader biological function in mESCs. We used genome wide gene expression and chromatin state data from a panel of 176 unique and genetically diverse mouse embryonic stem cell lines to investigate the presence, patterning and functional implications of statistically defined genetic-epigenetic interactions. We found individual interactions acting on single genes, and patterns of interactions defining regulatory regions. By linking genetic variation to epigenetic state, we defined interactions around genes that match with previously identified Topologically Associating Domains. We also uncovered genetic-epigenetic interactions at TAD boundaries flanking genes that are differentially expressed in mESCs from different genetic backgrounds, and that underlie known differences in pluripotency and differentiation propensity. These results were made possible by the greater specificity afforded by a well-characterized, genetically diverse sample population, providing us a means to better understand the molecular basis of ESC states. This provides a potential roadmap to those seeking to study complex developmental regulatory programs determined by epigenetic states.

Funding Source: NIGMS R01 GM115518

Keywords: genetically diverse model, epigenetic regulatory interaction, pluripotency and differentiation propensity

TWO DISTINCT MODES OF CIS REGULATION CONTROL CELL SPECIFICATION IN RESPONSE TO SHH DURING MOUSE SPINAL CORD DEVELOPMENT

Delas, M Joaquina, Demuth, Madeleine, Briscoe, James

Developmental Dynamics, The Francis Crick Institute, London, UK

Morphogen gradients act in a concentration-dependent manner in many developing tissues to organise the pattern of cellular differentiation. How a single signal is interpreted by responding cells to establish multiple cell-type specific gene expression programmes remains unclear. Ventral regions of vertebrate spinal cord serve as an example of this process. In this tissue, the morphogen Shh and its transcriptional effectors, the Gli family of proteins, control the expression of a set of transcription factors (TFs). These, in turn, regulate each another through repressive interactions to determine cell type identity. Using a cellular model of mouse spinal cord progenitor specification, we developed assays of cell type specific chromatin accessibility and gene regulation. These data revealed that the majority of cell types in the neural tube, whether or not they have been exposed to Shh signalling, share the same cis regulatory topology. This suggests that these progenitors use the same regulatory logic and that the accessible cis regulatory elements act as the information processing devices decoding cell type identity based on the TFs expressed in a cell. By contrast the ventral-most progenitor type, p3 cells, which are the only cell type to depend on Gli activation for their specification, display a dramatically different set of accessible regulatory elements. We propose that this indicates a distinct regulatory topology and consequently p3 cells interpret Shh differently. These data provide molecular evidence for the coexistence of two morphogenetic fields in the ventral spinal cord. This reveals an unexpected relationship between cis regulatory configuration and molecular identity and supports the existence of two different modes of Shh morphogen interpretation for the allocation of cell type identity in the spinal cord.

Keywords: cis regulation, chromatin, Shh

GENOME-WIDE CRISPR-CAS9 SCREENING UNCOVERS THE POLYCOMB COMPLEX PRC1.3 AS AN ESSENTIAL REGULATOR OF NAÏVE HUMAN PLURIPOTENT CELL REPROGRAMMING

Bendall, Adam[1], Collier, Amanda[2], Fabian, Charlene[1], Malcolm, Andrew[1], Tilgner, Katarzyna[3], Semprich, Claudia[1], Wojdyla, Katarzyna[1], Nisi, Paola[1], Kishore, Kamal[4], Franklin, Valar[5], D'Santos, Clive[5], Yusa, Kosuke[6], Rugg-Gunn, Peter[1]

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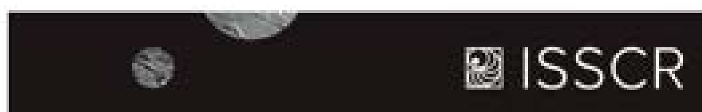
Human pluripotent stem cells (hPSCs) exist in naïve and primed states, which possess distinct transcriptomes, epigenomes and differentiation potential. As naïve hPSCs capture an earlier developmental state in culture than primed hPSCs, they possess several unique and sought-after properties including their ability to differentiate into extra-embryonic cell types and to model events such as X chromosome inactivation. Naïve hPSCs are most commonly generated by primed-to-naïve cell reprogramming, but this process is inefficient and creates heterogeneous cell populations. Our incomplete understanding of the biology underpinning naïve cell reprogramming means that unlocking the full potential of naïve hPSCs remains challenging. To overcome this gap, we



used genome-wide CRISPR-Cas9 loss of function screening to identify novel regulators of primed to naive hPSC reprogramming. Unexpectedly, amongst the top candidates identified as essential for naive cell reprogramming were all of the components of the Polycomb Repressive Complex 1 (PRC1) subtype PRC1.3. PRC1.3 is a chromatin modifying enzyme, and has not been studied previously in human pluripotency or reprogramming. To investigate the requirement for PRC1.3 in these contexts, we deleted the core PRC1.3 complex component PCGF3 in primed hPSCs and comprehensive characterisation showed that PRC1.3 is dispensable for human primed pluripotency, but is essential for primed-to-naive reprogramming, as predicted by our genetic screen. Mechanistic analyses using ChIP-sequencing, RNA-sequencing and proteomics identified a group of key genes that normally gain PRC1.3 occupancy during reprogramming and become transcriptionally repressed. This function is orchestrated by a naive-specific interaction between PRC1.3 and PRDM14. A failure to repress this cohort of genes is characteristic of a failure to successfully reprogramme. We also identified a new developmentally-regulated switch in PRC1.3 complex composition that occurs between the primed and the naive state, corroborated in single cell RNA-sequencing data in hPSCs and in vivo in early human embryos. These findings provide novel insight into regulators that establish naive pluripotency in human cells and the mechanisms that are employed to reconfigure cell identity during cell state transitions.

Funding Source: This project is funded by the Biotechnology and Biological Sciences Research Council (BBSRC) and Medical Research Council (MRC).

Keywords: Pluripotency, Reprogramming, CRISPR-Cas9

EPIGENETIC MECHANISMS OF CELLULAR PLASTICITY AND REPROGRAMMING TO TOTIPOTENCY

Torres-Padilla, Maria Elena

Institute of Epigenetics & Stem Cells, Helmholtz Zentrum München, Germany

Totipotency is a fundamental cellular feature. In mammals, the terminally differentiated sperm and oocyte fuse to create a totipotent zygote upon fertilisation. The mechanisms underlying the epigenetic reprogramming towards totipotency that follows fertilisation are not fully understood, and the molecular features of totipotent cells remain scarce. Embryonic cells remain totipotent only for a restricted time window. During this time, embryonic cells are characterised by an atypical chromatin structure and reactivation of specific families of retrotransposons. Recently, it was reported that totipotent-like cells arise in ES cell cultures in vitro. Like in the embryo, these cells are characterised by the expression of MERVL LTR retrotransposons. To address how the expression of these elements is regulated during the transition between totipotent and pluripotent states, we first examined histone modifications and chromatin structure in early mouse embryos. Remarkably, we have found that specific features of embryonic chromatin are also present in totipotent-like cells in vitro. Based on this analysis, we have begun to decipher key molecular regulators of repetitive elements in the embryo, and how they contribute to shaping the regulatory programme of the newly formed embryo. Our results have identified candidate pathways that regulate chromatin function and expression of these elements and show that they can promote totipotency. We will present our latest results that reveal a new role for chromatin integrity in promoting epigenetic reprogramming and sustaining molecular features of totipotent cells in vivo.

Keywords: epigenetics, reprogramming, totipotency

THEME SESSION MDD 3 (MODELING DEVELOPMENT AND DISEASE) MODELING DISEASE 7:30 - 9:15 EDT REBROADCAST WITH LIVE CHAT 16:30 - 18:15 AND 19:30 - 21:15 EDT

MECHANISMS DRIVING CARDIOMYOCYTE PROLIFERATION DURING ZEBRAFISH HEART REGENERATION

Bakkers, Jeroen

Cardiac Development, Regeneration and Disease, Hubrecht Institute, Utrecht, Netherlands

Myocardial infarction causes permanent scarring due to the irreversible loss of heart muscle tissue. Recent findings suggest that stimulating cardiomyocyte proliferation has the potential to repair the damaged myocardium and but very little is known about the mechanisms and cellular processes occurring when adult cardiomyocytes re-enter the cell cycle. Unlike mammals, zebrafish have the natural capacity to regenerate their hearts after injury without the formation of a permanent scar due to the induction of cardiomyocyte proliferate to replace the missing tissue. We are using the zebrafish model to identify mechanisms and cellular processes that that drive adult cardiomyocyte proliferation using single-cell RNA-sequencing in combination with genetic tools. We observed that proliferating cardiomyocytes represent embryonic cardiomyocytes and that they undergo neuregulin-induced metabolic reprogramming from oxidative phosphorylation (OXPHOS) to glycolysis, which is required for cell cycle re-entry. Interactions with other cell types such as fibroblasts play important roles in regulating cardiomyocyte proliferation and scar resolution, which I will discuss further.

Keywords: regeneration, heart, zebrafish

MOLECULAR AND FUNCTIONAL SIGNATURES ASSOCIATED WITH 16P11.2 RECIPROCAL GENOMIC DISORDER: INSIGHTS INTO NEURODEVELOPMENTAL DISORDERS

Tai, Derek[1], Erdin, Serkan[2], Gao, Dadi[2], Wang, Jennifer[2], Razaz, Parisa[2], Mohajeri, Kiana[2], Aneichyk, Tatsiana[2], Lim, Elaine T.[3], Nuttle, Xander[2], de Esch, Celine E.[2], Currall, Benjamin B.[2], O'Keefe, Kathryn[2], Yadav, Rachita[2], Ragavendran, Ashok[2], Stortchevoi, Alexei[2], Morini, Elisabetta[2], Ma, Weiyuan[2], Arbogast, Thomas[4], Hastie, Alex[5], Kelleher, Raymond J.[2], Perlis, Roy H.[2], Gusella, James F.[2], Talkowski, Michael E.[2]

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Recurrent copy number variants (CNVs) of chromosome 16p11.2 represent a reciprocal genomic disorder (RGD) associated with neurodevelopmental disorders and psychiatric phenotypes including intellectual disability and autism spectrum disorder (ASD). However, the pathogenic mechanisms and functional relevance of plausible individual genetic drivers within 593 kb 16p CNV region are not known. To systemically dissect molecular functions associated with 16p RGD, we performed transcriptome analyses of 101 mice with reciprocal CNVs of the syntenic chromosome 7qF3 region across cortex, striatum, and cerebellum, as well as three non-brain tissues. Furthermore, we generated neuron and

cerebral organoid derivatives (n = 46) of CRISPR-engineered human iPSC disease model and assessed functional and single-cell signatures associated with 16p RGD. Differentially expressed genes (DEGs) and pathway enrichment analysis revealed that the strongest and most consistent effects occurred within the CNV segment. Across various brain regions in adult mouse and human neuron models, DEGs were enriched for ASD-associated genes, synaptic vesicle exocytosis, regulation of translation at synapse. Overall, our results suggested some commonalities in transcriptional signatures between reciprocal CNVs, but most expression changes were tissue, genotype, and cell-type specific. Notably, DEGs across various models were broadly related to cell differentiation and fate commitment, cytoskeleton, and neuron development, suggesting neurogenesis and associated functional deficits are implicated in neuropathology of disease. To further address the underlying mechanisms, we focused on functional and developmental assessment of human neuron models. Cellular phenotyping revealed significant reduction of neurite length and branchpoints, as well as aberrant electrophysiological features in the neurons carrying 16p CNV. Cerebral organoid models displayed abnormal neurodevelopmental signatures with altered cell geometry and reciprocal responses to 16p CNVs, implicating neuron fate commitment and imbalanced brain signaling in disease pathogenesis. Collectively, our study suggests disease-associated molecular and functional signatures associated with this highly penetrant reciprocal genomic disorder.

Funding Source: NIH/NINDS 5R01NS093200-05 NIH/NICHHD 5R01HD096326-02

Keywords: 16p11.2, Transcriptome, copy number variation

SARS-COV-2 INFECTION OF HUMAN IPSC-DERIVED CARDIAC CELLS PREDICTS NOVEL CYTOPATHIC FEATURES IN COVID-19 PATIENTS

Rockwood, Sarah J.[1], Perez-Bermejo, Juan A.[2], Kang, Serah[2], Simoneau, Camille R.[3], Joy, David A.[2], Ramadoss, Gokul N.[2], Silva, Ana C.[2], Flanigan, Will R.[2], Li, Huihui[4], Nakamura, Ken[4], Whitman, Jeffrey D.[5], Ott, Melanie[3], Conklin, Bruce R.[2], McDevitt, Todd C.[2]

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Over 25% of hospitalized COVID-19 patients display evidence of acute myocardial injury, which correlates with up to ten-fold higher odds of in-hospital mortality, even in cases with no history of cardiovascular disease. Additionally, among survivors, many patients develop long-term cardiac dysfunction that persists months after infection. Together, these observations suggest that SARS-CoV-2 may cause de novo damage in the heart. However, the pathogenesis of COVID-19 in the heart, including cell-specific susceptibility to viral exposure, is unknown. Here, we show that SARS-CoV-2 productively and robustly infects human induced pluripotent stem cell (iPS)-derived cardiomyocytes (iPS-CMs), but fails to productively infect iPS-fibroblasts or iPS-endothelial cells. Through targeted inhibition assays, we determined that SARS-CoV-2 uses an ACE2-, CTSL-dependent endolysosomal route to infect iPS-CMs rather than the TMRPSS2 route common to other cell types. Microscopic analysis of infected iPS-CMs revealed a severe and distinct pattern of myofibrillar fragmentation, as well as numerous iPS-CMs lacking nuclear DNA. These morphological disruptions aligned with the transcriptional dysregulation of structural and nuclear proteins within infected cardiomyocytes. Human autopsy samples from COVID-19 patients displayed sarcomeric abnormalities similar to the sarcomeric disruption observed in vitro, as well as cardiomyocytes without nuclear DNA staining. These observations underlie the importance of developing cardioprotective therapies to reduce both acute and long-term consequences of COVID-19 in the heart, and to prevent a rise in heart disease in both severe and asymptomatic cases. The striking cytopathic features we observed provide key insights into the early pathology of SARS-CoV-2-induced cardiac damage and offer a high-throughput cell-based screening platform with in vivo relevance for the discovery and validation of therapeutic strategies to combat COVID-19.

Funding Source: This work was supported by U01 ES032673-03, R01-HL130533, R01-HL13535801, P01-HL146366, as well as gifts from the Roddenberry Foundation and from Pauline and Thomas Tusher.

Keywords: human induced pluripotent stem cells, cardiac, COVID-19

ORGANOID MODELS OF NORMAL AND MALIGNANT PULMONARY NEUROENDOCRINE CELLS REVEAL PATHWAYS IMPORTANT FOR NEUROENDOCRINE CELL GROWTH, DIFFERENTIATION, AND TRANSFORMATION

Dayton, Talya L.[1], Alcala, Nicolas[2], Den Hartigh, Lisanne[1], McFaline, José Luis[3], Moonen, Laura[4], Levy, Sonja[5], Mangiante, Lise[2], Derks, Jules[6], Hackeng, Wenzel[7], van Den Berg, José[8], Buikhuisen, Wieneke[9], Brosens, Lodewijk[7], Tesselaar, Margot E.[5], Valk, Gerlof D.[10], Vriens, Menno R.[11], Speel, Ernst Jan[4], Foll, Matthieu[2], Fernandez-Cuesta, Lynette[2]

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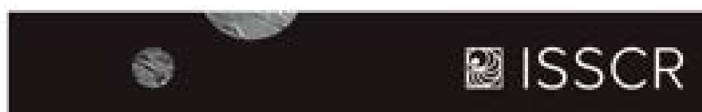
Organoid cultures are a powerful model system for the study of cell biology and human disease. Recently, we have applied the organoid culture system to study neuroendocrine (NE) cells and tumors of the lung. A paucity of in vitro and in vivo models has limited the study of NE cell derived tumors, Neuroendocrine neoplasms (NENs) and little is known about normal NE cells and how they contribute to NEN formation. We have generated the first patient derived tumor organoids (PDTOs) of low grade lung NENs, typical and atypical carcinoids. We have also generated PDTOs from high grade NENs and from a rare only recently identified lung NET subtype, a "supra-carcinoid." In parallel, we have established fetal airway organoids (AOs) that contain the putative cells of origin for lung NENs, pulmonary neuroendocrine cells (PNECs). The in vitro growth of our lung NEN PDTOs recapitulates what is observed for different lung NEN subtypes in patients. Genomic analysis of lung NEN PDTOs and matched primary tumors shows that PDTOs maintain the intratumor heterogeneity of the primary tumor. Ongoing analyses are aimed at elucidating the evolutionary



trajectory of lung NENs. Through modification of lung NEN PDTO growth media we have defined small molecule components that either promote or inhibit NEN cell growth in culture, suggesting potential therapeutic vulnerabilities. In parallel, we have identified signals that induce the differentiation PNEC precursors in fetal AOs to produce significant numbers of differentiated PNECs. To resolve transcriptional changes during PNEC differentiation and to further define PNECs, we are performing single cell RNA-seq of fetal AOs during the course of induced PNEC differentiation. These novel organoid culture systems are being used for further molecular and genetic analyses. Lung NEN PDTOs and PNEC-enriched fetal AOs are novel preclinical in vitro models for the study of neuroendocrine biology and disease.

Funding Source: NETRF Accelerator Award, MSCA Individual Fellowship, Longfonds BREATH Consortium

Keywords: pulmonary neuroendocrine cells, Neuroendocrine Neoplasms, Airway organoids

DISSECTING THE IMPACT OF REGIONAL IDENTITY IN A HUMAN ESC-BASED MODEL OF H3.3G34R-MUTANT HIGH-GRADE GLIOMA

Funato, Kosuke[1], Smith, Ryan C.[2], Saito, Yuhki[3], Tabar, Viviane[1]

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H3.3G34R is a recurrent mutation in the histone H3F3A gene in high-grade glioma (HGG). H3.3G34R-mutant HGGs mostly arise in the cortex of the brain, while H3K27M-mutant HGGs are confined to the brainstem. We hypothesized that oncogenic activity of the H3.3G34R mutation depends on the regional identity of the cell type of origin. To test this hypothesis, we took advantage of human embryonic stem cell (ESC) system, which enables us to derive various region-specific neuronal cell types, and developed a new ESC-based model of H3.3G34R-mutant HGG. Cancer genetics data indicate that the vast majority of H3.3G34R-mutant HGGs harbor loss-of-function mutations in both TP53 and ATRX. To emulate these genetic alterations, we developed a series of genetically engineered ESC lines that harbor different combinations of the three mutations (i.e., H3.3G34R, TP53, and ATRX). We next carried out computational analyses that demonstrated a significant enrichment of gene-expression signatures associated with inhibitory interneuron progenitors of the developing ventral forebrain. Based on these data, the ESC lines were differentiated into interneuron progenitors and, as a control cell type, ventral hindbrain progenitors using defined protocols developed in our lab. Our model shows that H3.3G34R, ATRX, and TP53 mutations cooperatively block differentiation and maintain proliferation in forebrain interneuron progenitors, but not in hindbrain progenitors, replicating the regional specificity of patient tumors. RNA-seq data show region-specific effects of the mutations on the transcriptional profile and alternative RNA splicing patterns that lead to the activation of the Notch signaling pathway only in the forebrain cells. In particular, our data demonstrate that human-specific NOTCH2NL genes are upregulated by the mutations and contribute to tumorigenicity. Additionally, we identified genomic amplification of the NOTCH2NL locus in H3.3G34R-mutant patient samples, which further supports that NOTCH2NL acts as an oncogene in HGGs. Taken together, our study sheds new insight into the context-dependent activity of the H3.3G34R mutation and suggest new mechanisms for the activation of key developmental pathways that contribute to gliomagenesis.

Funding Source: K.F. was supported by a Druckenmiller Fellowship from the New York Stem Cell Foundation and by Center for Stem Cell Biology at MSKCC. V.T. is supported by NIH/NCI R01 CA208405. R.C.S. was supported by NIH/NCI F31 CA210408.

Keywords: Pediatric and young adult brain tumor, Histone-mutant glioma, Pluripotent stem cell-based cancer models

CRISPR/CAS9-EDITED HUMAN IPSC-CM IN ENGINEERED HEART TISSUES REPRODUCE HALLMARKS OF PRIMARY CARNITINE DEFICIENCY

Loos, Malte[1], Klampe, Birgit[1], Schulze, Thomas[1], Behrens, Charlotta[1], Ulmer, Bärbel[1], Laufer, Sandra[1], Yin, Xiaoke[2], Mayr, Manuel[2], Eschenhagen, Thomas[1], Hansen, Arne[1]

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Primary carnitine deficiency (PCD) is a recessive monogenic disease due to mutations in the gene SLC22A5, encoding for the carnitine transporter OCTN2. Without life-long carnitine supplementation, PCD patients suffer from muscular weakness and dilated cardiomyopathy. Reduced carnitine uptake was accounted to cause decreased mitochondrial lipid energy reserve or cytosolic lipotoxicity. However, currently available PCD models were not able to distinguish causative from secondary pathomechanisms. The aim of this study is to utilize CRISPR/Cas9-edited human induced pluripotent stem cell- derived cardiomyocytes (hiPSC-CMs) to establish a human PCD model. Control hiPSC lines were genetically edited by CRISPR/Cas9 to introduce the missense mutation c.95A>G homozygously (p.N32Shom) and a knockout of the SLC22A5 gene (OCTN2-/-). Cardiomyocytes were differentiated with an embryoid body-based protocol and the iPSC-CM-based engineered heart tissue (EHT) technology served as a three-dimensional disease model. Video optical analysis of OCTN2-/- and p.N32Shom showed significantly lower force of contraction compared to the isogenic control. OCTN2-/- and p.N32Shom were instable in medium only containing long-chain fatty acids and had a higher glucose consumption in medium containing serum and glucose. Carnitine supplementation (high concentration) led to a significant increase in force and reduction in glucose uptake. Quantitative proteomic analysis revealed an upregulation of proteins involved in cholesterol metabolism and the hexamine biosynthetic pathway as well as a paradoxical down regulation of pyruvate oxidation and glycolysis. The theory of an impaired pyruvate metabolism was supported by an increase of pyruvate dehydrogenase kinase 4 on mRNA level. Taken together, OCTN2-deficient EHTs reproduce the phenotype of PCD patients, what was reverted by carnitine supplementation, underscoring the value of these CRISPR-edited hiPSC-CM as human PCD disease model in a dish. Markedly metabolic alteration in PCD-EHTs demonstrated by higher glucose uptake as well as proteomic analysis gave first mechanistic insight. With further experiments, like Seahorse Mito Stress Test and assessment of acylcarnitine and ceramide tissue content, we will utilize this model to gain a better understanding of PCD.

Keywords: IPSC-Cardiomyocytes, Metabolism, Disease modeling

MODELING INTER- AND INTRA-TUMOR HETEROGENEITY USING PATIENT-DERIVED GLIOBLASTOMA ORGANOIDS

Ming, Guo-li

Neuroscience and Psychiatry, University of Pennsylvania, Philadelphia, PA, USA

Glioblastoma is the most prevalent malignant brain tumor in adults and remains almost invariably lethal due to its aggressive and invasive nature. It is well known that glioblastomas exhibit vast inter- and intra-tumoral heterogeneity. We have developed a novel approach to rapidly generate glioblastoma organoids (GBOs) in a defined culture medium directly from fresh tumor specimens. We found that these GBOs recapitulate the histological features, cellular diversity, gene expression, and mutational profiles of their corresponding parental tumors. Furthermore, these GBOs can be efficiently xenografted into the adult mouse brain, displaying rapid and aggressive infiltration and maintaining key driver mutation expression.

Keywords: glioblastoma, glioblastoma organoids, CART

THEME CI (CELLULAR IDENTITY)
PLENARY V: CELLULAR IDENTITY
9:30 - 11:00 EDT
REBROADCAST WITH LIVE CHAT 21:30 – 23:00 EDT

PRESERVING CELLULAR IDENTITY AND EPIGENETIC MEMORY THROUGH MITOSIS

Fisher, Amanda G.[1], Djeghloul, Dounia[2], Cheriyamkunnel, Sherry[1], Dimond, Andrew[1], Brown, Karen[2], Patel, Bhavik[3], Kramer, Holger[4], Merkenschlager, Matthias[2]

[1]*Institute of Clinical Sciences, Imperial College London, UK,* [2]*Lymphocyte Development, UKRI MRC London Institute of Medical Sciences, London, UK,* [3]*Flow Cytometry, UKRI MRC London Institute of Medical Sciences, London, UK,* [4]*Proteomics and Metabolomics, UKRI MRC London Institute of Medical Sciences, London, UK*

As cells enter mitosis, chromosomes condense prior to nuclear envelope breakdown. Although some DNA-associated factors are displaced, others remain chromosome-bound and may serve to 'bookmark the genome' and ensure that cellular identity is accurately conveyed to daughter cells. We have developed convergent technologies that enable the factors bound to individual mitotic chromosomes to be quantified and compared in different cell types, and to probe their functional relevance. We are using these approaches to examine the mitotic profiles of stem cells and their differentiated progeny, to understand the dependency of mitotic bookmarking on underlying chromatin features, and to examine differences between the active and inactive X chromosomes in mitosis. Long-term we will use such information to design novel strategies aimed at epigenetically altering cellular identity or changing gene expression, through targeted intervention during mitotic cell division.

Funding Source: MRC core funding MC_A652_5PY20

Keywords: epigenetic memory, active and inactive X chromosome, mitotic bookmarking

ILLUMINATING NEURAL STEM CELL TRAJECTORIES AT SINGLE CELL RESOLUTION

Ngai, John

NINDS, National Institutes of Health, Bethesda, MD, USA

The generation of neuronal diversity in the nervous system requires the specification and differentiation of a multitude of cellular lineages. The regulatory programs governing the differentiation of mature neurons from their progenitors remain incompletely characterized, however, in part because of the difficulty in studying neuronal progenitor cells in their native environments. In the vertebrate olfactory system, primary sensory neurons are continuously regenerated throughout adult life via the proliferation and differentiation of multipotent neural stem cells. Upon severe injury, these adult tissue stem cells are activated and go on to reconstitute all of the cellular constituents of this sensory epithelium. The regenerative capacity of the olfactory epithelium therefore presents a powerful and experimentally accessible paradigm for elucidating the mechanisms regulating neural stem cell function. I will present recent studies employing single cell transcriptomic and epigenomic analyses that give insights into the genetic and epigenetic programs that both define and regulate olfactory neurogenesis during regeneration.

Keywords: olfactory, neural stem cell, regeneration

NEW SINGLE-CELL TECHNOLOGIES TO DISSECT REPROGRAMMING AND DEVELOPMENT

Morris, Samantha

Genetics and Developmental Biology, Washington University School of Medicine, Saint Louis, MO, USA

Direct lineage reprogramming involves the remarkable conversion of cellular identity. Single-cell technologies aid in deconstructing the considerable heterogeneity in transcriptional states that typically arise during lineage conversion. However, lineage relationships are lost during cell processing, limiting accurate trajectory reconstruction. We previously developed 'CellTagging', a combinatorial cell indexing methodology, permitting the parallel capture of clonal history and cell identity, where sequential rounds of cell labeling enable the construction of multi-level lineage trees. CellTagging and longitudinal tracking of fibroblast to induced endoderm progenitor (iEP) reprogramming reveals two distinct trajectories: one leading to successfully reprogrammed cells, and one leading to a dead-end state. Here, I present two new methods to enable the molecular mechanisms underlying reprogramming outcome to be dissected. The first is an experimental method, 'Calling Cards', enabling transcription factor binding to be recorded, in individual cells, in the earliest stages of reprogramming. The second method is a new computational platform, called 'CellOracle', that uses single-cell transcriptome and chromatin accessibility data to reconstruct changes in GRN configurations across the reprogramming process. Together, these tools provide new mechanistic insights into how transcription factors can drive changes in cell identity, and help reveal new factors to enhance the efficiency and fidelity of reprogramming.

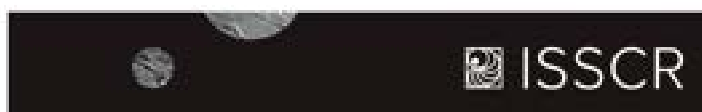
Keywords: Single-cell lineage tracing, Direct lineage reprogramming, Gene regulatory networks

TRANSCRIPTIONAL AND EPIGENETIC BASIS OF INTESTINAL CRYPT CELL PLASTICITY

Shivdasani, Ramesh A.

Medicine, Dana Farber Cancer Institute and Harvard Medical School, Boston, MA, USA

Stem cells in continuously self-renewing digestive epithelia provide one paradigm to understand cellular identities and intermediate cell states. Ablation of the intestinal stem cell (ISC) compartment has surprisingly few immediate consequences because new stem cells regenerate rapidly. Although a dedicated pool of 'reserve' stem cells was previously regarded as the principal source of this restoration, the weight of evidence now indicates that new ISCs arise by dedifferentiation of their recent



progeny. Our laboratory studies the cis-regulatory and transcriptional underpinnings of forward ISC differentiation into absorptive or secretory cell lineages and of those specified daughter cells' reverse differentiation into ISCs. These studies are considerably facilitated by genetic mouse models for reliable lineage tracing, coupled with sensitive methods to map tissue-restricted cis-regulatory elements at high resolution. In forward differentiation, these approaches reveal the likely actions of key transcription factors at lineage branch points, where selected incipient cis-regulatory (enhancer) modules are stabilized and others are concomitantly suppressed. The sum of these activities establishes specific cell types and preserves their identities, while maintaining the latent plasticity that readily allows dedifferentiation when stem cells are lost. The growing recognition of cell plasticity in diverse adult epithelia suggests that the underlying mechanisms are broadly conserved. The presentation will discuss how findings in intestinal crypts reveal underlying principles. We will discuss experimental models, key methodological advances, and findings from highly dynamic adult intestinal crypt cells that modulate or reverse their identities under conditions of stem cell loss or withdrawal of crucial transcription factors.

Keywords: intestinal stem cells, epithelial plasticity, gene regulatory elements

ISSCR MOMENTUM AWARD SESSION 13:15 - 13:45 EDT

PRINCIPLES OF REGENERATION CAPTURED BY IMAGING THE SKIN OF LIVE MICE

Greco, Valentina

Department of Genetics, Yale Medical School, New Haven, CT, USA

My lab and I are honored to receive the ISSCR Momentum Award for the scientific discoveries we have made. It takes a team of scientists to make discoveries, and this award recognizes our team. Although often a polite custom to acknowledge lab members, I want to emphasize that the work this award recognizes was possible because of the talent and continuous intellectual contributions of my lab members. The ability of my lab to make innovative discoveries arises from my lab members' willingness to offer feedback on my ability to create and maintain a culture of inclusivity, which in turn supports their drive, creativity and scientific discoveries. Through this iterative process, together we tackle fundamental principles of organ regeneration during homeostasis and the emergence of aberrant tissue growth. For a long time, a major challenge in the mammalian stem cell field was the inability to follow the same cells in vivo. This limitation obscured insight into the dynamics of these processes, the contributions of intercellular interactions to tissue growth, and the initial events leading to malignancy. To overcome this challenge, my lab established the ability to visualize skin epithelial stem cells in an intact animal over prolonged periods of time by two-photon microscopy. Looking at the skin epithelium, we have studied regeneration in real time by labeling, manipulating and tracking stem cells. Our studies have expanded to understanding the skin as a whole organ and the crucial functions that other cells, such as fibroblasts and immune cells, play in supporting skin function. These approaches have repeatedly led us to a significant number of discoveries, including but not limited to 1) stem cell position dictates their fate in the hair follicle, 2) a stem cell-mediated phagocytic clearance mechanism that regulates the size of the hair follicle stem cell pool and 3) the unanticipated plasticity of the skin epithelium to correct aberrant tissue growths induced by mutational and non-mutational insults. Moving ahead, we continue our commitment to a holistic approach to both scientists and science in order to understand how different tissue types in the skin both sustain their homeostasis as well as interact with epithelial stem cells that harbor cancerous mutations.

Keywords: skin, live imaging, regeneration

THEME SESSION MDD 4 (MODELING DEVELOPMENT AND DISEASE) MODELING DEVELOPMENT 14:00 - 15:45 EDT REBROADCAST WITH LIVE CHAT 02:00 – 03:45 EDT

CHARTING HUMAN DEVELOPMENT USING A MULTI-ORGAN ENDODERMAL ATLAS AND ORGANOID MODELS

Camp, Jarrett[1], Yu, Qianhui[1], Kilik, Umut[1], Holloway, Emily[2], Spence, Jason[2]

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Organs are composed of diverse cell types, which traverse transient states during organogenesis. To interrogate this diversity during human development, we generate a single-cell transcriptome atlas from multiple developing endodermal organs of the respiratory and gastrointestinal tract. We illuminate cell states, transcription factors, and organ-specific epithelial stem cell and mesenchyme interactions across lineages. We implement the atlas as a high-dimensional search space to benchmark pluripotent stem cell-derived human intestinal organoids (HIOs) in multiple culture conditions. We show that HIOs recapitulate reference cell states, and use HIOs to reconstruct the molecular dynamics of intestinal epithelium and mesenchyme emergence. We show that the mesenchyme-derived niche cue NRG1 enhances intestinal stem cell maturation in vitro and that the homeobox transcription factor CDX2 is required for regionalization of intestinal epithelium and mesenchyme in humans. Collectively, this work combines cell atlases and organoid technologies to understand how human organ development is orchestrated.

Keywords: Human Intestinal Organoids, Epithelial Stem Cell-Mesenchyme Crosstalk, Human Developmental Cell Atlas

EX UTERO DEVELOPMENT OF MOUSE EMBRYOS FROM PRE-GASTRULATION TO ADVANCED ORGANOGENESIS

Aguilera Castrejon, Alejandro, Oldak, Bernardo, Shani, Tom, Hanna, Jacob H.

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Establishment of the mammalian body plan occurs shortly after the embryo implants into the maternal uterus, and our understanding of post-implantation developmental processes remains limited. While methods for in vitro culture of pre- and peri-implantation mouse embryos are routinely utilized, approaches for robust culture of post-implantation embryos from egg cylinder stages until advanced organogenesis remain to be established. We develop herein highly stable ex utero post-implantation mouse embryo culture platforms, that enable appropriate development of embryos before gastrulation (E5.5) until the hind limb formation stage (E11). Late gastrulating embryos (E7.5) are grown in 3D rotating bottles settings, while extended culture from pre-gastrulation stages (E5.5 or E6.5) requires a combination of novel static and rotating bottle culture protocols. Histological, molecular, and single cell RNA-seq analysis validate that the ex utero developed embryos recapitulate precisely in utero development.

This culture system is amenable to introducing a variety of embryonic perturbations and micro-manipulations that can be followed ex utero for up to 6 days. Establishment of a system to robustly grow normal mouse embryos ex utero from pre-gastrulation to advanced organogenesis represents a valuable tool to investigate post-implantation embryogenesis, eliminating the uterine barrier to mechanistically interrogate morphogenesis and tissue specification in mammals.

Keywords: Mouse embryo, In vitro development, Organogenesis

NEURO-IMMUNE ORGANOIDS FOR MODELING EARLY BRAIN DEVELOPMENT AND DISEASE

Popova, Galina[1], Schmunk, Galina[1], Kim, Chang[1], Soliman, Sarah S.[1], Keefe, Matthew G.[1], Shin, David[1], Jain, Samhita[2], Li, Tao[2], Tejera, Dario[3], Hengen, Keith[4], Ransohoff, Richard R.[5], Piao, Xianhua[2], Nowakowski, Tomasz J.[1]

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Many fundamental questions about microglia function during brain development in humans remain unanswered as we lack experimental strategies to interrogate their function and responses ex vivo. Xenotransplantation of microglia into brain organoids that develop largely without myeloid lineage cells allows a systematic comparison of neurodevelopmental processes in the presence or absence of microglia. By developing a 'neuro-immune' cerebral organoid model, we systematically characterized 3D brain organoids transplanted with primary human microglia and showed that microglia integrate into the organoids and affect brain development. By performing scRNAseq analysis between transplanted and control organoids, we detected subtle but statistically significant differences at transcriptional level in a cell type-dependent manner. Unexpectedly, we found widespread expression of genes involved in type I interferon response in organoid radial glia, which becomes attenuated in the presence of microglia. In contrast, neurons appear transcriptionally invariant to microglia transplantation, but instead undergo synaptic remodeling and an accelerated maturation of network properties. Functional network activity recordings in brain organoids identified a role for human microglia in modulating synaptic density and accelerating the emergence of synchronized oscillatory network activity, similarly to what has been reported in maturing cortical organoids. In agreement with the role of microglia in synaptic pruning, we detected synaptic material inside of the microglia cells and overall reduction of synaptic clefts in microglia-transplanted organoids. Together, our work reveals transcriptional and functional responses of the developing human neural tissue to the presence of microglia and establishes a novel model for studying neuro-immune interactions and developmental diseases in a context of human tissue.

Funding Source: NRSA F32 1F32MH118785 to Galina Schmunk Chan Zuckerberg Biohub Intercampus Investigator Award to Tomasz Nowakowski

Keywords: organoids, microglia, neuro-immune

CARDIOIDS REVEAL SELF-ORGANIZING PRINCIPLES OF HUMAN CARDIOGENESIS

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Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Vienna, Austria

Organoids capable of forming tissue-like structures have transformed our ability to model human development and disease. With the notable exception of the human heart, self-organizing organoids have been reported for all major organs. Here, we established self-organizing cardioids from human pluripotent stem cells that intrinsically specify, pattern and morph into chamber-like structures containing a cavity. Cardioid complexity can be controlled by signaling that instructs the separation of cardiomyocyte and endothelial layers, and by directing epicardial spreading, inward migration and differentiation. We find that cavity morphogenesis is governed by a mesodermal WNT-BMP signaling axis and requires its target HAND1, a transcription factor linked to developmental heart chamber defects. Upon cryoinjury, cardioids initiated a cell type-dependent accumulation of extracellular matrix, a pathological hallmark of heart disease. Thus, human cardioids represent a powerful platform to mechanistically dissect self-organization, congenital heart defects, and serve as a foundation for future translational research.

Funding Source: Austrian Academy of Sciences (OEAW) and Austrian Technology Fund (FFG)

Keywords: Cardioids, Cardiac organoids, Cardiac defects and injury

GENERATION OF FUNCTIONAL HUMAN KIDNEY ORGANOIDS FROM METANEPHRIC NEPHRON PROGENITORS AND URETERIC BUD CELLS SEPARATELY DIFFERENTIATED FROM HUMAN IPS CELLS

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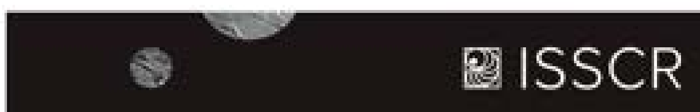
About 700 million patients suffered from chronic kidney disease (CKD) worldwide. The lack of effective curative options has led to research on regenerative therapies using stem cells. Accordingly, recent studies using human induced pluripotent stem cells (hiPSCs) have developed protocols to induce kidney-lineage cells and reconstruct kidney organoids. However, no reports have generated human kidney tissues by recapitulating nephrogenesis using metanephric nephron progenitors (NPs) and ureteric bud (UB) cells separately induced from hiPSCs, in which NP-derived glomeruli and renal tubules and UB-derived collecting ducts are interconnected. Furthermore, no in vivo imaging studies have directly demonstrated that hiPSC-derived kidney organoids produce urine. We separately and efficiently induced metanephric NPs and UB cells from hiPSCs in the original 2D differentiation culture conditions, co-cultured these two progenitors using bioreactors and performed immunofluorescent analysis using the CUBIC tissue clearing method. In addition, we transplanted mixed aggregates from the two progenitors into immunodeficient mice and examined them using in vivo multiphoton microscopy. After co-culture of the two progenitors, NPs constructed SIX1(+) active nephrogenic niches close to UB tips and S-shaped body-like structures. They further organized kidney structures that contained glomeruli, proximal and distal tubules, Henle's loops and collecting ducts in vitro and in vivo. By using two hiPSC lines that constitutively express fluorescent reporter proteins (GFP or mCherry), we demonstrated that the connecting points of GFP(+)

SPEAKER ABSTRACTS ▲ POSTER ABSTRACT GUIDE



NP-derived distal tubules and mCherry(+) UB-derived collecting ducts showed a marker expression pattern consistent with their counterparts in human embryonic kidneys, indicating that they were functionally interconnected. Furthermore, the intravenous injection of fluorescent-conjugated dextran confirmed that the hiPSC-derived glomeruli were functionally integrated with the host vasculature. Moreover, we observed urine-like dextran accumulation in the hiPSC-derived Bowman's space in vivo. Our culture system should contribute to the mechanistic elucidation of human nephrogenesis and the development of regenerative therapies against kidney diseases.

Keywords: kidney organoid, ureteric bud, collecting duct

VASCULARIZATION OF CARDIAC ORGANOIDS CONTROL THE EXTRACELLULAR MATRIX ENVIRONMENT AND REGULATES FUNCTIONALITY

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Crosstalk between cardiac cells is critical during heart development but its role in organ maturation is still largely uncharacterised. Here, we show that endothelial cells increase the force of contraction and enhance the expression of mature sarcomeric proteins and extracellular matrix (ECM) components in human pluripotent stem cell derived cardiac organoids (hCO). Endothelial cells regulate cardiac maturation and function both directly through secretion of ECM molecules and indirectly via paracrine signaling. Laminin α5, an endothelial enriched ECM protein, was identified as a key regulator of cardiac maturation and contractility in vitro. Loss-of-function studies in mice confirmed that Lama5 was required for myocardial expansion during heart development in vivo. In addition, paracrine PDGF signaling was identified as a mediator of increased ECM deposition and cardiac contractility in hCO. This study uncovers matrix regulatory functions of endothelial cells governing cardiac maturation and highlights the importance of multicellularity for organoid models.

Keywords: cardiac organoids, tissue engineering, endothelial cells

USING HUMAN IPSC-MICROGLIA AND CHIMERIC MICE TO STUDY THE GENETICS OF ALZHEIMER'S DISEASE

Blurton-Jones, Matt

University of California, Irvine, USA

Genome wide association studies (GWAS) strongly implicate microglia in the development and progression of Alzheimer's Disease (AD). Yet, murine homologues of AD-linked microglial genes often exhibit substantial differences from their human counterparts, limiting the potential utility of traditional mouse models. To address this challenge, we and others developed methods to differentiate human induced pluripotent stem cells (iPSCs) into microglia. By combining fully-defined microglial differentiation with CRISPR gene editing of patient-derived iPSC lines, studies can now examine the impact of AD risk genes on human microglial function in vitro. However, microglia are highly sensitive to their environment, exhibiting transcriptomic deficiencies when kept in isolation from the brain. To further study the transcriptomic and functional impact of AD risk genes in vivo, we recently developed a chimeric approach by transplanting iPSC-derived hematopoietic-progenitors into the brain of hCSF1-expressing immune-deficient mice. This approach results in context-dependent differentiation into microglia, acquisition of an ex vivo human microglial gene signature, and responsiveness to both acute and chronic insults. Notably, transplanted microglia also exhibit robust transcriptional responses to A-plaques. By combining this model with CRISPR-edited iPSCs, we have begun to examine the impact of two microglial-enriched AD risk genes (TREM2 and PLCG2). Taken together, our findings suggest that mutations associated with increased AD risk led to impaired induction off a disease-associate microglial (DAM) state. In contrast, an AD protective mutation promotes the induction of numerous MHC-II antigen presentation genes, a transcriptional state that appears to immediately proceed the development of the DAMs. Ongoing studies seek to further define the mechanisms by which these genes alter this important microglial response and whether Neurofibrillary Tangle pathology elicits a similar or differing transcriptomic program.

Keywords: Chimeric, Alzheimer's Disease, Microglia

THEME SESSION CI 4 (CELLULAR IDENTITY) PLURIPOTENCY DYNAMICS
14:00 - 15:45 EDT
REBROADCAST WITH LIVE CHAT 02:00 – 03:45 EDT

INTERCONVERSIONS BETWEEN NAIVE AND PRIMED PLURIPOTENCY AS MODELS TO STUDY CELL FATE CONTROL

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Naive and primed states of pluripotency, represented for example by mouse ESCs and EpiSCs, recapitulate two early stages of mammalian development. We have taken advantage of the interconversions between naive and primed states to investigate mechanisms controlling cell fate. We have shown recently that BMPs can convert mEpiSCs to mESCs through a network of TFs activated by BMPs. Through single cell sequencing, we show that BMPs drives mEpiSCs to a PGC-like state which can then become ESC-like under 2iL conditions. By analyzing the chromatin states of naive and primed states, we were surprised by the completely different architectures of these two states. Notably, the naive open chromatin loci are primarily enriched with the expected pluripotency TF motifs such as those for Oct4-Nanog-Sox families. On the other hand, the primed specific chromatin loci appear to be enriched with motifs from AP1 family, in addition to those of pluripotent TFs. To probe this further, we further show that cJUN, a archetypal AP1, can drive a very rapid exit from the naive state in an inducible system. This serves as a model to investigate mechanisms that regulate the pluripotent-somatic interface. One such mechanism appears to be driven by BAF complexes. Unpublished results in these interconnected areas will be presented and a model presented to show that naive and primed states are governed very differently.

Keywords: Naive pluripotency, primed pluripotency, AP1 transcription factors

COMPREHENSIVE MULTI-OMIC PROFILING REVEALS THE POLYCOMB REPRESSOR COMPLEX PRC2 RESTRICTS HUMAN NAIVE EPIBLAST TO TROPHOBLAST STEM CELL FATE INDUCTION

Pasque, Vincent[1], Zijlmans, Dick[2], Talon, Irene[1], Verhelst, Sigrid[3], Bendall, Adam[4], Van Nerum, Karlien[1], Janiszewski, Adrian[1], To, San Kit[1], Jansen, Joop[5], Knops, Ruth[5], Admiraal, Danielle[2], Panda, Amitesh[1], Bhanu, Natarajan[6], Deforce, Dieter[3], Garcia, Benjamen A.[6], Vermeulen, Michiel[2], Dhaenens, Maarten[3], Marks, Hendrik[2]

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Chromatin-based processes govern cell identity and developmental gene expression programs. Human naive and primed pluripotent states possess unique transcriptional and chromatin properties that reflect their distinct developmental identities. Their differentiation potential also differs as naive cells uniquely give rise to early extraembryonic trophectoderm cell types. Defining how chromatin-associated pathways control pluripotent cell identity and capacity for differentiation is important for the future applications of pluripotent cells. However, the characterization and functional assessment of chromatin-bound proteins and associated modifications in naive and primed human pluripotent states have not been performed in depth. Here, we apply an integrated multi-omics approach to comprehensively map the transcriptome and chromatin-associated proteome and histone post-translational modifications of naive and primed human pluripotent stem cells. We identify key chromatin regulators, transcription factors and DNA modifiers that define each pluripotent state. Integrating the chromatin-bound proteome and histone modification data sets reveals differences in the relative abundance and activities of distinct chromatin modules. We also find that the regulation of several major histone modifications is well-conserved between human and mouse pluripotent states, including the strong enrichment of H3K27me3 in naive chromatin, and we additionally identify human-specific regulators. Importantly, inhibiting Polycomb Repressive Complex 2 (PRC2) in naive cells results in substantial alterations in histone modification patterns but without inducing global changes in chromatin-associated proteins or in gene expression. Excitingly, we discover that PRC2 activity is a chromatin barrier that restricts human naive cell to trophoblast differentiation, and that PRC2 inhibition promotes trophoblast induction. Taken together, we have identified major differences in chromatin regulators that enforce the distinct identities of naive and primed human pluripotent states. Moreover, because chromatin-associated proteins are major regulators of embryo development and often perturbed in diseases, our findings will contribute to understanding these processes and their misregulation.

Funding Source: This work is supported by the FWO, KU Leuven, BBSRC, MRC, ERC and the Dutch Cancer Society.

Keywords: human pluripotency, chromatin, trophoblast

THE ETS TRANSCRIPTION FACTOR ERF CONTROLS THE EXIT FROM THE NAÏVE PLURIPOTENT STATE

Vega Sendino, Maria, Olbrich, Teresa, Tillo, Desiree, Tran, Andy D., Domingo, Catherine N., Franco, Mariajose, FitzGerald, Peter C., Kruhlak, Michael J., Ruiz, Sergio

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The naive epiblast undergoes a transition to a pluripotent primed state during embryo implantation. Despite the relevance of the FGF pathway during this period, little is known about the downstream effectors regulating this signaling. Here, we examined the molecular mechanisms coordinating the naive to primed transition by using inducible ESC to genetically eliminate all RAS proteins. We show that differentiated RASKO ESC remain trapped in an intermediate state of pluripotency with naive-associated features. Elimination of the transcription factor ERF overcomes the developmental blockage of RAS-deficient cells by naive enhancer decommissioning. Mechanistically, ERF regulates NANOG expression and ensures naive pluripotency by strengthening naive transcription factor binding at ESC enhancers. Moreover, ERF negatively regulates the expression of the de novo methyltransferase DNMT3B, which participates in the extinction of the naive transcriptional program. Collectively, we demonstrated an essential role for ERF controlling the exit from naive pluripotency during the progression to primed pluripotency.

Funding Source: Intramural Research Program (NIH).

Keywords: Naive pluripotency, Primed pluripotency, MAPK

EPITHELIAL TISSUE STRUCTURE REGULATES NAIVE AND PRIMED STATES IN HUMAN PLURIPOTENT STEM CELLS

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In their developmental progression from naive (inner cell mass) to primed (late epiblast) states during the peri-implantation stages, human pluripotent cells undergo significant morphological and epigenetic changes. Naive pluripotent cells in the pre-implantation blastocyst are unpolarized, and retain the ability to give rise to both germ and somatic lineages. By contrast, primed pluripotent cells in the late epiblast form a highly polarized epithelial bilaminar disk, and lose potency for germ cell differentiation. It is unknown whether changes in morphology are purely correlative to changes in pluripotency state, or whether they play a more significant regulatory role in defining pluripotent cell potency. In vitro, human pluripotent stem cells (PSCs) are cultured as epithelial sheets and recapitulate morphological and transcriptomic characteristics of primed pluripotent cells in the late epiblast. To test whether epithelial tissue structure impacts pluripotency state, we used CRISPR-interference to knock down (KD) the tight junction protein Zonula occludens-1 (ZO1), a critical regulator of apical/basolateral polarity, in human induced PSCs. ZO1 KD cells exhibited significantly reduced expression of the apical polarity marker Ezrin, and loss of barrier function (measured through FITC-Dextran transwell permeability studies), suggesting effective disruption of

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epithelial structure. Furthermore, ZO1 KD cells displayed transcriptomic signatures more similar to naive PSCs (significant increases in NANOG and DPPA3 expression, decrease in OTX2), along with a marked bias towards primordial germ cell specification upon differentiation with bone morphogenic protein-4 (BMP4). Our results indicate that tissue structure can directly regulate pluripotency state and may provide new insights into the mechanisms which guide exit from naive pluripotency.

Funding Source: Emergent Behaviors of Integrated Cellular Systems (CBET 0939511) California Institute of Regenerative Medicine (LA1_C14-08015)

Keywords: pluripotent stem cell, primordial germ cell, epithelium

CAPTURING PLURIPOTENT CELLS IN 3D SELF-RENEWING EPITHELIAL SPHEROIDS

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Pluripotent epiblast cells are the founder population of the embryo. When mammalian embryos implant in the uterus, pluripotent cells form an epithelial tissue that surrounds a central cavity. Epithelialization is fundamental for developmental progression and is conserved in all amniotes. However, current methodologies to culture pluripotent cells in vitro do not recapitulate the morphological features of pluripotent cells in vivo. Here, we report the establishment of a 3D culture method that captures pluripotent cells in epithelial self-renewing spheroids. 3D self-renewing spheroids closely recapitulate the organization and transcriptional profile of the epiblast of early post-implantation embryos. In terms of gene expression, single-cell sequencing analyses revealed that approximately 90% of the cells retain a post-implantation pluripotent identity, with the remaining 10% showing mesendoderm marker expression. In terms of tissue organization, approximately 75% of the spheroids are organized as a simple epithelial tissue. Immunofluorescence analyses uncovered a close correlation between spheroid disorganization and mesendoderm specification, highlighting the importance of epithelial tissue architecture for pluripotency maintenance. Moreover, epithelial organization also preserved genome integrity, as we observed a higher degree of chromosomal stability in our 3D spheroids in comparison to standard 2D pluripotent cultures. Our work establishes the 3D self-renewing spheroids as a tractable model of the post-implantation epiblast. We are currently applying this system to deconstruct the complexity of mammalian development.

Keywords: pluripotency, epithelial tissue, mouse embryo

AMNION-SPECIFIC MARKERS DEMARCATE THE REALM OF VARIOUS HUMAN PLURIPOTENT STATES

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Development proceeds through successive choices of alternative cell fates. Mammals share most of the fundamental design of cell lineage diversification during early development: from fertilized eggs to the trophectoderm (TE) and inner cell mass (ICM), from the ICM to epiblast (EPI) and hypoblast. Although the mouse EPI directly segregates into somatic lineages, other mammals, including primates, have an inserted diversification of the early EPI into the somatic lineage-segregating late EPI and amniotic epithelial (AME) cells. As most mammalian cell lineages were molecularly defined in the mouse, this pauses a complication for identifying the amnion lineage in other mammals. Here, by exploring deposited single-cell RNA-seq data of the human blastocyst's expanded cultures, we surprisingly found that most genes expressed in AME cells were also expressed in the TE-lineage cells. By avoiding AME/TE-dual markers, we imputed authentic human AME-specific markers such as PITX1, ACTC1, IGF2, UFC1, SMDT1, HAND1 and CDH2. CDH2 is also expressed in hypoblast cells, and CDH2-positive hypoblast-like cells were proposed to act as founder cells of human pluripotent cells in culture. However, its presence in amnion would merit revisiting the hypoblast-founder issue. Next, we searched the single-cell RNA-seq profiles of in-house-made human iPS cell (hiPSC) lines. We found that hiPSCs fell into two categories: one only expressed early EPI markers, and the other co-expressed early EPI and AME markers. We invariably found that hiPSC lines of the former EPI-only category have higher differentiation capabilities toward various cell types. Together, we deduced that human pluripotent stem cells best fit the early EPI stage of development, and the failure to repress the AME fate in these cells may lead to the bearers' developmental defects. Other research lines describe the "resetting" of EPI-stage human pluripotent stem cells toward blastocyst-stage epiblast (naive) or TE. We also discuss these findings in light of the novel markers found in this study.

Funding Source: This research was supported by AMED under Grant Number JP20bk0104090.

Keywords: Human pluripotency, Amnion, iPS cell

PLURIPOTENCY IN THE HUMAN GERMLINE

Clark, Amander T.

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Pluripotency is a major feature of cells in the germline and is functionally defined as the ability of cells to develop into the three major embryonic layers of the early embryo. In recent years the concept of pluripotency has changed to a spectrum of pluripotent states particularly for pluripotent cells found in the pre-implantation and early post-implantation human embryo. These pluripotent states can be stabilized in vitro using a variety of culture conditions. However, the pluripotent state of newly specified post-implantation human germline cells called primordial germ cells (PGCs) is enigmatic, as human PGCs share cellular and molecular features with naive pluripotent cells, yet have only one fate and that is to differentiate into male and female germ cells and gametes. In this talk, I will discuss how the naive pluripotent identity of human PGCs is threaded into the pluripotency network through local re-wiring of chromatin regions associated with primate-specific retrotransposons, and that this rewiring is essential for human PGC biology. In addition, I will also show how the primate-specific long non-coding RNA XACT is expressed in hPGCs, and that repression of XACT is coincident with loss of naive-like pluripotency and commitment to male and female germ cell fate. Taken together, the ability to differentiate male and female germ cells and gametes in vitro from stem cells (in vitro gametogenesis) relies upon understanding the major developmental transitions during human germ cell formation and differentiation, with a major transition involving a successful step into and out of the naive-like pluripotency program inherent to PGCs.

Keywords: Pluripotency, Germline, Embryo

SATURDAY, JUNE 26

THEME SESSION CA 1 (CLINICAL APPLICATIONS) COMPLEX 3D SYSTEMS FOR THERAPY AND DRUG DISCOVERY
7:30 - 9:15 EDT
REBROADCAST WITH LIVE CHAT 16:30 - 18:15 AND 19:30 - 21:15 EDT

ETHICS OF HUMAN BRAIN ORGANOID RESEARCH FROM THE PERSPECTIVE OF SOCIAL SCIENCE SURVEY

Fujita, Misao

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Organoids are three-dimensional structures which reproduce the formation processes of tissues and organs is in vitro using somatic and pluripotent stem cells. Because they can reproduce the structures and functions of human tissues and organs, widespread use of organoids for the development of drugs and therapeutic methods is anticipated. However, ethical considerations are required for conducting organoid research, depending on the type of tissues and organs produced. Among those, issues related to informed consent of cell donors, biobanking, consciousness, moral status, transplantation into animal brains, translational research, and regulations are all areas of concern in brain organoid research. In this study, we mostly focus on consciousness and moral status, as well as transplantation into animal brains, and report the results of social science surveys conducted mainly by our research team. We introduce exploratory research that summarizes trends in newspaper coverage on how brain organoids and consciousness are reported in society. In addition, we report the results of a survey questionnaire which investigates the concerns of the general public and researchers about chimeric animals with brains derived from human-induced pluripotent stem cells. The results are not intended to directly lead to the development of research regulations or guidelines. However, social reactions and public concerns cannot be ignored, as societal trust is essential for the development of science and technology. Therefore, it is important to monitor them on a continuing basis and share concerns with the research community.

Funding Source: Institute for the Advanced Study of Human Biology (WPI-ASHBi), Kyoto University and Uehiro Foundation on Ethics and Education

Keywords: ethics, human brain organoid, social science

3D ORGANOIDS GENERATED FROM HUMAN TROPHOBLAST STEM CELLS MODEL EARLY PLACENTAL DEVELOPMENT AND SUSCEPTIBILITY TO EMERGING VIRAL INFECTIONS

Karvas, Rowan M.[1], Verma, Sonam[2], Kumar, Deepak[2], Dong, Chen[1], Dietmann, Sabine[1], Mysorekar, Indira[2]

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Trophoblast organoids grown in 3D provide a powerful tool to study human placental development, but obtaining trophoblasts from the first trimester is complicated due to ethical and legal restrictions. Here we report that human trophoblast stem cells (hTSCs) obtained from primary cytotrophoblasts (CTBs), human blastocysts, and naive human pluripotent stem cells (hPSCs) can efficiently give rise to 3D trophoblast organoids. Trophoblast organoids derived from naive and primary hTSCs recapitulate the villous architecture of placenta-derived organoids containing an epithelial CTB shell with a syncytiotrophoblast (STB) core. Single cell RNA-sequencing demonstrated that organoids derived from naive and primary hTSCs contained a similar cellular composition, which includes two discrete CTB clusters, two discrete STB clusters, and one extravillous trophoblast (EVT) population. Comparison with scRNA-seq data from in vitro cultured human embryos showed a high degree of alignment with post-implantation CTB, STB, and EVT populations. Given emerging evidence regarding pregnancy complications due to COVID-19 infection, we investigated the susceptibility of hTSC-derived organoids to SARS-CoV-2 infection. Our scRNA-seq data indicate that the common entry receptors for the SARS-CoV-2 virus, ACE2 and TMPRSS2, are expressed in STBs. In accordance with these expression data, infection of human trophoblast organoids with a pseudovirus expressing the SARS-CoV-2 spike protein on its surface (VSV-eGFP-SARS-CoV-2-S) resulted in limited infection in STBs. By comparison, infection of trophoblast organoids with Zika virus resulted in more robust infection in multiple trophoblast cell types. Our results demonstrate that SARS-CoV-2 has the ability to infect a subset of STBs, yet the underlying CTB population appears resilient to SARS-CoV-2 infection, potentially limiting the vertical transmission of the virus to the fetus. The generation of 3D trophoblast organoids from hPSCs provides a means of deriving patient-specific organoids to study placental disease and the response to emerging viral infections.

Funding Source: T32 EB028092 DP2 GM137418 Shipley Foundation Program for Innovation in Stem Cell Science Edward Mallinckrodt, Jr. Foundation Children's Discovery Institute of Washington University

Keywords: Organoid, Trophoblast, SARS-CoV-2

PREDICTING ARRHYTHMOGENIC DRUG RISK IN A METABOLICALLY MATURED CARDIAC MICROPHYSIOLOGICAL SYSTEM

Charwat, Verena[1], Charrez, Bérénice[1], Huebsch, Nathaniel[1], Siemons, Brian[1], Finsberg, Henrik[2], Lee-Montiel, Felipe T.[1], Wall, Sam[2], Telle, Aashild[2], Jæger, Karoline[2], Cleres, David[1], Jeffreys, Nicholas C.[1], Boggess, Steven C.[3], Edwards, Andrew G.[4], Miller, Evan[3], Tveito, Aslak[2], Healy, Kevin E.[1]

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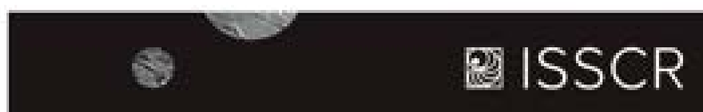
Evaluation of arrhythmogenic drug properties is required by regulatory agencies before any new compound can obtain market approval. Despite these precautions, cardiac side effects remain the 2nd most common cause for safety-related market withdrawal. On the other hand, false-positive preclinical findings can prohibit potentially beneficial candidates from moving forward in the development pipeline. Complex in vitro models using cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CM) have been identified as a useful approach that allows for rapid and cost-efficient screening of proarrhythmic drug risk. Most currently available hiPSC-CM models employ simple 2D culture formats with limited structural and functional relevance. Here we use our miniaturized 3D cardiac microphysiological system (MPS), a micro-heart muscle created from hiPSC-CMs and hiPSC stromal cells, as a platform for arrhythmia risk assessment. We hypothesized that mimicking post-natal switching of the heart's primary ATP source from glycolysis to fatty acid oxidation could enhance electrophysiological maturation of hiPSC-CM and demonstrate



enhanced maturity of the microtissues when treated with our recently developed maturation media. Our results using two different hiPSC lines suggest that systematic combination of biophysical stimuli and metabolic cues can enhance the electrophysiological maturation of hiPSC-CM. However, they also reveal that maturation-inducing cues can have differential effects on electrophysiology depending on the baseline phenotype of hiPSC-CM. We have also analyzed the data with proprietary computational analyses to identify which ion channels mechanistically contribute to enhanced phenotypic maturation of the micro-heart muscle. Finally, we used the matured version of the MPS to test pro-arrhythmic properties of 7 drugs with known ion channel effects and known clinical risk: Dofetilide and Bepridil (high risk); Amiodarone and Terfenadine (intermediate risk); Nifedipine, Mexiletine and Lidocaine (low risk). Our MPS successfully predicted the risk for most candidates, and outperformed existing hiPSC-CM 2D models, attributed to higher maturity of the 3D tissues.

Funding Source: This work was funded in part by the California Institute for Regenerative Medicine DISC2-10090, NIH-NHLBI HL130417 and the Research Council of Norway INTPART Project 249885.

Keywords: Cardiac microphysiological system, metabolic maturation, drug arrhythmia risk prediction

BIOPRINTED 3D HUMAN OUTER BLOOD RETINAL BARRIER UNCOVERS RPE-DEPENDENT CHOROIDAL PHENOTYPE IN ADVANCED MACULAR DEGENERATION

Quinn, Russell L.[1,2], Song, Min Jae[3], Nguyen, Eric[1], Hampton, Christopher[1,4], Park, Tea Soon[1], Sharma, Ruchi[1], Singh, Anju[5], Voss, Ty[3], Bose, Devika[1], Koster, Celine[1], Malley, Claire[1], Dejene, Roba[6], Tristan, Carlos[3], Derr, Paige[3], Derr, Kristy[3], Michael, Sam[3], Barrone, Francesca[1], Maminishkis, Arvydas[1], Singec, Ilyas[3], Ferrer, Marc[3], Bharti, Kapil[1]

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Age-related macular degeneration (AMD), a leading cause of blindness in the industrialized world, and occurs due to damage and/or dysregulation of the outer-blood-retina-barrier (OBRB). The mechanism of AMD initiation and progression to advanced stages (termed wet-form and dry-form AMD) remain poorly understood due to the lack of physiologically relevant OBRB models tailored for human physiology. Here, we demonstrate a native-like human 3D-Bioprinted OBRB (3D-OBRB) model which is comprised of a fully-polarized induced pluripotent stem cell (iPSC) retinal pigment epithelium (RPE) monolayer and an underlying dense capillary-bed resembling the choroidal vasculature created from primary human cells. Endothelial cells, choroidal fibroblasts, and ocular pericytes were encapsulated in a collagen-derived gel and 3D-printed on a degradable scaffold to facilitate choroidal capillary formation, while iPSC-RPEs were seeded on the apical side of the scaffold 7 days after bioprinting. Tissues were then harvested and/or analyzed over the course of several weeks via Confocal and Electron microscopy, quantitative cytokine analysis, single cell RNA sequencing (sc-RNAseq), and Trans Electrical Epithelial Resistance Measurements (TEER). This model was able to recapitulate physiological traits unique to the OBRB, such as fenestrated capillaries and apical processes on RPE. 3D-OBRB tissues were able to be implanted into the eyes of rats as well, where they integrated into the rat choroid and maintained their integrity. Finally, AMD-related pathological processes such as choroidal neovascularization (CNV) and RPE-choroidal degeneration were able to induced after introducing appropriate antagonists to mature tissues. This was achieved via complement activation in the 3D-OBRB (which triggers dry-AMD phenotypes such as subRPE drusen and choriocapillaris degeneration), and hypoxia activated HIF-a which induced wet-AMD phenotypes (choriocapillaris neovascularization). Anti-VEGF drugs suppressed neovascularization in the wet AMD model- validating this model for clinical translation and drug discovery. Our model provides a ground-breaking tool for performing in-vitro experiments capable of capturing complex tissue behaviors which are typically reserved for animal models or clinical studies.

Keywords: Tissue Engineering, Bioprinting, Retinal health

COMPLEX ACTIVITY AND SHORT-TERM MEMORIES IN RECIPROCALLY CONNECTED CEREBRAL ORGANOIDS

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Institute of Industrial Science, The University of Tokyo, Japan

Macroscopic axonal connections in the human brain distribute information and neuronal activity across the brain. Although this complexity previously hindered elucidation of functional connectivity mechanisms, brain organoid technologies have recently provided novel avenues to investigate human brain function by constructing small segments of the brain in vitro. Here, we describe the neural activity of reciprocally connected human cerebral organoids cultured on a multi-electrode array. Compared to conventional organoids, connected organoids produced significantly more intense and complex oscillatory activity. Optogenetic manipulations revealed that the connected organoids maintained temporal patterns of external stimuli, indicating that the connected organoids were able to retain temporal memory. Our findings suggest that connected organoids provide a useful approach to understand the roles of macroscopic circuits in the human brain.

Keywords: Brain organoid, Microfluidics, Optogenetics

HUMAN LENS REGENERATION VIA TRANSPLANTATION OF PLURIPOTENT STEM CELL-DERIVED LENS EPITHELIAL CELLS; A POTENTIAL NEW TREATMENT FOR CHILDHOOD CATARACT

O'Connor, Michael[1], Shparberg, Rachel[1], Stait-Gardner, Timothy[2], Harman, David[1], Watson, Stephanie[3]

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Cataracts decrease light transmission through the eye's lens. They are a leading cause of low vision and blindness in children. Cataracts must be surgically removed by 12 weeks of age to avoid permanent blindness due to light deprivation. Unlike adults, implantation of an artificial lens is avoided in young children as their eye growth changes the requirements of the artificial lens. This leads to years of traumatic vision management using contact lenses or thick glasses. Even worse, >50% of treated eyes develop low vision or become legally blind. Routine complications include monovision (76% of patients), turned eye (>46% of patients) and uncontrolled eye movements (71% of patients). Unsurprisingly, childhood cataract patients report quality of life as low as child cancer patients. The poor vision outcomes impair motor, cognitive, language and social development, causing lifelong social, educational and employment disadvantage – including malnutrition and higher mortality rates in developing countries. Lens regeneration, from cataract mutation-free lens epithelial cells, might avoid these problems by providing a replacement biological lens capable of growing with the patient. Towards this end we first established robust methods to reproducibly mass-produce

human lens epithelial cells from pluripotent stem cells. We then identified a cell attachment substrate to facilitate transplantation of the human lens cells. To test human lens regeneration in vivo, we assessed lens regeneration 3 months after transplanting human lens cells into rabbits. MRI and histology showed more lens material present after transplantation of human lens cells vs the attachment substrate alone. Mass spectrometry showed that only transplantation of human lens cells produced human lens tissue. These data indicate continued development could lead to human lens cell transplantation providing a much-needed, improved treatment for childhood cataract.

Funding Source: Stem Cells Australia

Keywords: cataract, human pluripotent stem cell, transplantation

ORGANS-ON-A-CHIP: A NEW TOOL FOR THE STUDY OF HUMAN PHYSIOLOGY

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Micro-engineered cell culture models, termed Organs-on-Chips, have emerged as a new tool to recapitulate human physiology and drug responses. Multiple studies and research programs have shown that Organs-on-Chips can capture the multicellular architectures, vascular-parenchymal tissue interfaces, chemical gradients, mechanical cues, and vascular perfusion of the body. Accordingly, these models can reproduce tissue and organ functionality and mimic human disease states to an extent thus far unattainable with conventional 2D or 3D culture systems. In this talk, we will present two approaches of using this technology. The first, will demonstrate how drug can be tested by linking of 8 human-Organ-on-a-Chip and showing results that are comparable to clinical data. Furthermore, we demonstrate how to exploit the micro-engineering technology in a novel system-level approach to decompose the integrated functions of the neurovascular unit into individual cellular compartments, while retaining their paracellular metabolic coupling. Using individual, fluidically-connected chip units, we have created a system that models influx and efflux functions of the brain vasculature and the metabolic interaction with the brain parenchyma. This model reveals a previously unknown role of the brain endothelium in neural cell metabolism: In addition to its well-established functions in metabolic transport, the brain endothelium secretes metabolites that are directly utilized by neurons. This discovery would have been impossible to achieve using conventional in vitro or in vivo measurements.

Funding Source: the Azrieli Foundation, Israel Science Foundation 2248/19, ERC SweetBrain 851765, TEVA and the Aufzien Center for Prevention of Parkinson.

Keywords: Organ-on-a-Chip, In vitro models, Human physiology

THEME SESSION CA 2 (CLINICAL APPLICATIONS) ENGINEERING TISSUE AND ORGANS 7:30 - 9:15 EDT REBROADCAST WITH LIVE CHAT 16:30 - 18:15 AND 19:30 - 21:15 EDT

STANDARDIZATION OF A SCALABLE HUMAN NEURAL ROSETTE ASSSAY FOR ASSESSMENT OF NEURAL TUBE DEFECT RISK & DEVELOPMENTAL NEUROTOXICICTY

Ashton, Randolph S.[1,2], Knight, Gavin T.[1], Lundin, Brady[2], Fedorchak, Nikolai[1], Iyer, Nisha[1], Robinson, Joshua[3], Iskandar, Bermans[4], Ashton, Lydia[5], Sethares, William[6], Willette, Rebecca[6]

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Neural organoids derived from human pluripotent stem cells (hPSCs) are becoming powerful tools for investigating CNS development, physiology, and disease. However, the innate and spontaneous emergent properties of neurally differentiating hPSC aggregates, which make neural organoids possible, also limits their application due to inconsistencies in organoid tissue cytoarchitecture. We hypothesized that this is caused by the absence of biophysical and biochemical cues normally present within the developing embryo. To reinstate control in vitro, we endeavor to develop culture methods and platforms that enable facile spatiotemporal control of microenvironmental cues to standardize early neural organoid morphogenesis. In this talk, our success in exerting biophysical control over microscale tissue morphology to standardize the derivation of singularly polarized, forebrain through spinal neuroepithelial rosette tissues will be presented. This mimics the earliest stage of CNS morphogenesis, i.e., neural tube formation. Thus, scaling of this culture platform to create an "off-the-shelf" screen for quantitatively assessing a chemicals' risk of causing congenital neural tube defects and general developmental neurotoxicity will also be discussed.

Funding Source: NSF CCF-1418976 & IIS-1447449 (RMW) and CBET-1651645 (RSA) grants, NIH1U54 AI117924-01 (RMW), R21NS082618, R33NS082618, and R21HD103111 (RSA) grants, and EPA STAR Award 83573701 (RSA), Burroughs Wellcome Fund Award 1014150 (RSA)

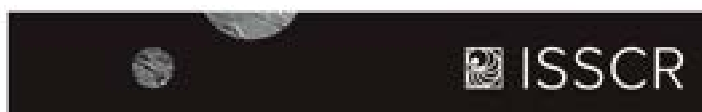
Keywords: Neural Organoid, Tissue Morphogenesis, Developmental Neurotoxicity

IDENTIFYING GENETIC REGULATORS OF ENDOCRINE AND BETA CELL IN VITRO DIFFERENTIATION VIA GENOME-WIDE LOSS-OF-FUNCTION SCREENING

Veres, Adrian, Faust, Aubrey L., Bushnell, Henry L., McGunnigle, Helen M., Schissler, Luke M., Melton, Douglas A.

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

Regenerative biology promises cures for human diseases through engineered cells and tissues. Transplantation of pancreatic beta cells is a cure for Type 1 diabetes whose application is limited by tissue availability and immunocompatibility. Directed differentiation of human stem cells to beta cells can address these problems, but deriving pure beta cells remains an elusive goal. Here,



we ask whether cell fate decisions can be controlled via genetic alteration to enhance the efficiency of beta cell differentiation. To identify such edits, we create and differentiate a pooled stem cell population in which each individual cell carries a distinct genetic perturbation. We completed a genome-wide knock-out screen in two rounds, initially targeting 19,144 genes with 4 sgRNAs per gene and then the top 806 first round hits using a custom library with 10 sgRNAs per gene. Sorting cells at differentiation day 45, we measure the effect of each sgRNA as a multi-dimensional effect on the ratio of 4 cell types: beta, glucagon-producing alpha, serotonin-producing enterochromaffin (EC), and unlabelled. Our screen finds 244 genes that significantly alter endocrine cell formation when knocked-out. 67 TFs are among these, recapitulating both the importance of known key TFs such as NEUROG3, PDX1, PAX4 and ARX and identifying novel TFs. Beyond TFs, we find several groups of genes with closely-related functions showing similar effects upon perturbation including three related MAPKs, enzymes involved in heparan sulfate biosynthesis, and ubiquitin targeting machinery such as F-box proteins. We identify two F-boxes whose perturbation can respectively increase beta cell formation by 50% and decrease EC cell formation by 30%. Notably, many hits including these two F-boxes are not differentially expressed across populations sampled via scRNA-seq, highlighting the value of our unbiased approach. Our screen provides the first genome-wide characterization of genes that control beta cell formation. This approach can be applied broadly to directed differentiation systems, providing a powerful layer of control over cell fate. Our work paves the way to tailored human cell lines whose pluripotency is intentionally curtailed via precise genome edits to restrict the emergence of undesired fates and produce optimized therapeutic products.

Keywords: genome-wide screen, beta cell therapeutics, cell fate control

IGF1R DELETION IN A HOST EMBRYO AUGMENTS DONOR CONTRIBUTION TO HOST TISSUES IN BOTH INTRA- AND INTER-RODENT CHIMERAS

Nishimura, Toshiya[1], Suchy, Fabian P.[2], Bhadury, Joydeep[2], Igarashi, Kyomi J.[3], Charlesworth, Carsten C.[2], Nakauchi, Hiromitsu[3]

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Although successful interspecies organ generation has been demonstrated in rodents using blastocyst complementation, similar success is yet to be observed in other species. A major reason is that the early developmental arrest hinders the formation of high chimeric fetus, leaving chimeric fetuses having not enough chimerism to complement a developmental niche. Here, we demonstrate that the deletion of Insulin-like growth factor 1 receptor (Igf1r) in mouse embryos creates what we have termed the "cell competitive niche", which significantly increases donor chimerism in both intra (mouse→mouse) and inter-species (rat→mouse) rodent chimeras. We have found that Igf1r deletion augments donor chimerism starting from embryonic day 11 (E11) onward in several organs except blood. Remarkably, donor chimerism in Igf1r null chimeras increased approximately 3 times higher than wild-type chimeras at E18.5. The enhanced donor chimerism continuously increased and even took over the whole organ in some intra-species chimeras. Although not the case in all organs, donor chimerism in kidney, brain, and lung approached 100%. Since chimerism was consistently higher in Igf1r null kidneys than in other Igf1r null organs, we further investigated the structure and function of these almost entirely donor-derived organs. The donor-derived kidneys were morphologically normal, with unremarkable tissue architecture, and the level of serum blood urea nitrogen and creatinine in these chimeras were in normal range indicating these kidneys functioned. Immunohistochemistry revealed all renal component of the kidneys were derived from donor cells. Donor chimersm significantly increased in rat→mouse interspecies chimeric embryos and it reached almost 70% in lung in these chimeras. Since the Igf1r deletion increases donor chimerism from the mid to late developmental stages, highly chimeric fetuses can also evade the early developmental arrest observed in interspecies chimera formation. This observation should facilitate donor cell contribution to host tissues, resulting in whole-organ generation via blastocyst complementation across wide evolutionary distances.

Funding Source: This work was supported by grants from CIRM (LA1_C12-06917, DISC1-10555) and the Ludwig Foundation, and Leading Advanced Projects for Medical Innovation, Japan Agency for Medical Research and Development.

Keywords: Pluripotent stem cell, Organ regeneration, Chimera

3D-PRINTED ABCB5-POSITIVE STEM CELLS FOR TREATING BILATERAL LIMBAL STEM CELL DEFICIENCY

Lee, Catherine[1], Frank, Markus[2], Frank, Natasha[1], Ksander, Bruce[3], Kunes, Jennifer[4], Lee, Wonhye[4], Sasamoto, Yuzuru[1], Yoo, Seung-Schik[4]

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Limbal stem cells (LSC) residing in the limbus continually repopulate the corneal epithelium. Limbal stem cell deficiency (LSCD) occurs when these LSC are damaged (due to trauma such as burns) or missing (due to genetic conditions). Patients with LSCD are unable to regenerate the corneal epithelium, resulting in blindness due to invasion of the conjunctiva and neovascularization. For patients with unilateral LSCD, transplantation of autologous limbal tissue or ex vivo expanded limbal cells from the unaffected eye can be used to treat LSCD. However, patients with inflammation as well as those with severe pathologies resulting in total, bilateral LSCD have no source of autologous LSC and must rely on allogeneic transplants, associated with poor outcomes and requiring lifelong immunosuppression. Such patients would greatly benefit from an alternative autologous source of stem cells and reconstructed LSC niche to sustain donor stem cells. We previously demonstrated that human ABCB5+ LSC were capable of restoration of the corneal epithelium in an NSG mouse model of LSCD. We found that ABCB5 is also expressed by skin stem cells and hypothesized that these dermal cells could provide an alternative source of stem cells for corneal epithelial regeneration. Human ABCB5+ dermal stem cells (DSC) expanded in vitro and purified by cell sorting were cultured in corneal differentiation media to determine their ability to transform into corneal epithelial cells. ABCB5+ DSC were also transplanted onto NSG mice with mechanically induced LSCD. In vitro, a 3D bioprinter was used to layer collagen and precisely deliver ABCB5+ DSC to the peripheral rim of human central corneas, their normal anatomical location, which we predict will enhance reconstruction of the LSC niche. Human ABCB5+ DSC were induced in vitro to express significant levels of PAX6 and KRT12 and mice transplanted with human purified ABCB5+ DSC had clearer corneas compared to mice transplanted with carrier only or ABCB5- cells. Bioprinted ABCB5+ DSC surrounding a human central cornea. Our results support the use of 3D-printed ABCB5+ DSC as an alternative au-

tologous source of stem cells to regenerate the corneal epithelium and reconstruct the LSC niche in patients with bilateral LSCD.

Funding Source: Organ Design and Engineering (ODET) T32 EB016652-05

Keywords: Cornea, Bioprinting, Limbal stem cell deficiency

ELECTRICAL PROPERTIES AND OPTOGENETIC STIMULATION OF HUMANIZED CHAMBER-SPECIFIC ENGINEERED HEART TISSUES COMBINING DECELLULARIZED HEARTS WITH INDUCED PLURIPOTENT STEM CELL DERIVED CARDIOMYOCYTES

Ghiringhelli, Matteo, Abboud, Yousef, Gruber, Amit, Arbel, Gil, Edri, Oded, Huber, Irit, Gepstein, Amira, Gepstein, Lior

Physiology, Sohnis Research laboratory for Cardiac Electrophysiology and Regenerative Medicine, the Rappaport Faculty of Medicine and Research Institute, Technion-Israel Institute of Technology, Haifa, Israel

Construction of three-dimensional ventricular and atrial patches using decellularized extracellular matrix could allow the creation of "organ-like" structures for disease modeling, drug testing and regenerative medicine applications. To engineer functional artificial anatomical hearts several obstacles need to be overcome including chamber specific cells, prevention of immune reaction and donor/ host tissue electrical coupling In order to tackle these challenges we aimed to combine human induced pluripotent stem cell (hiPSC) technology, development biology inspired differentiation system to generate chamber-specific cardiomyocytes, decellularization/recellularization processes, and optogenetics utilizing light-sensitive ion channels to generate light-controllable atrial/ ventricular tissue engineered patches. Previously described robust differentiation protocols were used to derive atrial or ventricular cells from hiPSCs. Atrial and ventricular patches of adult rats were decellularized using 1% SDS, 3% triton-X. The decellularized scaffolds were then recellularized with hiPSC- derived cardiomyocytes (2x107 atrial or ventricular cells). Adenoviral transduction was used to express the light-sensitive cationic channel ChR2 in the tissue engineered constructs. Eight days after cell seeding, we observed the development of spontaneous contraction of the atrial/ventricular patches. Immunostaining for atrial (Cx40) or ventricular (MLC-2V) markers, gene expression, action-potential morphology, and the response to chamber-specific pharmacology confirmed the atrial/ventricular specific identity of the patches. Optical mapping, using an EM-CCD camera, was used to characterize the conduction and repolarization properties of the generated tissues. The engineered patches could be paced and their electrical activity controlled by either electrical or optogenetic stimulation. Finally, arrhythmogenic reentrant could be induced in the tissue models. Three-dimensional light-sensitive chamber-specific engineered heart patches could be generated that could be controlled and manipulated through electrical and light pacing. These tissue could be used for several pathophysiological, drug testing, disease modeling and regenerative medicine applications.

Keywords: decellularization, human induce pluripotent stem cell, optogenetics

HYPOIMMUNE IPSC-DERIVED CELL PRODUCTS TREAT CARDIOVASCULAR DISEASES IN IMMUNOCOMPETENT ALLOGENEIC MICE

Deuse, Tobias[1], Tediashvili, Grigol[1], Hu, Xiaomeng[1], Gravina, Alessia[1], Tamenang, Annika[1], Wang, Dong[1], Agbor-Enoh, Sean[2], Jang, Moon[3], Alawi, Malik[4], Saygi, Ceren[1], Liu, Yuan[3], Valantine, Hannah[5], Lanier, Lewis L.[6], Schrepfer, Sonja[1]

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We report on the existence and functionality of the immune checkpoint signal regulatory protein-α (SIRPα) in NK cells and describe its relevance for cell therapy. NK cell SIRPα is upregulated upon cytokine stimulation, interacts with target cell CD47 in a threshold-dependent manner, and counters other stimulatory signals including IL-2, CD16, or NKG2D. Elevated CD47 on engineered graft cells was therefore assumed to protect engineered MHC knockout cells against innate immune killing and make them sources for universal cell products for regenerative medicine. To challenge our hypothesis, we engineered hypoimmune MHC class I- and class II-deficient and CD47 overexpressing C57BL/6 iPSC derivatives. Their ability to evade both macrophage and NK cell killing was confirmed in vitro. We then tested the ability of hypoimmune endothelial cells and cardiomyocytes to treat major diseases in mice. Outcome parameters were adopted from clinically relevant endpoints commonly used in human trials. Endothelial cells were injected into the thigh muscle in mice with critical hindlimb ischemia. The graft cells were able to survive in immunocompetent, fully allogeneic BALB/c recipients as assessed by quantitative in vivo bioluminescence imaging. Injections of hypoimmune endothelial cells improved leg perfusion over a 28-day study period and markedly increased the likelihood for limb preservation. A mixture of endothelial cells and cardiomyocytes was injected into infarcted mouse hearts and both cell types orthotopically engrafted in the heart and showed unrestrained survival. Cell therapy led to an improvement in heart failure parameters, including improved stroke volume and cardiac output. Our study supports the concept of hypoimmune, universally-compatible, regenerative cell therapy products for cost-efficient treatments for major ischemic diseases.

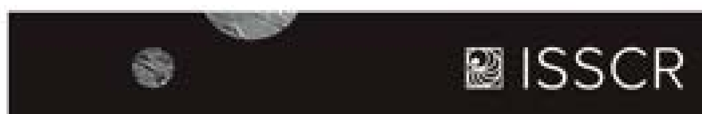
Keywords: cardiac regeneration, iPSC-derived cardiomyocytes, hypoimmune

BROMODOMAIN AND EXTRATERMINAL INHIBITION BLOCKS INFLAMMATION-INDUCED CARDIAC DYSFUNCTION

Hudson, James E.

Cell and Molecular Biology, QIMR Berghofer, Brisbane, Australia

Cardiac injury and dysfunction occur in inflammatory diseases and increase the risk of mortality. Additionally, cardiovascular risk factors such as diabetes and obesity can cause chronic inflammation. The causes of inflammation-driven cardiac effects are ill defined, but could be damage caused by heightened immune system activity and/or dysfunction caused directly by inflammatory factors. As our human cardiac organoid platform does not contain immune cell populations it provides a unique platform to screen inflammatory factors potentially driving dysfunction. We identify an inflammatory 'cytokine-storm', a cocktail of interferon gamma, interleukin 1β and poly(I:C), induced diastolic dysfunction. To identify mechanisms, we use a state-of-the-art pipeline combining



human cardiac organoids with phosphoproteomics and single nuclei RNA sequencing. STAT1 and BRD4 are activated along with a viral response that is consistent in both human cardiac organoids and hearts of SARS-CoV-2 infected K18-hACE2 mice. Using drug screening we identify that bromodomain and extraterminal family inhibitors (BETi) recover dysfunction in hCO and completely prevent cardiac dysfunction and death in a mouse cytokine-storm model. Additionally, BETi decreases transcription of genes in the viral response which could also be key targets as therapeutics as well. Together, BET mediated responses are promising therapeutic targets for inflammation driven cardiac dysfunction. Currently, the FDA breakthrough designated drug apabetalone, is a promising candidate to prevent mediated cardiac damage.

Funding Source: Berghofer and Brazil family donations, National Health and Medical Research Council of Australia, the Medical Research Future Fund (MRFF9200008), Queensland Health, Dynomics Inc. and a Snow Medical Fellowship.

Keywords: Heart, Pluripotent Stem Cells, Drug discovery

THEME CA (CLINICAL APPLICATIONS) PLENARY VI: CELLULAR THERAPY AND TISSUE ENGINEERING 9:30 - 11:00 EDT REBROADCAST WITH LIVE CHAT 21:30 – 23:00 EDT

MECHANISMS OF OLIGODENDROCYTE REGENERATION

Tesar, Paul J.

Genetics & Genome Sciences, Case Western Reserve University, Cleveland, OH, USA

Understanding the developmental regulators that ultimately produce functional cell types is a central goal of biology and medicine. While the mechanisms controlling early differentiation have been well-studied for many important cell lineages, the regulation of subsequent maturation timing remains unknown. Here we show that the timing of cellular maturation is governed by a transient form of transcriptional condensates. During oligodendrocyte development we find that the transcription factor SOX6 dramatically re-localizes from nearly all super enhancers in oligodendrocyte progenitor cells (OPCs) to form developmental condensates across a small set of gene bodies in immature oligodendrocytes. Genes in condensate loci are highly expressed in immature cells but turn off upon maturation. CRISPR-, RNAi, or miRNA-mediated suppression of SOX6 reduced developmental condensate gene activation and accelerated maturation directly to mature myelinating oligodendrocytes. Collectively, this work describes how Sox6-regulated developmental condensates govern processivity along the continuum of oligodendrocyte formation from OPCs. This highlights the potential for biomolecular condensates to control maturation rate during development and may inform new approaches to accelerate the regeneration of mature cell types in numerous diseases.

Keywords: oligodendrocyte progenitor cells, regeneration, myelin

HPSC-DERIVED ORGANOIDS FOR COVID-19 DISEASE MODELING AND DRUG SCREENING

Chen, Shuibing

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hPSC-derived cells/organoids provide a platform to systematically evaluate the tropism and cellular response upon viral infection, which can be adapted to screen for anti-viral drugs. In response to the COVID-19 pandemic, we assembled a large consortium team to create a panel of hPSC-derived cells/organoids to study SARS-CoV-2 tropism. By screening twelve different types of cells and organoids, we found that lung, colon, heart, liver, pancreatic cells and neurons can be infected by SARS-CoV-2. This work presented the first stem cell model to understand the tropism of SARS-CoV-2. In addition, we reported the first organoid-based screen and identified several drug candidates blocking SARS-CoV-2 entry. This work not only facilities understanding the pathogenesis of COVID-19 patients, but also identified novel drug candidates to treat COVID-19 patients. In summary, hPSC-derived cells/organoids provide useful models to systematically investigate the host response to viral infection, immune-mediated host damage, and for anti-viral drug discovery.

Keywords: Organoids, SARS-CoV-2, viral infection

TISSUE ENGINEERED HEART REPAIR: FROM NON-HUMAN PRIMATES TO A FIRST-IN-PATIENT CLINICAL TRIAL

Zimmermann, Wolfram-Hubertus[1,2,3]

[1]*Institute of Pharmacology and Toxicology, University Medical Center, Georg-August-University, Göttingen, Germany,* [2]*DZHK (German Center for Cardiovascular Research), partner site Göttingen, Germany;* [3]*Cluster of Excellence "Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells" (MBExC), Georg-August-University, Göttingen, Germany*

Remuscularization of the failing heart can be achieved by implantation of engineered heart muscle (EHM). We make use of well characterized induced pluripotent stem cells (iPSCs) for the derivation of cardiomyocytes and stromal cells, which are subsequently combined in collagen type I hydrogels to prepare EHM with contractile properties of native myocardium under defined conditions. EHM can be scaled as needed for individual in vivo applications with no apparent requirement for pre-vascularization. In pivotal preclinical studies in non-human primates (Rhesus macaque model) with and without chronic heart failure, evidence for safety and efficacy of EHM allografting under immune suppression could be obtained. Key findings of these studies with 6 months follow-up include: (1) retention of EHM allografts with no evidence for critical cell loss; (2) EHM vascularization and perfusion as evidenced by histopathology and MRI-perfusion analyses; (3) a dose dependent thickening of the target heart wall with evidence for enhanced local contractility aligned with the proposed mode of action; (4) improved left ventricular geometry and ejection fraction. Unwanted effects, such as arrhythmia and tumor growth were not observed. Collectively, these data constituted a pivotal element for the regulatory approval of a first-in-patient / first-in-class early clinical trial, the Biological Ventricular Assist Tissue in terminal Heart Failure (BioVAT-HF) trial (NCT04396899). Conflict of Interest: WHZ is founder, equity holder and advisor of Repairon GmbH.

Funding Source: DZHK (German Center for Cardiovascular Research), partner site Göttingen, Germany; German Federal Ministry for Science and Education (IndiHEART: 161L0250A); the German Research Foundation (SFB 1002 C04/S01).

Keywords: tissue engineering, heart repair, induced pluripotent stem cells

IDENTIFYING AND OVERCOMING THE IMMUNOLOGICAL HURDLE IN CELLULAR THERAPY FOR REGENERATIVE MEDICINE

Schrepfer, Sonja[1,2]

[1]*Surgery, University of California, San Francisco, CA, USA,* [2]*Sana Biotechnology Inc, South San Francisco, CA, USA*

Dr. Schrepfer's research career has been dedicated to making fundamental discovers in transplant and stem cell immunobiology. Work by Dr. Schrepfer is at the forefront of stem immunobiology and paves the way for treatment of a wide range of diseases – from supporting functional recovery of failing myocardium to the derivation of other cell types to treat diabetes, blindness, cancer, lung, neurodegenerative, and related diseases. She spent many years examining in detail the fetomaternal interface for application to the envisioned cell therapy. Her work demonstrates that hypo-immunogenic cells reliably evade immune rejection in allogeneic recipients from various species that are entirely mismatched in their major histocompatibility complex profile, and further, these cells show long-term survival without immunosuppression in mice and humanized mice (published in Nature Biotechnology in 2019, JEM 2021). These findings - truly hypo-immunogeneic iPSCs - achieve the "holy grail" of stem cell immunobiology. Dr. Schrepfer, Professor at the University of California San Francisco (UCSF), and Scientific Founder and SVP from Sana Biotechnology, Inc. was trained as cardiac surgeon in the Cardiothoracic Surgery Departments in Munich and Hamburg, Germany before pursuing a career as a full-time scientist in transplant immunology. She received her PhD in transplant immunology and joined Stanford University to start her own research group in 2005 in the Department of Cardiothoracic Surgery. Dr. Schrepfer's findings have been highlighted in leading journals such as Nature and Science and she received numerous prestigious awards for her work on the immunobiology in pluripotent stem cell transplantation.

Keywords: immunogenicity, hypo-immune pluripotent stem cells, immune hurdle

THEME SESSION CA 3 (CLINICAL APPLICATIONS)
ROAD TO CLINIC I (REGENERATIVE MEDICINE)
12:00 - 13:10 EDT
REBROADCAST WITH LIVE CHAT 00:00 – 01:10 EDT 27 JUNE

STEM CELL DERIVED ISLETS TO TREAT DIABETES

Kieffer, Timothy J.

Cellular & Physiological Sciences, University of British Columbia, Vancouver, BC, Canada

Diabetes results from insufficient production of the hormone insulin from beta cells in pancreatic islets. Islet transplantation can replace the lost beta cells in patients but is limited by the scarcity of available donor organs. Our aim is to differentiate pluripotent stem cells into functional islets that can serve as an unlimited source for transplantation to treat diabetes. We have investigated the therapeutic potential of pancreatic endoderm cells derived from human embryonic stem cells. Several weeks following transplant into diabetic rodents, these cells mature and secrete sufficient human insulin, in a regulated manner, to reverse diabetes. In rats, we observed inconsistent survival of pancreatic endoderm cells implanted subcutaneously in macroencapsulation devices designed to be immunoprotective via use of a cell impermeable layer, but this was rectified by the addition of portals to enable direct capillary vascular permeation into the device interior. In contrast both device types supported cell survival, differentiation and function in mice, with more rapid C-peptide release and better glucose tolerance observed using the devices containing portals. Kidney capsule grafts often contained ductal cells and cysts, whereas cells implanted subcutaneously within macroencapsulation devices differentiated predominantly to endocrine cells. As part of a ViaCyte clinical trial (clinicaltrial.gov identifier: NCT03163511), we investigated the safety and efficacy of pancreatic endoderm cells implanted in non-immunoprotective macroencapsulation devices for the treatment of patients with type 1 diabetes and hypoglycemic unawareness. Patients underwent subcutaneous implantation of cell products combined with an immunosuppressive regimen. After implant, patients had increased fasting C-peptide levels, increased glucose-responsive C-peptide levels, and developed mixed meal-stimulated C-peptide secretion. Patients had reduced insulin requirements, increased time in target blood glucose range, and improved hypoglycemic awareness. Explanted grafts contained cells with a mature beta cell phenotype that were immunoreactive for insulin, islet amyloid polypeptide, and MAFA. Collectively, these findings support future investigation into optimizing cell therapies for diabetes.

Funding Source: This work was supported by: Stem Cell Network, JDRF, Canadian Institutes of Health Research, ViaCyte, Inc.

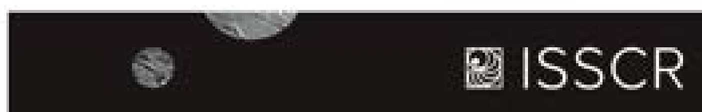
Keywords: diabetes, stem cells, clinical trial

TWO STEP WNT SIGNALLING ACTIVATION FACILITATES THE INDUCTION OF HUMAN PLURIPOTENT STEM CELL DERIVED MIDBRAIN DOPAMINERGIC NEURONS FOR TRANSLATIONAL USE

Kim, Tae Wan[1], Koo, So Yeon[2], Piao, Jinghua[3]

[1]*Developmental Biology, Memorial Sloan Kettering Cancer Center, New York, NY, USA*, [2]*Neuroscience Graduate Program, Weill Cornell Graduate School of Biomedical Sciences, New York, NY, USA*, [3]*Department of Neurosurgery, Memorial Sloan Kettering Cancer Center, New York, NY, USA*

Parkinson's disease (PD) is the second most common neurodegenerative disorder. The pathological hallmark of PD is the progressive degeneration of specific pigmented midbrain dopaminergic (mDA) neurons, resulting in the motor dysfunction, such as rigidity, tremor, and dyskinesia. Due to a specific cell-type loss of mDA neuron in the midbrain, PD was one of the initial candidates for cell replacement therapy. Human pluripotent stem cells (hPSC) s have shown considerable promise for applications in regenerative medicine including the development of dopamine neuron replacement paradigms for the treatment of Parkinson's disease. Robust protocols have been developed to generate authentic mDA neurons capable of reversing dopamine-related deficits in animal models of Parkinson's disease. However, the generation of mDA neurons at clinical scale under conditions suitable for human application remains an important challenge. Here we present an optimized mDA neuron derivation protocol based on a biphasic WNT signaling activation strategy that improves expression of midbrain markers such as EN1 while minimizing expression of contaminating posterior (hindbrain) and anterior (diencephalic) lineage as well as non-neuronal contaminants. Molecular, biochemical and electrophysiological profiling exhibit the resultant progenitor mature into mDA neurons. This WNT boosting protocol shows highly reproducible across differentiations and hPSC lines. Using EN1 knock-out and isogenic control hPSCs, we observed off-target diencephalic/STN fates are suppressed by EN1 in biphasic WNT activation conditions, implying a pivotal role for EN1 in mediating induction of mDA neuron and suppression of alternative markers in response to WNT boosting condition. Cryopreserved, "off-the-shelf" mDA products established via biphasic WNT activation can be successfully transplanted into 6OHDA lesioned rats to induce recovery of amphetamine induced rotation behavior. These studies take advantage of our ability to generate bona fide mDA neurons in a clinically relevant culture system and are the basis for clinical grade dopamine neuron production and preclinical safety and efficacy studies to offer the best possible



cell therapy to PD patients in the near future. It was recently approved by FDA for a phase 1 trial.

Keywords: Cell replacement therapy, Dopaminergic neuron, Parkinson's disease

CELL FUSION TO COMBINE THERAPEUTIC PROPERTIES

Lye, Kathryn[1], Harding, Jeffrey[2], Nagy, Kristina[3], Monetti, Claudio[3], Nagy, Andras[2]

[1]*Institute of Medical Science, University of Toronto, ON, Canada,* [2]*Research, Lunenfeld Tanenbaum Research Institute, Toronto, ON, Canada,* [3]*Research, Former, Lunenfeld Tanenbaum Research Institute, Toronto, ON, Canada*

Cell fusion is essential for tissue development, physiology, and regeneration. The exploitation of this process in research has led to ground-breaking advancements including the fusion of pluripotent cells with somatic cells to induce reprogramming and the fusion of B cells with myeloma cells to generate hybridomas for monoclonal antibody production. The creation of new cell types in both examples relies on the inheritance of parental properties in fused cells. This study strives to generate a better candidate source of cells for universal cell therapy using cell fusion as a cell engineering tool. We engineered a genetic change in mouse and human embryonic stem cells, which allows for stringent selection of fusion with another cell without the need for chemical fusogens. With this platform, we have previously shown that diploid mouse embryonic stem cells (mESCs) fuse into tetraploid cells that remain tetraploid beyond 31 passages, express markers of pluripotency, and form teratomas in vivo that contain structures from all three embryonic germ layers. Here we show the design and development of a Fusion Partner cell with the potential to transfer safety and immune tolerance technology to any therapeutic cell type. This study will investigate the inheritance of SafeCell (SC) technology and induced Allogeneic Cell Tolerance (iACT) in fused cells. In theory, inheritance of these technologies will give any cell the potential to overcome the two biggest hurdles of most emerging cell-based therapies: tumorigenicity and allogeneic immune rejection. This cell fusion platform makes critical therapeutic properties accessible to any current or future candidate cells for therapy.

Keywords: Cell fusion, Cell therapy, Graft tolerance

INFORMING IN VITRO STEM CELL DIFFERENTIATION THROUGH SINGLE-CELL RNASEQ ANALYSIS OF THE DEVELOPING HUMAN FETAL STRIATUM

Cattaneo, Elena[1,2]

[1]*Department of Biosciences, University of Milan, Italy,* [2]*National Institute of Molecular Genetics, Milano, Italy*

Current protocols for stem cell differentiation in striatal medium spiny neurons (MSNs) that are lost in Huntington's disease (HD) suffer from a lack of information about their normal development in the fetal human brain. Single-cell RNA sequencing and bioinformatics have made it possible to study human fetal samples with unprecedented resolution and to extract high-quality information from large transcriptomic data sets. Following this approach here we have combined bulk and single-cell RNA-seq to decode the unique gene signature of the developing human fetal striatum. The generated map was used to verify the quality and composition of our current stem cell differentiation protocol. We show that we are able to generate authentic D1 and D2-MSNs that highly mimic their in vivo counterpart in terms of gene composition and cell-fate acquisition steps. This information is expected to improve protocols for differentiation into distinct MSN states and increase our understanding of diseases affecting the striatum along with advancing the possibility of cell therapy treatments for HD.

Keywords: Huntington, Single cell transcriptomics, Stem cells

THEME SESSION CA 4 (CLINICAL APPLICATIONS)
ROAD TO CLINIC II (DRUG DISCOVERY)
12:00 - 13:10 EDT
REBROADCAST WITH LIVE CHAT 00:00 – 01:10 EDT 27 JUNE

PHASE 1/2A CLINICAL ASSESSMENT OF A BIOENGINEERED, RPE CELL-BASED IMPLANT FOR THE TREATMENT OF ADVANCED DRY AGE-RELATED MACULAR DEGENERATION

Lebkowski, Jane S.[1], Kashani, Amir H.[2], Rahhal, Firas[3], Avery, Robert[4], Chen, Sanford[5], Chan, Clement[6], Palejwala, Neal[7], Ingram, April[8], Dang, Wei[9], Mitra, Debbie[10], Lin, Chin-Min[9], Zhu, Danhong[11], Martinez-Camarillo, Juan Carlos[10], Bailey, Jeff[12], Arnold, Cassidy[12], Faynus, Mohamed[12], Pennington, Britney[12], Johnson, Lincoln[12], Clegg, Dennis[13], Hinton, David[14], Humayun, Mark[10]

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Retinal pigment epithelium (RPE) dysfunction and loss along with deterioration of the Bruch's membrane are a hallmark of the advanced form of dry age-related macular degeneration, called geographic atrophy (GA). Without the RPE, overlying photoreceptors ultimately degenerate, leading to severe, progressive vision loss. Previous studies using macular translocation or RPE sheet transplantation suggest that RPE replacement strategies may delay disease progression or improve vision. Enrollment and one-year follow-up of an interventional, U.S. FDA-cleared Phase 1/2a clinical trial to assess the safety and efficacy of a subretinal implant in subjects with GA has been completed. The composite implant consists of polarized allogeneic human embryonic stem cell-derived RPE cells on an ultrathin parylene substrate designed to mimic the diffusion properties of the Bruch's membrane. In this study, the worst-seeing eye, with a baseline best-corrected visual acuity (BCVA) of ≤ 20/200, and hence legally blind, was implanted subretinally. Subjects received a well-tolerated 68-day course of tacrolimus in the peri-implantation period. Subjects were followed for changes in BCVA along with ophthalmologic exams. The implantation procedure was completed in the outpatient setting and in all 15 treated patients, the implant was properly positioned over the area of GA and did not deteriorate or deform during long-term follow-up. On average, 86.9% of the area of GA was covered by the implant and pigmentation remained evident upon long-term

follow-up even post- immunosuppression withdrawal. There were no unanticipated serious ocular adverse events. Ocular SAEs related to hemorrhage and edema were confined to 3 of the first seven subjects during the first year of follow-up with better hemorrhage prevention resulting in no such SAEs in the last 8 subjects. As assessed by optimal coherence tomography, there was good preservation and retinal architecture even in areas where the implant was placed over intact host RPE cells. At one year of follow-up, 67% (10/15) of subjects had stable or improved BCVA, an important finding in subjects with such advanced disease. The concurrent clinical structural and functional assessments support the potential therapeutic use of the implant for the treatment of GA and further trials are planned.

Funding Source: Funding Source; California Institute of Regenerative Medicine, Santen Pharmaceuticals

Keywords: macular degeneration, implant, clinical trial

COMBINED GENETIC AND CHEMICAL SCREENS USING HUMAN NEURAL STEM CELLS IDENTIFY ZIKA VIRUS RESISTANCE FACTORS AND NEW DRUG CANDIDATES

Chu, Pei-Hsuan[1], Braisted, John[2], Cheng, Ken[2], Dorjsuren, Dorjbal[2], Eastman, Richard[2], Lal, Madhu[2], Simeonov, Anton[2], Chen, Lu[2], Whitt, Nadia[2]

[1]*Stem Cell Translation Laboratory, National Center for Advancing Translational Sciences (NCATS), Rockville, MD, USA,* [2]*Division of Pre-Clinical Innovation, NCATS, Rockville, MD, USA*

Zika virus (ZIKV) is a flavivirus that can cause serious conditions in humans such as microcephaly and Guillain-Barré syndrome. Identification of host factors that enhance or protect from ZIKV infection may provide clues for therapeutic intervention. To identify host factors that confer virus resistance, we performed functional genomics studies by genome-wide siRNA screens using neural stem cells (NSCs) derived from human pluripotent cells. PAX6-expressing NSCs were generated using an automated cell culture system, which cultured cells enough for 185 of 384-well plate in one batch to perform comprehensive knockdown screens comprising 21,584 human genes (targeted by 64,755 unique siRNAs). We identified diverse hits indicating different cellular mechanisms/processes such as viral entry, heparan sulfate modifications, ER protein complex, RNA replication/splicing, and protein synthesis. To better understand viral entry mechanisms, we focused on cell surface protein analysis and confirmed previously reported hits such as AXL, FURIN and SCARB1, as well as novel candidates. Other surface proteins which may play a role in ZIKV resistance were identified as immune response and cytokine related receptors. Moreover, functional enrichment analysis indicated possible interventions at later stages of virus replication, such as RNA splicing/maturation process. Next, we established pairwise siRNA combination matrix screening for dual gene knockdown and identified combinatorial effects in gene perturbation. To eliminate false signals due to unspecific siRNA targeting, two siRNAs per gene were used producing 1040 combinations for 28 selected genes. Lastly, we applied a genomic data-driven compound selection approach to identify small molecules targeting hits from genetic screen by utilizing multiple databases (NCATS small molecule libraries, DrugBank, Pharos) and prioritized 1032 compounds to perform pharmacological inhibition of ZIKV infection. Collectively, our integrated strategy of functional genomics followed by targeted chemical screening represents a novel approach to develop rational therapies against ZIKV and may also be useful for the current COVID-19 pandemic.

Keywords: genome-wide screen and small molecule screen, virus resistance, neural stem cell

ROPALS TRIAL: PHASE 1/2A, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY OF ROPINIROLE HYDROCHLORIDE FOR ALS PATIENTS BASED ON THE IPSC DRUG REPOSITIONING

Takahashi, Shinichi[1], Morimoto, Satoru[1], Okada, Kensuke[2], Daté, Yugaku[2], Ito, Daisuke[2], Nakahara, Jin[2], Okano, Hideyuki[1]

[1]*Physiology, Keio University School of Medicine, Shinjuku-ku, Japan,* [2]*Neurology, Keio University School of Medicine, Shinjuku-ku, Japan*

No effective treatment has been established for amyotrophic lateral sclerosis (ALS) because of a limitation of animal model for drug development. Thus, we performed drug screening using motor neurons (MNs) derived from disease-specific-induced pluripotent stem cells (iPSC) for ALS and we found that ropinirole hydrochloride inhibited reactive oxygen species and the abnormal aggregation of TDP-43 or FUS, improved mitochondrial function, and prevented MN death. In December 2018, we started an investigator-initiated clinical trial (UMIN000034954, JMA-IIA00397) testing ropinirole hydrochloride for ALS. The aims are to assess the safety and tolerability as well as efficacy of ropinirole hydrochloride in patients with ALS. This is a phase 1/2a randomized, double-blind, placebo-controlled, single-center, open-label continuation clinical trial. The major inclusion criteria were: 1) "clinically possible and laboratory-supported ALS", "clinically probable ALS" or "clinically definite ALS" according to the criteria for the diagnosis of ALS (El Escorial revised) and within 60 months after disease onset; 2) each ALSFRS-R score ≧2 points; 3) change in total ALSFRS-R score of -2 to -5 points during the 12-week run-in period. The primary aim is to assess the safety and tolerability of ropinirole hydrochloride. Secondary outcomes include: Combined Assessment of Function and Survival (CAFS), ALSFRS-R score, quantitative muscle strength and volume, and an efficacy evaluation using subjects-derived iPSCs/MNs. A total of 29 patients have been recruited; 21 of these patients (13 men) are enrolled in the 24-week double-blind phase. At enrollment, the mean ± SD disease duration was 20 ± 11 months. ALSFRS-R score was 40 ± 3 (3 ± 1 reduction during the run-in period). The whole trial was completed in July 2020 and the results will be known after April 2021. Our trial will be a touchstone trial for iPSC-based drug repositioning and will provide promising data.

Funding Source: Japan Agency for Medical Research and Development (AMED): Grant No. JP 18ek0109329h0001, 19ek0109329h0002 (H.O.) and, in part, K Pharma, Inc. All test drugs and part of the comparator supplied free-of-charge by GlaxoSmithKline K.K.

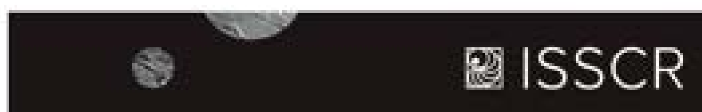
Keywords: amyotrophic lateral sclerosis, induced pluripotent stem cells, ropinirole hydrochloride

APPLICATION OF DISEASE-SPECIFIC IPS CELLS FOR DISCLOSING THE PATHOMECHANISM AND DISCOVERING THERAPEUTIC CHEMICALS FOR INTRACTABLE DISEASES

Toguchida, Junya

Department of Regeneration Sciences and Engineering, Institute for Frontier Life and Medical Sciences, Kyoto University, Kyoto, Japan

One of main clinical applications of induced pluripotent stem cells (iPSCs) is that for the disease modelling and drug discovery using disease-specific iPSCs. We have conducted this approach for a number of hereditary skeletal diseases, and the results lead us to the clinical trial in one disease, fibrodysplasia ossificans progressive, FOP. FOP is characterized by progressive heterotopic ossification, which is frequently caused after inflammatory episodes (flare-up). The inflammation induces the proliferation of myofibroblastic cells, which differentiate to chondrocytes and then



heterotopic bones are produced through endochondral ossification. We analysed this process using iPSC-derived mesenchymal stromal cells and found that activin-A is a key molecule to trigger this process at both inflammatory and differentiation stages. We also found that this induction of HO is via the activation of mTOR, and mTOR inhibitors can effectively inhibit HO by Activin-A. Sirolimus is one of mTOR inhibitors and has been used in clinics for several types of diseases such as kidney transplantation as an immunosuppressant. Because it can be used in clinic also in Japan, we planed the clinical trial using sirolimus. The study consisted of two phases, and the initial phase was the double-blinded randomized comparison test for 24 weeks, and the second phase was the open-label continuous administration test for more than 52 weeks. Most of patient were able to finish both phases, and now we are evaluating the therapeutic effect by several points such as the number of new HO. We have identified candidate drugs for other monogenic and also multifactorial diseases and disease-specific iPSCs are useful tools to develop new treatments for rare intractable diseases.

Funding Source: This study was supported by AMED in Japan, Sumitomo Dainippon Pharma Co., and Nobelpharma.

Keywords: disease-specific iPSC, fibrodysplasia ossificans progressiva, sirolimus

PLENARY VII: BREAKTHROUGHS IN THERAPY DEVELOPMENT
13:15 - 15:20 EDT
REBROADCAST WITH LIVE CHAT 01:15 – 03:20 EDT, 27 JUNE

THE GENESIS OF A PHASE 1 CLINICAL TRIAL OF HUMAN ES-DERIVED MIDBRAIN DOPAMINE NEURON GRAFTS FOR PARKINSON'S DISEASE

Viviane, Tabar

Neurosurgery, Memorial Sloan Kettering Cancer Center, New York, NY, USA

Parkinson's disease is characterized by the loss of dopaminergic neurons in the substantia nigra leading to disabling deficits. Dopamine neuron grafts may provide a significant therapeutic advance over current therapies. Here I will present our team's approach to the derivation of midbrain dopamine neurons from human embryonic stem cells, the manufacture of large-scale cryopreserved dopamine neurons from human ES cells under cGMP conditions, and the preclinical datasets that were required for regulatory approval. The product, MSK-DA01 underwent an extensive set of biodistribution, toxicity, and tumorigenicity assessments in mice under GLP conditions. A large-scale efficacy study was also performed in rats with the same lot of cells intended for potential human use and demonstrated survival of the grafted cells and behavioral amelioration in 6-hydroxydopamine lesioned rats. There were no adverse effects attributable to the grafted cells, no obvious distribution outside the brain, and no cell overgrowth or tumor formation. I will discuss some of the challenges on the path to regulatory approval and highlights of the Phase 1 clinical trial that was just open for patients with Parkinson's disease.

Funding Source: New York Stem Cell

Keywords: Dopamine Neurons, Parkinson's disease, Human Embryonic Stem Cells

FROM STEM CELLS TO BRAIN ASSEMBLOIDS: CONSTRUCTING AND DECONSTRUCTING THE HUMAN NERVOUS SYSTEM

Pasca, Sergiu P.

Psychiatry and Behavioral Sciences, Stanford University, Stanford, CA, USA

A critical challenge in understanding the molecular rules underlying the development, assembly and dysfunction of the human brain is the lack of direct access to intact, functioning human brain tissue for direct investigation and manipulation. In this talk, I will describe efforts in my laboratory to build functional cellular models and to capture previously inaccessible aspects of human brain development. To achieve this, we have been using instructive signals to derive, from human pluripotent stem cells, self-organizing 3D tissue structures named neural spheroids or region-specific brain organoids that resembles domains of the developing central nervous system. We have shown that these cultures, such as the ones resembling the cerebral cortex, recapitulate many features of neural development, can be derived with high reliability across dozens of cell lines and experiments and, when maintained as long-term cultures, recapitulate an intrinsic program of maturation that progresses towards postnatal stages. The formation of specific brain regions is, however, shaped by interactions with other regions through long-distance projections as well as by wiring locally in microcircuits with neurons that have migrated from other niches. To study migration and circuits in vitro and in vivo, we developed a next-generation experimental paradigm that we termed brain assembloids. As devised by us, assembloid generation is modular, relying on producing brain region-specific organoids that are subsequently fused in 3D to allow formation of neural circuits. I will illustrate how these patient-derived models can be applied to study the cellular and molecular consequences of genetic mutations or copy number variants associated with neuropsychiatric disease and to acquire a deeper understanding of human physiology.

Funding Source: National Institute of Health, Chan Zuckerberg Initiative, New York Stem Cell Foundation

Keywords: assembloids, organoids, brain disorders

DELIVERING ON THE PROMISE OF CELL AND GENE THERAPY

Bradner, James E.

Novartis Institutes for Biomedical Research, Cambridge, MA, USA

Convergent advances in stem cell biology and disease biology afford an immediate opportunity to engineer a new science of therapeutics for debilitating diseases. First examples of impactful cell and gene therapies are translating to broad and efficacious use in cancer and numerous non-malignant diseases. We, at the Novartis Institutes for BioMedical Research, have placed significant strategic emphasis on innovating stem-progenitor cell therapies and AAV-based gene therapies. We brought to patients the first approved CART therapy for the treatment of leukemia (Kymriah) and the first systemic AAV gene therapy for spinal muscular atrophy (Zolgensma). Kymriah reprograms a patient's immune cells to recognize and fight cancers such as acute lymphoblastic leukemia (ALL) and B-cell lymphomas. Zolgensma is a one time, transformative therapy that directly treats SMA by replacing the missing or defective SMN1 gene with a functional copy. Today, our research and early development pipeline includes over 25 cell and gene therapies for life-threatening diseases of the bone marrow, eye, and brain. This research benefits from sustained focus, a web of productive collaborations, originator agnostic technology platforms, and a culture of bravery and curiosity in drug hunting. In this lecture, I will share our progress in regenerative biology,

stem-progenitor cell therapy, and gene therapy – highlighting early successes as well as humbling challenges along the path to reimaging medicine.

Keywords: AAV, CART, stem-progenitor cell therapy

KEYNOTE ADDRESS: RECENT PROGRESS IN IPS CELL RESEARCH AND APPLICATION

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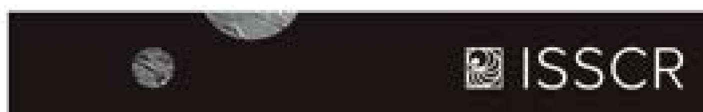
Induced pluripotent stem cells (iPSCs) can proliferate almost indefinitely and differentiate into multiple lineages, giving them wide medical application. As a result, they are being used for new cell-based therapies, disease models and drug development around the world. We are proceeding with an iPSC stock project in which clinical-grade iPSC clones are being established from healthy donors with homologous HLA haplotypes to lower the risk of transplant rejection. We started distributing the iPSC stock to organizations in Japan, and related clinical studies have begun for age-related macular degeneration (AMD), Parkinson's disease, corneal epithelial stem cell deficiency and other diseases, giving expectation that iPSC-based regenerative medicine will be widely used in the future. However, donors with HLA homozygous are rare. Genome editing technology could be used to reduce the transplant-rejection risk. Indeed, we reported HLA gene-edited iPSCs that could expand the range of patients who benefit from iPSC therapies faster than the homologous HLA haplotype strategy. This technology also has the potential to prevent or treat genetic diseases and gives great hope to patients. Other applications of iPSCs are drug screening, toxicity studies and disease modeling. In 2017, a new drug screening system using iPS cells for fibrodysplasia ossificans progressiva (FOP) was reported, revealing one drug candidate, Rapamycin, which is now undergoing a clinical trial to treat FOP patients. Additionally, Bosutinib, a drug for leukemia was revealed to be efficacious for amyotrophic lateral sclerosis (ALS) using a disease-specific iPSC model. Accordingly, we initiated a new clinical trial for Bosutinib to treat ALS at Kyoto University Hospital and other centers in 2019. Most recently, we launched a project to study COVID-19 by establishing several lines of iPSCs from recovered COVID-19 patients of different severity. We expect the models made from these cells to improve diagnosis, prevention and treatment for COVID-19. Over the past decade, iPSC research has made great progress, moving toward innovative therapeutics for people with intractable diseases by the application of new findings from basic science and reverse translation from clinics.

Keywords: induced pluripotent stem cells (iPSCs), cell-based therapies, genome editing technology



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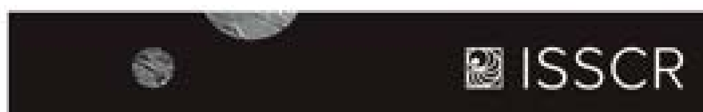

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