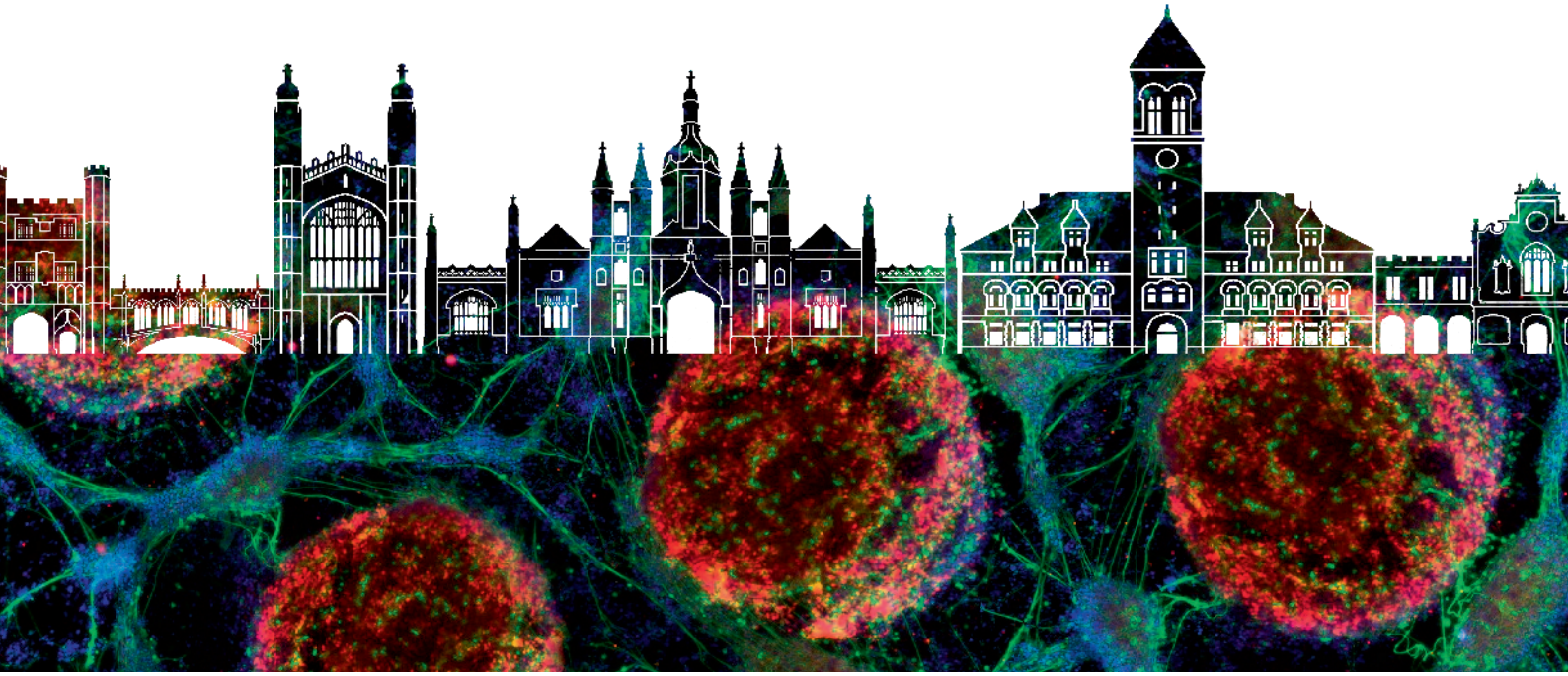


Cambridge International Stem Cell Symposium

19th - 21st September 2018

Organised by:

wellcome-MRC cambridge stem cell institute

PRACTICAL INFO

Poster Presenters

Please display your poster on the day of your poster presentation, when you arrive at the venue. Your poster numbers can be found in the event booklet poster insert.

Gala Dinner

The Gala Dinner will be held at Trinity College, accessed from West Road Concert Hall via the Queen's Road entrance. Only delegates who have registered and paid for dinner can attend. Delegate passes must be worn to access Trinity College.

Poster Prizes

Poster prizes will be awarded on Friday 21 September, with prizes generously provided by STEMCELL Technologies.

Registration

Please check in at the registration desk when you arrive at West Road Concert Hall. You will receive a delegate pass which must be worn at all times throughout the event.

Photography

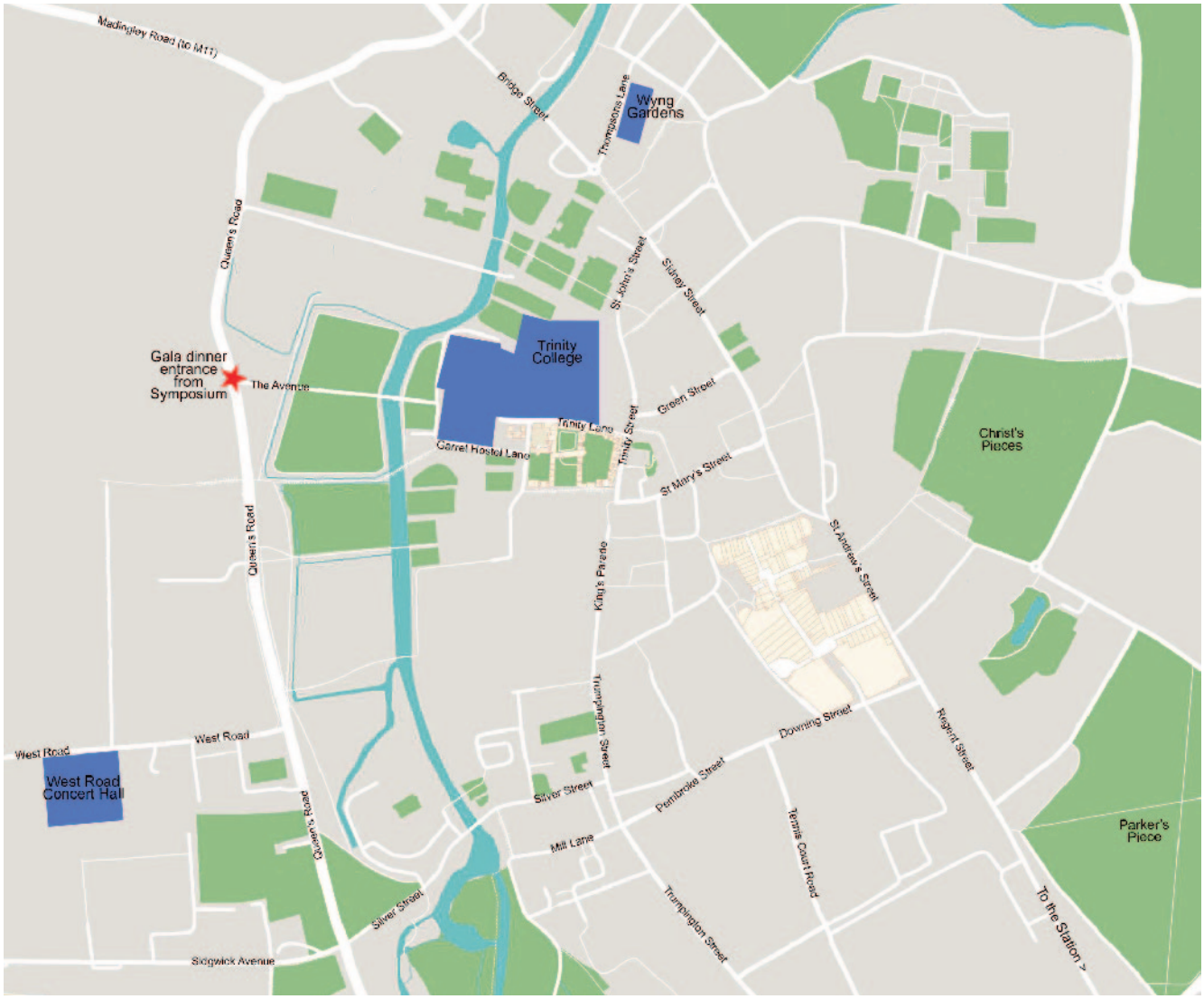
Photography and filming will take place throughout the Symposium. The photographs and films will be used by the University of Cambridge for the purpose of promoting its activities and may be published on the University's websites and social media, and circulated to the press and other media organisations for publication, transmission or broadcast. If you have any questions please speak to a member of staff.

Social Media

The hashtag for the Cambridge International Stem Cell Symposium is #CamStemCell. We will be liking and re-tweeting content from the Cambridge Stem Cell Institute Twitter account @SCICambridge throughout the event.

WiFi

If you do not have access to eduroam, please collect a WiFi code from the registration desk.



WELCOME

On behalf of the Cambridge Stem Cell Institute, I am delighted to welcome you to the 2018 Cambridge International Stem Cell Symposium, which brings together stem cell scientists from around the world.

This Symposium celebrates a step change for the Cambridge Stem Cell Institute - our move to a new state of the art building on the Cambridge Biomedical Campus. The relocation will bring together our faculty, currently based in six different locations around Cambridge, and will result in an integrated, vibrant and cohesive stem cell hub, well placed to capitalise on its scientific and clinical environment. We look forward to being able to welcome you to our new home soon.

As you all know, stem cells are fundamentally important for metazoan evolution and their dysfunction underlies many of the global health challenges that face us today. By co-locating biological, physical and clinical stem cell scientists, working across multiple cell types and at different scales, we aim to transform human health through a deep understanding of stem cell biology. I for one feel fortunate to be living in such exciting times.

I hope you enjoy the Symposium and your visit to Cambridge.

Prof Tony Green

Director, Wellcome – MRC Cambridge Stem Cell Institute

The new home of the Wellcome - MRC Cambridge Stem Cell Institute on the Cambridge Biomedical Campus

Credit: Sébastien Ricoult

Wednesday 19th September

11:00	**Registration & Refreshments**	
	Session 1 Chaired by Tony Green	Supported by Olympus
12:40	**Opening Address**	Patrick Maxwell, Head of the School of Clinical Medicine Chris Abell, Pro-Vice-Chancellor for Research
12:50	Allon Klein *Harvard University*	State and lineage maps of embryonic and adult tissues
13:20	Cristina Lo Celso *Imperial College London*	Healthy and malignant haematopoiesis: active cells in a dynamic environment
13:50	William Richardson *Wolfson Institute for Biomedical Research*	Glial and neuronal plasticity, learning and memory
14:20	Michaela Frye *University of Cambridge*	RNA methylation as a regulator of gene expression
14:40	**Poster Pitches**	
14:50	**Refreshments & Trade Exhibition**	
	Session 2 Chaired by Robin Franklin	Supported by STEMCELL Technologies
15:30	David Scadden *Harvard University*	Cancer through a stem cell lens
16:00	Kate Storey *University of Dundee*	Mechanisms regulating neural differentiation
16:30	Kevin Chalut *University of Cambridge*	Niche stiffness underlies the aging of central nervous system progenitor cells
16:50	**Keynote: Malin Parmar** *Lund University*	Developing a stem cell based therapy for Parkinson's Disease
17:30	**Poster Session 1 & Drinks Reception**	
19:00	**End of Day 1**	

Thursday 20th September

08:00	**Conference Opens**	
	Session 3 Chaired by Ben Simons	Supported by ZEISS
08:45	Fiona Watt *King's College London*	Cell state transitions in mammalian epidermis
09:15	Dirk Schübeler *Friedrich Miescher Institute for Biomedical Research*	Accessing the genome: transcription factors as sensors and modifiers of chromatin
09:45	Adrian Thrasher *University College London*	Evolving haematopoietic stem cell gene therapy
10:15	Andreas Trumpp *Heidelberg Institute for Stem Cell Technology*	Metabolic and epigenetic control in stem cells and cancer
10:45	**Refreshments & Trade Exhibition**	
	Session 4 Chaired by Anna Philpott	Supported by Geneflow and Biological Industries
11:25	Tom Rando *Stanford University*	Molecular regulation of stem cell quiescence
11:55	Elisa Laurenti *University of Cambridge*	Molecular and functional heterogeneity within the human haematopoietic stem cell compartment
12:15	Roger Barker and Malin Parmar	**Topical Debate:** Stem Cell therapies will never be curative for adult onset degenerative conditions
12:50	**Lunch & Trade Exhibition**	
13:15 - 13:35	STEMCELL Technologies Lunchtime Seminar Adam Hirst	Human pluripotent stem cell quality: essential considerations for gene editing, cloning, maintenance and disease modeling

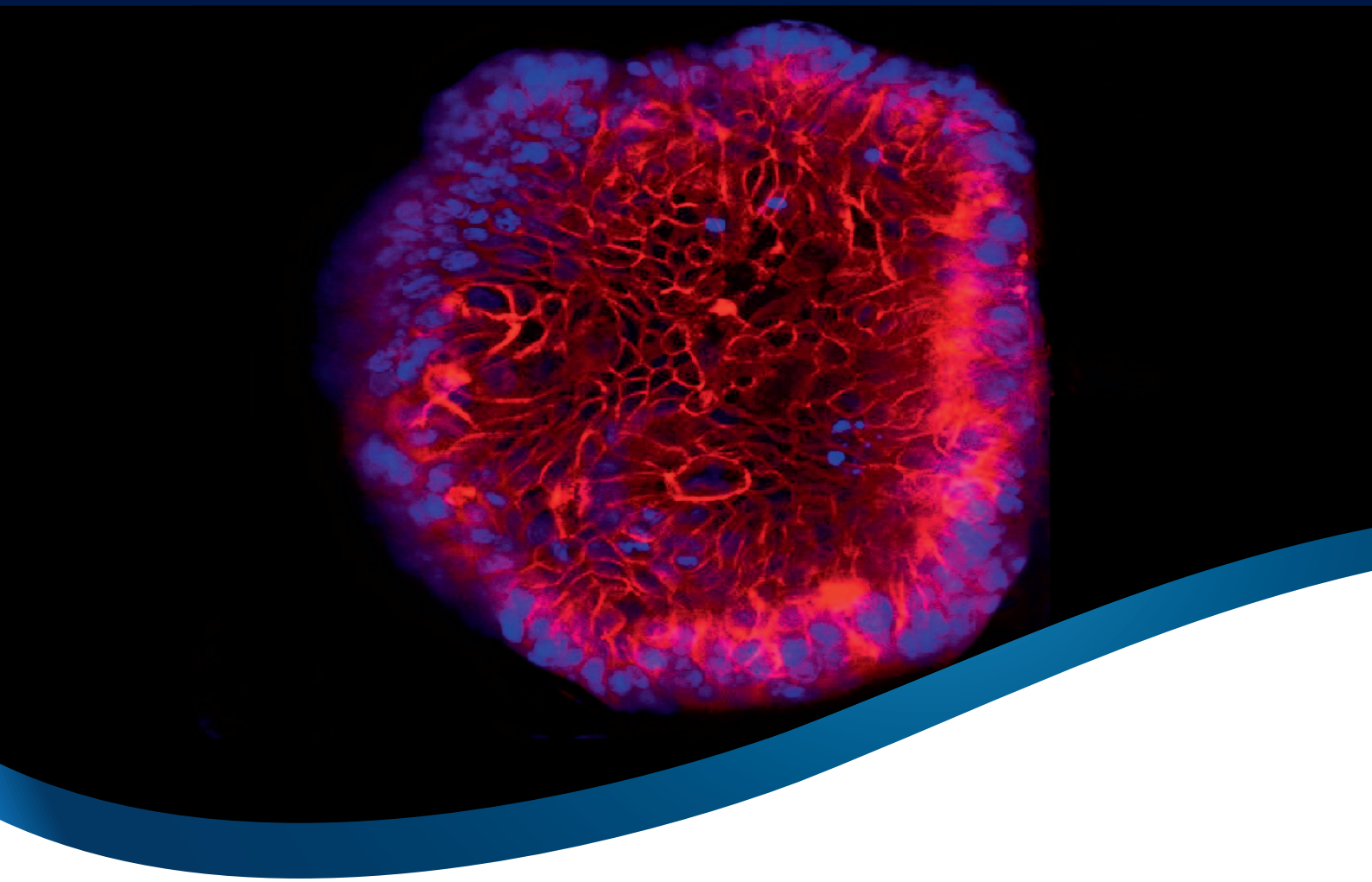
	Session 5 Chaired by Brian Huntly	Supported by Olympus
14:00	Peter Reddien *Massachusetts Institute of Technology*	The cellular and molecular basis for regeneration in planarians
14:30	Masayo Takahashi *RIKEN Center for Biosystems Dynamics Research*	Retinal cell therapy - now & future
15:00	David Kent *University of Cambridge*	Tracking human hematopoietic stem cell clonal dynamics using somatic mutations
15:20	**Poster Pitches**	
15:30	**Refreshments & Trade Exhibition**	
	Session 6 Chaired by Bertie Göttgens	Supported by DefiniGEN
16:10	Emanuelle Passegué *Columbia University*	TNFα coordinates hematopoietic stem cell survival and myeloid regeneration
16:40	Meritxell Huch *University of Cambridge*	Liver organoids for the study of liver regeneration and disease
17:00	**Keynote: Leonard Zon** *Harvard University*	Pathways regulating hematopoietic stem cell self-renewal and migration
17:40	**Poster Session 2 & Drinks Reception**	
19:00	**End of Day 2**	
19:30	Gala Dinner at Trinity College	

Friday 21st September

08:00	**Conference Opens**	
	Session 7 Chaired by Roger Barker	Supported by STEMCELL Technologies
08:45	Stuart Forbes *University of Edinburgh*	Drivers of liver failure and regeneration - can we steer the way to therapy?
09:15	Elena Cattaneo *University of Milan*	Huntingtin's biology and pathology in neurons derived from human pluripotent stem cells
09:45	Frederic de Sauvage *Genentech Inc - San Francisco*	Targeting intestinal stem cells in cancer
10:15	Cédric Ghevaert *University of Cambridge*	Producing platelets in vitro for transfusion: truth and dare
10:35	**Poster Prize Presentation**	
10:45	**Refreshments & Trade Exhibition**	
	Session 8 Chaired by Austin Smith	Supported by Axol Bioscience
11:25	Cédric Blanpain *Université Libre de Bruxelles*	Cell of origin in cancer and tumor heterogeneity
11:55	Duanqing Pei *Guangzhou Institutes of Biomedicine and Health*	Cell fate decisions during somatic cell reprogramming
12:25	Emma Rawlins *University of Cambridge*	Cell-cell interactions controlling normal human lung development
12:45	**Keynote: Hiromitsu Nakauchi** *Stanford University*	Stable ex vivo HSC expansion: dream or reality?
13:25	**Closing Remarks**	
13:30	**Refreshments**	
14:00	**End of Symposium**	

DefiniGEN

Disease modelling in iPS-derived human cells



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Speaker Profiles

Cédric Blanpain, Université Libre de Bruxelles

Cédric Blanpain is full Professor, WELBIO investigator and director of the laboratory of stem cells and cancer at the Université Libre de Bruxelles. His research group uses lineage-tracing approaches to study the role of SCs during development, homeostasis and cancer. His group uncovered the existence of stem cells and progenitors acting during homeostasis and repair of the epidermis, and uncovered a novel paradigm of lineage segregation in the mammary gland and prostate. His lab pioneered in using mouse genetics to identify the cell of origin of epithelial cancers. They identified the cancer cell of origin and the mechanisms regulating the early steps of tumor initiation in skin basal cell carcinoma, skin squamous cell carcinoma and mammary tumors. His lab developed novel genetic approaches to unravel the mode of tumour growth within their natural environment and to understand the mechanisms regulating cancer stem cell functions and tumor heterogeneity.

Cédric Blanpain received several prestigious awards including EMBO Young investigator award, ERC starting and ERC consolidator grants, the outstanding young investigator award of the ISSCR 2012, the Liliane Bettencourt award for life sciences 2012, Joseph Maisin Award for basic biomedical Science 2015. He has been elected member of the EMBO in 2012, the Belgian Royal Academy of Medicine 2015, and the Academia Europa 2016.

Elena Cattaneo, University of Milan

Elena Cattaneo is a Professor at University of Milan. She is director of the "Laboratory of Stem Cell Biology and Pharmacology of Neurodegenerative Diseases" at the Department of Biosciences, and is co-founder and director of UniStem, the University of Milan's Centre for Stem Cell Research (www.unistem.it). On August 30th 2013, the President of Italian Republic, Giorgio Napolitano, appointed her Senator for Life on account of her scientific and social merit. Following the appointment, Elena Cattaneo has continued to lead the lab. The aim remains to find breakthroughs that may accelerate a cure for Huntington's disease

Kevin Chalut, University of Cambridge

Kevin Chalut is a biophysicist with a PhD in Physics from Duke University. Since 2011 he has been a Royal Society University Research Fellow. Kevin's post-graduate background is in biotechnology and imaging, particularly with regards to detecting cancer and characterising stem cells. He is currently a group leader at both the Cavendish Laboratory and the Wellcome-MRC Cambridge Stem Cell Institute.

His work focuses on developing novel biotechnology to investigate physical states of cells such as mechanics and subcellular structure; in the last few years he has focused almost exclusively on the biophysics of embryos and embryonic stem cells. The ultimate goal of his laboratory is to discover physical mechanisms and their importance to pluripotency, differentiation and reprogramming.

Frederic de Sauvage, Genentech

Frederic de Sauvage obtained his PhD summa cum lauda from the Catholic University of Louvain in Belgium. He joined the laboratory of David Goeddel at Genentech as a postdoctoral fellow in 1990 and was hired as a Scientist in 1992. In 1994, Dr. de Sauvage's team at Genentech discovered Thrombopoietin (TPO), the long sought physiological regulator of platelet production. He switched his focus to the Hedgehog pathway in the late 1990s. His work led to the development of vismodegib, a Hedgehog Pathway Inhibitor recently approved for the treatment of metastatic or locally advanced basal cell carcinoma (BCC). Dr. de Sauvage has recently turned his attention to studying the role of intestinal stem cells in tumorigenesis. In 2011 he received the Achievement in Advancing Targeted Therapies for Cancer & Melanoma Award from the American Skin Association in recognition for his work for patients with BCC. He was elected fellow of the American Association for the Advancement of Science in 2016. Dr. de Sauvage is now the vice president of Research-Molecular Oncology at Genentech.

Stuart Forbes, University of Edinburgh

Stuart Forbes (SF) is Professor of Transplantation and Regenerative Medicine, at the University of Edinburgh. SF's research focuses on how the chronically damaged liver regenerates and how these mechanisms become deranged in liver and bile duct cancer (Group Website: http://www.crm.ed.ac.uk/research/group/liver-stem-cells-regeneration). SF's research studies how the epithelial cells in the liver (hepatocytes and biliary cells) interact with the inflammatory cells of the liver during injury and regeneration. The group is seeking to use these findings to develop novel therapies for liver failure and cancer.

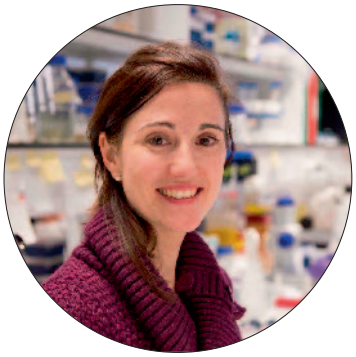
SF is Director of the MRC Centre for Regenerative Medicine, (http://www.crm.ed.ac.uk/) which houses 230+ scientists and clinicians. SF is Director of the UK Regenerative Medicine Platform Hub for the Engineered Cell Environment (https://www.ukrmp.org.uk/hubs/engineered-cell-environment/). SF is a consultant Hepatologist at the Scottish Liver Transplant Unit and has clinical interests in liver failure, cancer and liver transplantation.

Michaela Frye, University of Cambridge

Michaela Frye completed her PhD in Frankfurt/Main in Germany in 2000 studying the role of epithelial defensins in Cystic Fibrosis. In 2001, she joined Cancer Research UK (CRUK) in London as a Postdoctoral Fellow, where she developed her fascination for the question how stem cells form and maintain adult tissues. In 2007, Michaela started her independent research group at the Wellcome–MRC Cambridge Stem Cell Institute. She received a CRUK Career Development Fellowship in 2007 and a CRUK Senior Fellowship and an ERC Consolidator Grant in 2013 to study how dysregulation of stem cell function contributes to human diseases and cancer. She currently holds a Readership in Stem Cell Biology at the Department of Genetics.

Cédric Ghevaert, University of Cambridge

Dr Ghevaert is a senior Lecturer in Transfusion Medicine at the University of Cambridge and Consultant Haematologist for the NHS Blood and Transplant. He obtained his MD in 1998 from the University Libre de Bruxelles. After qualifying as a Consultant Haematologist, he did his PhD in Cambridge to develop recombinant antibodies for the treatment of fetomaternal alloimmune thrombocytopenia which culminated in a first-in-man study. Upon obtaining his PhD, Dr Ghevaert obtained a personal fellowship from the British Heart Foundation to work on megakaryopoiesis in the context of myeloproliferative diseases at the University of Birmingham. He was appointed as a Principal Investigator at the University of Cambridge in 2010 and is currently a group leader at the Wellcome-MRC Cambridge Stem Cell Institute. His group focuses its work on the production of blood cells from pluripotent stem cells with the declared aim to produce novel cellular therapies for transfusion to patients. His approach combines the manipulation of key transcriptional regulators to efficiently forward programme stem cells into mature blood cells, and uses 3D bioengineered scaffolds to recreate the bone marrow niche and increase the production efficiency and purity of the manufactured blood cells. He still has a keen interest in inherited platelet disorders (such as Thrombocytopenia with Absent Radii) and using the pluripotent stem cell technology for disease modelling. His expertise lies at the hinge of basic bench-based science and translational studies and as such will be supervising a clinical trial of in vitro-produced red cells in human volunteers in 2018.



Meritxell Huch, University of Cambridge

After obtaining her PhD degree at the Center for Genomic Regulation in Barcelona, Spain, Meritxell moved to the Netherlands to join the laboratory of Professor Hans Clevers in order to redirect the focus of her research into Adult Stem Cell Biology. In her postdoctoral stay, she isolated adult mouse stomach and mouse and human liver cells and proved these can be expanded in culture, forming stomach and liver organoids in vitro. In 2014 Dr Meritxell Huch established her independent lab at the Gurdon Institute, University of Cambridge, where she works on elucidating the replicative potential of adult stem cells during tissue regeneration and disease.

David Kent, University of Cambridge

David Kent earned a B.Sc. in Genetics and English Literature at the University of Western Ontario, Canada (1999-2003) and obtained his Ph.D. in normal adult blood stem cell biology at the University of British Columbia, Canada (2003-2009). His postdoctoral research was at the University of Cambridge where he primarily studied malignant blood stem cell biology. His research group studies fate choice in single blood stem cells and how changes in their regulation lead to cancers.

David is currently the Wellcome-MRC Cambridge Stem Cell Institute's Public Engagement Champion and has a long history of public engagement and outreach including the creation of The Black Hole, a website and blog that provides information on and analysis of issues related to the education and training of scientists.

Allon Klein, Harvard University

Dr. Klein carried out his graduate training in physics with Prof. Ben Simons (Cambridge University), and his postdoctoral training in experimental systems biology with Prof. Marc Kirschner (Harvard Medical School). He is currently an Assistant Professor in the Department of Systems Biology at Harvard Medical School. Dr. Klein studies how cells make fate choices in developing and adult tissues, specifically specializing in mapping proliferative hierarchies and quantifying the phenomenon of fate control. He has developed droplet microfluidics for high-throughput single-cell transcriptomics (inDrop RNA-Seq), and theoretical/ computational methods for analyzing single-cell data sets and for quantitative clonal analysis. He focuses on the early embryo, the hematopoietic system, and epithelial tissues as model systems

Elisa Laurenti, University of Cambridge

Elisa Laurenti's career has so far been dedicated to the study of the very first steps of haematopoiesis both in mouse and human. She trained with Prof. Andreas Trumpp (ISREC, Lausanne, Switzerland) for her PhD, studying Myc function and dormancy in mouse haematopoietic stem cells (HSCs). During her post-doc with Dr John Dick (University Health Network, Toronto, Canada), she became interested in the molecular control of human HSC quiescence and differentiation towards lymphoid fates, building comprehensive transcriptional resources and establishing novel in vitro single cell and in vivo assays to study the function of the earliest lymphoid progenitors and of the purest human HSC population described so far. Since 2014, she is a principal investigator and Wellcome Trust Sir Henry Dale Fellow at the Wellcome-MRC Cambridge Stem Cell Institute. Research in her laboratory combines single cell tools, at the transcriptomic, genomic and functional level to dissect the heterogeneous behaviours and molecular programmes of human HSCs at all stages of human life, including during the early stages of leukemia initiation. The goal is to understand how the dynamic molecular networks and functional properties of human HSC control blood formation in health and disease.

Cristina Lo Celso, Imperial College London

Dr Lo Celso graduated from Torino University in Italy. She obtained her PhD from UCL, working with Fiona Watt at the CRUK London Research Institute, where she studied epidermal stem cells. She started performing intravital microscopy of the haematopoietic stem cell (HSC) niche during her postdoctoral training at Harvard University with David Scadden. In 2009 she started her independent research group at Imperial College London, where she is now a reader in the department of Life Sciences, and network lead of the Imperial Stem Cell and Regenerative Medicine Network. Dr Lo Celso recently established a satellite laboratory at the Sir Francis Crick Institute. Her research aims to understand the mechanisms regulating HSC function during steady state and in the presence of stress, such as infections and leukaemia development. Her approach combines mouse bone marrow intravital microscopy techniques, computational image analysis, molecular profiling and mathematical modelling of the HSC niche. Dr Lo Celso is the first woman to receive the Foulkes Medal award (2017).

Hiromitsu Nakauchi, Stanford University

After earning M.D. and a Ph.D. in Japan, Dr. Nakauchi went to Stanford University as a postdoctoral scholar and isolated CD8 genes. After returning to Japan, he started working on hematopoietic stem cells in his laboratory at RIKEN. In 1994, he became a Professor of Immunology at the University of Tsukuba where he demonstrated that a single hematopoietic stem cell could reconstitute the entire hematopoietic system, a definitive experimental proof for the "stemness". Since April 2002, he has been a Professor of Stem Cell Therapy in the Institute of Medical Science at The University of Tokyo (IMSUT). In 2008, he was appointed Director of the newly established Center for Stem Cell Biology and Regenerative Medicine at IMSUT. In 2014, he returned to Stanford University as faculty to continue his stem cell research at the Institute of Stem Cell Biology and Regenerative Medicine. Goals of his work are to translate discoveries in basic research into practical medical applications.

Malin Parmar, Lund University

Malin Parmar is a Professor in cellular neuroscience at Lund University. Together with her lab she has shown in a series of high profile publications how human fibroblasts can be converted into neurons, how glial cells can be reprogrammed into neurons in vivo, and how functional dopamine neurons can be generated from human embryonic stem cells. She is the recipient of an ERC starting grant and an ERC Consolidator grant and was recently awarded a New York Stem Cell Foundation – Robertson investigator. Malin's research has a strong translational focus and she collaborates within European and International networks as well as Industry partners to develop new, cell based therapies for Brain Repair with focus on Parkinson's Disease

Emanuelle Passegué, Columbia University

Emmanuelle Passegué, Ph.D., is the Alumni Professor of Genetics & Development and the Director of the Columbia Stem Cell Initiative (CSCI) at Columbia University Irving Medical Center (CUIMC) in New York City. Dr. Passegué received her Ph.D. from the University Paris XI (France), and trained with Dr. Erwin Wagner (Institute for Molecular pathology, Vienna, Austria) and Dr. Irv Weissman (Stanford University, USA) before joining the University of California San Francisco (UCSF) in 2005. Dr. Passegué was a Professor of Medicine in the Hematology/Oncology Division and the Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research at UCSF until 2016 before joining CUIMC in January 2017. Her research investigates the biology of blood-forming hematopoietic stem cells in normal and deregulated contexts such as hematological malignancies and physiological aging. Dr. Passegué has received a number of awards and prizes including a Scholar Award from the Lymphoma and Leukemia Society and an Outstanding Investigator Award from the NHLBI.

Duanqing Pei, Guangzhou Institutes of Biomedicine and Health

Duanqing Pei is Professor and Director General of Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, in Guangzhou, China. He is the founding executive director of the newly established Guangzhou Regenerative Medicine and Health Guangdong Laboratory, a new initiative in response to the National Lab System in China. Dr. Pei joined the Medical Faculty at Tsinghua University in Beijing China in 2002 and moved to the Guangzhou Institutes of Biomedicine and Health in 2004. Prior to this appointment, he served as a faculty member at the University of Minnesota School of Medicine. Dr. Pei studied the transcription regulation of hepatitis B virus (HBV) for his PhD thesis and worked on extracellular matrix remodeling as a postdoctoral fellow and faculty member. Upon returning to China, he started to work on stem cell pluripotency first and then reprogramming. The Pei lab in Tsinghua began to publish on the structure and function of Oct4, Sox2, FoxD3, Essrb, and Nanog, and their interdependent relationship towards pluripotency. The Pei lab was the first in China to create mouse iPSCs using a non-selective system, and then improved the iPS process systematically. The Pei lab subsequently disseminated the iPS technology in China by providing not only resources, but also training workshops. Besides basic research, he has a strong interest in translational research. His lab has used ZFNs initially, then Talen, and CRISPR to correct mutations in stem cells with hope to cure diseases through the combined approach of gene editing and stem cell technology. Now, his lab continues to explore new ways to understand and manipulate cell fate, utilize these cutting edge technology to cure diseases.

Tom Rando, Stanford University

Dr. Rando received his AB, MD, and PhD degrees from Harvard University and then completed a residency in neurology at UCSF and postdoctoral training at Stanford. He is currently Professor of Neurology and Neurological Sciences and Director of the Glenn Center for the Biology of Aging at Stanford. Dr. Rando directs the Center for Tissue Regeneration, Repair, and Restoration (CTR3), a program in regenerative medicine combining expertise in stem cell biology and bioengineering, at the VA Palo Alto Health Care System where he is also Chief of Neurology. Dr. Rando's research focuses on stem cell biology, with particular interest in stem cell aging. He has pioneered the field of systemic factors as regulators of cellular aging based upon seminal studies done in his laboratory using the technique of heterochronic parabiosis. His laboratory has also pioneered studies of the regulation of stem cell quiescence, with a focus both on the basic biology and therapeutic applications.

Dr. Rando has received numerous awards including the "Breakthroughs in Gerontology" Award from the American Federation for Aging Research, the NIH Director's Pioneer Award, and a Transformative Research Award from the NIH. Dr. Rando is a member of the National Academy of Medicine and a Fellow of the American Association for the Advancement of Science.

Emma Rawlins, University of Cambridge

Emma Rawlins is an MRC Senior non-clinical fellow based at the Gurdon Institute, University of Cambridge and her laboratory works on lung developmental and stem cell biology and regeneration. Specific questions addressed include: How are our lungs built and maintained? How does this go wrong in disease? Can we use our insights from developmental biology to induce effective lung regeneration? Or to promote improved maturation of premature lungs? The laboratory uses a combination of human embryonic lung organoids and mouse genetics as model systems. They perform multiple techniques including, in vitro and mouse genetics, lineage-tracing, microscopy, live-imaging, cellular and molecular techniques.

Peter Reddien, Massachusetts Institute of Technology

Peter W. Reddien is focused on understanding how regenerative organisms regrow body parts. Using planarians (flatworms), his lab has identified important cellular and molecular components of the process of regeneration. Peter developed and performed the first RNAi screen in planarians, identifying genes important for regeneration. The Reddien lab has shown that regeneration in planarians is driven by pluripotent stem cells called cNeoblasts. They also found that positional information in the form of molecular signals to surrounding tissues is harbored in muscle. Together, muscle-derived positional information and stem cells help explain regeneration. Peter Reddien is Professor of Biology and Associate Head of the MIT Department of Biology, Associate Director at the Whitehead Institute, and an HHMI Investigator.

William Richardson, Wolfson Institute for Biomedical Research

Bill Richardson obtained his BSc (Physics) from Manchester University in 1973, and his PhD (Biophysics) from King's College London in 1978. He spent postdoc time at NIH (molecular virology) and NIMR (molecular cell biology) before winning his first tenured job as Lecturer in the Department of Zoology (later Biology) at UCL. From then on, he switched to developmental neuroscience, strongly influenced by Prof Martin Raff, and spent the next 30 years at UCL working on the development of glial cells – especially oligodendrocytes, the myelinating cells of the central nervous system (CNS). In the past 10 years or so he has shown that oligodendrocytes continue to be generated from their precursors throughout young adult life, and that the newly-forming oligodendrocytes are required for mice to learn new motor skills. He is continuing to investigate the role of adult oligodendrogenesis (and neurogenesis) in learning and memory, through a combination of genetic and behavioural experiments in mice. He was elected a Fellow of the Academy of Medical Sciences in 2011 and of the Royal Society in 2013. He indulges his interest in Development outside the lab, keeping bees in his North London garden.

David Scadden, Harvard University

David Scadden is the Gerald and Darlene Jordan Professor of Medicine at Harvard University. He is Professor and Chair of the Harvard University Department of Stem Cell and Regenerative Biology. With Douglas Melton, he co-founded and co-directs the Harvard Stem Cell Institute. He is a hematologist/oncologist at the Massachusetts General Hospital where he founded and directs the Center for Regenerative Medicine and previously led the Hematologic Malignancies Program of the MGH Cancer Center. He is a member or fellow of the National Academy of Medicine, the American Academy of Arts and Sciences, the American Association for the Advancement of Science, the American College of Physicians, the Board of External Experts for the National Heart, Lung and Blood Institute and a former member of the National Cancer Institute's Board of Scientific Counselors. He is an Affiliate Member of the Broad Institute of Harvard and MIT. He co-founded Fate Therapeutics and Magenta Therapeutics and is a Director of Agios Pharmaceuticals. His work emphasizes using multidisciplinary approaches to define novel therapies for blood diseases.

Dirk Schübeler, Friedrich Miescher Institute for Biomedical Research

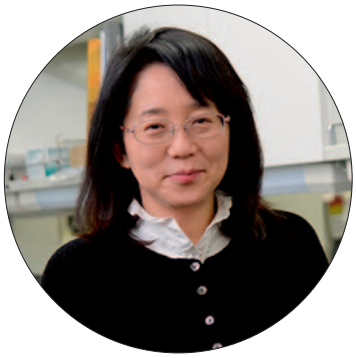
Dirk Schübeler obtained his PhD from the University of Braunschweig, Germany for work on cis-regulatory sequences that bind to the nuclear matrix. His postdoctoral studies with Mark Groudine in Seattle, USA focused on epigenetic phenomena involved in mammalian gene regulation.

In 2003 he established his own group at the Friedrich Miescher Institute in Basel continuing to study the regulation of chromatin and DNA methylation at the level of the genome. In 2008 he became a senior group leader and in 2011 Professor at the University of Basel. He previously obtained a ERC starting grant and currently holds an ERC advanced investigator grant.

The work of his lab has lead to comprehensive views on the genomic pattern of histone modifications, replication timing and DNA methylation in stem cells and during cellular differentiation. Current work is aimed at further understanding, at the level of individual gene loci and genome-wide, how these nuclear events influence the expression phenotype in higher eukaryotes and how they are propagated during cell division and differentiation.

Kate Storey, University of Dundee

Kate Storey is Head of the Division of Cell & Developmental Biology, Chair of Neural Development and co-director of the Light Microscopy Facility, in the School of Life Sciences, at the University of Dundee, Scotland, UK. She investigates cellular and molecular mechanisms regulating vertebrate neural differentiation in embryos and embryonic stem cells. Her work includes the development of live imaging approaches for monitoring individual cell behaviour and cell signalling in tissues as well as investigation of the molecular basis of the signalling switch that controls neural differentiation onset in the elongating vertebrate embryonic axis.



Masayo Takahashi, RIKEN Center for Biosystems Dynamics Research

Masayo Takahashi M.D., Ph.D. is Project Leader of the Laboratory for Retinal Regeneration at RIKEN. She received her M.D. from Kyoto University in 1986, and her Ph.D. in Medicine at the same institution in 1992. After serving as an assistant professor in the Department of Ophthalmology, Kyoto University Hospital, she moved to the Salk Institute in 1995, where she first learned of the potential of stem cells as a tool for retinal therapy. She returned to the same hospital in 1997, and since 2001 has served as an associate professor at the Translational Research Center. She joined RIKEN as a team leader of the retinal regeneration team in 2006. Her team started a pilot clinical study of autologous iPS cell-derived RPE cell sheets for exudative aged-related macular degeneration (AMD) in 2013. The first RPE cell sheet graft was transplanted in September 2014. In 2017, the team started using allogeneic iPS cells suspension in the clinical study. Her clinical specialty is retinal disease—macular diseases and retinal hereditary diseases in particular. Her aim is to gain a better understanding of these diseases at a fundamental level and develop retinal regeneration therapies.

Adrian Thrasher, University College London

Adrian Thrasher is Professor of Paediatric Immunology and Wellcome Trust Principal Research Fellow at the UCL Great Ormond Street Institute of Child Health (UCL GOSICH), and Honorary Consultant Paediatric Immunologist at Great Ormond Street Hospital for Children NHS Foundation Trust. He is the Programme Head of Infection, Immunity and Inflammation at UCL GOSICH and has a long standing research and clinical interest in development and application of gene therapy. He is Director of the Clinical Gene Therapy Programme, and Theme Leader of the Gene Stem and Cellular Therapies theme of the Biomedical Research Centre, at UCL GOSICH/GOSH. Adrian is PI on several clinical trials for immunodeficiency and is Director of the clinical gene therapy GMP facility, managing a team of trial coordinators, clinical scientists, and quality systems personnel.

His clinical interests are the diagnosis and treatment of patients with primary immunodeficiency. His specialist interests are in the Wiskott-Aldrich Syndrome (WAS), disorders of innate immunity, and Autoimmune Lymphoproliferative Syndrome. His team at UCL GOSICH/GOSH are conducting trials of somatic gene therapy for various forms of PID including SCID-X1, CGD, ADA-SCID, and WAS. Research interests include the pathophysiology of primary immunodeficiency syndromes especially WAS, the actin cytoskeleton in haematopoietic cells, the development of somatic gene therapy, and thymus transplantation.

Andreas Trumpp, Heidelberg Institute for Stem Cell Technology

Professor Andreas Trumpp is heading the Division of "Stem Cells and Cancer" at the German Cancer Research Center (DKFZ) in Heidelberg, Germany and is Managing Director of the "Heidelberg Institute for Stem Cell Technology and Experimental Medicine" (HI-STEM GmbH). After obtaining his PhD at the European Molecular Biology Laboratories (EMBL) in Heidelberg, in 1994 he moved to the University of California at San Francisco to work on the oncogene MYC with Prof. J. Michael Bishop. After starting his independent laboratory at the Swiss Institute for Experimental Cancer Research (ISREC) in Epalinges, Lausanne in 2000, he became Professor at the EPFL in Lausanne in 2005. Since 2008 his lab is located in Heidelberg.

Dr. Trumpp is internationally renowned for his contribution to the molecular and cellular basis of normal and malignant stem cell self-renewal as well as the role of MYC in stem cells and cancer. His work established the concept of dormancy in hematopoietic and pluripotent stem cells and his team recently identified a novel pathway in leukemic stem cells linking metabolic traits to epigenetic alterations in AML stem cells. The group has also isolated and characterized circulating metastasis stem cells in the blood of breast cancer patients and identified a novel mechanism that mediates resistance of pancreatic tumors to clinically used drugs. He has published more than 120 peer-reviewed papers, among them numerous senior authorships in Nature, Cell, Science, Cell Stem Cell, Nature Cell Biology and Nature Medicine (H-index: 55).

Fiona Watt, King's College London

Fiona Watt obtained her first degree from the University of Cambridge and her DPhil, in cell biology, from the University of Oxford. She was a postdoc at MIT, where she first began studying differentiation and tissue organisation in mammalian epidermis. She established her first research group at the Kennedy Institute for Rheumatology and then spent 20 years at the CRUK London Research Institute. She worked in Cambridge from 2007 to 2012. In 2012 she moved to King's College London to found the Centre for Stem Cells and Regenerative Medicine. She is internationally recognised for her work on stem cells and their interactions with the niche in healthy and diseased skin. She is currently on secondment as Executive Chair of the Medical Research Council.

Leonard Zon, Harvard University

Dr. Zon is the Grousbeck Professor of Pediatric Medicine at Harvard Medical School, an Investigator at Howard Hughes Medical Institute, and the Director of the Stem Cell Program at Boston Children's Hospital. He is internationally-recognized for his pioneering work in stem cell biology and cancer genetics, and has been the preeminent figure in establishing zebrafish as an invaluable genetic model for the study of the blood and hematopoietic development.

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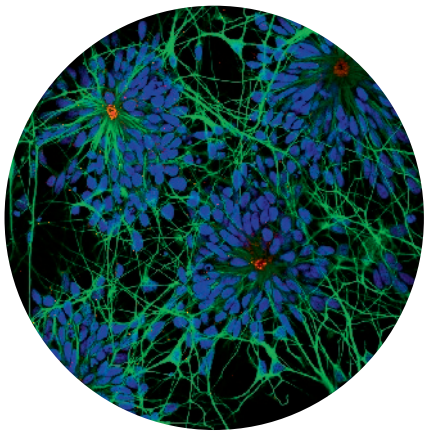

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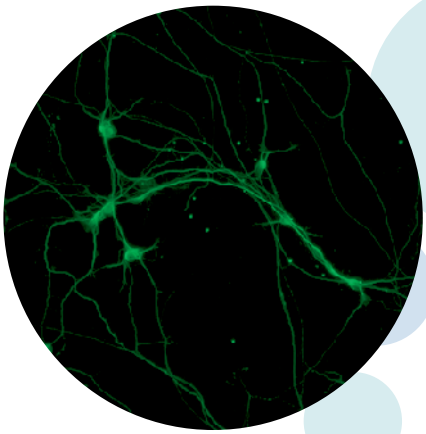

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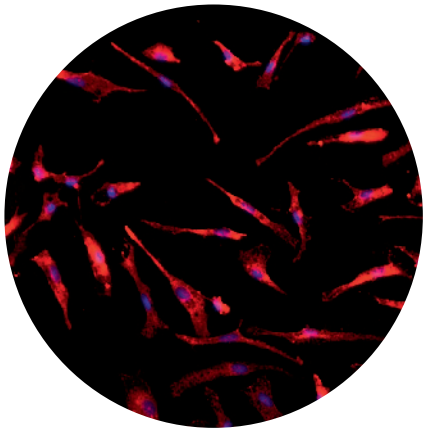

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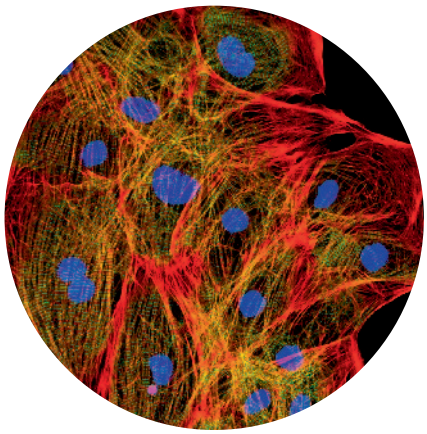

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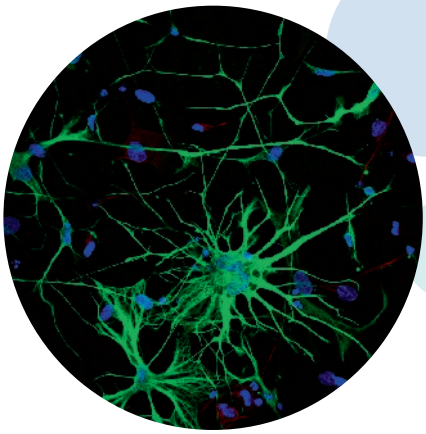

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Poster Abstracts

Session 1: Wednesday 19 September, 17:30 - 19:00

Session 2: Thursday 20 September, 17:40 - 19:00

Poster Session 1
Wednesday 19 September, 17:30 - 19:00

Theme 1: Adult Stem Cells

1 Cellular dynamics and crosstalk during intestinal tumour initiation

Yum MK, Han S, Dabrowska C, Fink J, Chatzeli L, Koo B-K, Simons B

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: intestine; early tumorigenesis; cell-cell interaction

Complex interactions between cancer cells and their surrounding microenvironment contribute to tumour progression metastasis and recurrence. However how cancer cells manipulate neighbouring normal epithelium remains elusive. Here we show the cellular and molecular mechanism of cancer cell - normal cell interaction during early intestinal tumourigenesis. Oncogene-associated multi-colour reporters (Red2Onco) were generated for fate-mapping of individual tumourigenic and normal clones. Through quantitative analysis of clonal distribution we find that KRAS or PI3K activation leads to acceleration in monoclonal conversion of not only tumourigenic clone itself but also normal clone in proximate crypt. Single cell transcriptomic profiling and loss-of function genetics will be carried out to elucidate molecular bases of this phenomenon. Our work reveals a novel mechanism of tumour propagation where cancer cells modulate its microenvironment dynamically which may contribute to field cancerisation.

2 The role of the protocadherin CDHR5 in colorectal cancer

Awad M, Krieger S, Milovanovic D, Svinka J ,Timelthaler G, Kenner L, Eferl R

Medical University Vienna, Austria

Keywords: colorectal cancer; secretory phenotype; metastasis suppressor; stemness

The protocadherin CDHR5 is a transmembrane protein that is located in the microvillar brush border of enterocytes, cholangiocytes and kidney epithelial cells. CDHR5 crosslinks microvilli and has been implicated in regulation of β-Catenin activity. Protocadherins are frequently downregulated in human cancers suggesting a negative role in oncogenesis. We are interested in CDHR5 functions in colorectal cancer (CRC). We found that CDHR5 expression is downregulated in altered crypt foci, adenomas, carcinomas and CRC liver metastasis. Transplantation experiments into immunocompromised host mice suggested a tumor-suppressive role of CDHR5 in CRC. Therefore, we generated CDHR5 knock-out mice (CDHR5Δ/Δ) to investigate CDHR5 functions in autochthonous colorectal tumors. Knock-outs were viable and did not show an overt intestinal phenotype but displayed shortening of microvilli, mislocalization of brushborder proteins, a mucosal barrier defect and a secretory phenotype determined by an increase of goblet, paneth and enteroendocrine cells in the intestine. Application of the Azoxymethane/Dextransulfate protocol for induction of CRC led to increased tumor load and formation of aggressive carcinomas that invaded the muscularis mucosa in CDHR5Δ/Δ mice. Tumors of CDHR5Δ/Δ mice showed increased nuclear β-Catenin levels and evidence for epithelial to mesenchymal transition. 3-dimensional BBE spheroids with shRNA-mediated knock-down of CDHR5 displayed increased expression of crypt base columnar stem cell markers. These data suggest that downregulation of CDHR5 in tumors promotes cancer stemness and metastasis. We are currently using 3-dimensional cultures and RNAs to investigate the crosstalk between CDHR5, Wnt and Notch signaling in normal tissue homeostasis and CRC.

3 Deciphering prostate progenitors compartment complexity as a route of targeting clinically significant prostate cancer

Baena E

Cancer Research UK
Manchester Institute, UK

Keywords: luminal progenitor; castration-resistant; stratification; prostate cancer

Personalized treatment for prostate cancer remains a challenge because there are no clear molecular subtypes to guide patient response. Imaging, PSA levels and pathological assessment of biopsies through the Gleason grading system remain the gold standard for diagnosis and risk stratification. Moreover, most genomic campaigns analysed single biopsies with reduce analysis of their cellular landscape, which limit the value of this analysis in what is know to be a multifocal disease. By combining genomic and multiparametric imaging analysis of high-risk prostate cancer patients, we have characterized the radiogenomic landscape of multifocal prostate cancer (Parry, Srivastana, Ali et al, in press). Moreover, coupling single-cell profiling and functional characterization by organoid-culture and in situ lineage-tracing analysis in mouse models, we have identified inherently castration-resistant cellular subpopulations in the prostate defined by their unique cell-surface markers. In particular, our studies define LY6D as a marker for prostate progenitors and castration-resistant luminal cells, which may serve as prognostic maker for advanced prostate cancer (Barros-Silva, Linn, Steiner, in revision). Further functional characterisation of the identified therapy-resistant prostate luminal subpopulations will highlight their contribution to tumour subtypes thereby advancing patient stratification and setting a pipeline to develop novel therapeutics.

4 Postnatal Cell Dynamics; Finding the path towards Oesophageal Homeostasis

McGinn J, Hallou A, Simons B, Alcolea M

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: Homeostasis, Development, Epithelial

The resilience of epithelial tissues in response to disruption has traditionally been thought to rely upon a stem cell population, enabling repair and maintenance. However, recent works suggest that cell fate is highly dynamic, providing the capacity for differentiating or lineage-committed cells to reacquire regenerative potential in response to increased tissue demands. The simple architecture of the mouse oesophagus represents an ideal model for studying cellular dynamics and provides insights into the remarkable plasticity of epithelial cells. Combining this model with genetic lineage-tracing and methods of statistical physics, it has been found that cells present an extraordinary resilience, being able to adapt their behavior in response to tissue perturbations maintaining tissue integrity. In this study we investigate post-natal development, where the mouse oesophagus must expand in coordination with the animal, as a convenient model to study changes in epithelial cell fate during the transition from development to homeostasis, and its potential relevance for tumor development. Observations throughout post-natal development have revealed that this tissue expansion occurs in a biphasic pattern with a fast initial growth that slows down before reaching adult size. This results in a tissue 7-times its size at birth, which is able to halt expansion upon achieving homeostasis. During earlier stages of post-natal development cells show a bias towards progenitor cell fate. This tilt in fate bias is progressively lost as the tissue matures, acquiring a greater number of stratified cell layers and an established epithelial barrier by increasing levels of terminal differentiation. During the transition from development to homeostasis, the co-localisation of markers specific to progenitor and differentiating cells provides critical insight into the mechanisms controlling early cell-fate decisions, and may be utilized to unveil the mechanisms of epithelial cell dynamics.

5 Tracing the early cellular events of gastric cancer

Chatzeli L, Dabrowska C, Koo B-K, Simons B

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: cancer stem cells; clonal dynamics; Kras

Gastric cancer is the third most common cancer worldwide and one of the leading causes of cancer mortality. Despite the efforts to identify common mutations occurring in gastric cancer the prognosis is still poor. This could be attributed to our limited knowledge of the early transformation events which includes characterisation of how a given oncogenic mutation perturbs the clonal dynamics of stem cells. To address this question, we developed a novel modified version of the confetti mouse line which expresses the oncogene KRasG12D exclusively in cells expressing Red Fluorescent Protein. The recombination is driven specifically in the stomach epithelium using the novel Anxa10creERT2 mouse line. We found that the cellular dynamics of oncogenic transformation depend on the gland compartment in which the mutation is activated within the corpus epithelium. Mutations in the highly dividing isthmus compartment give rise to clones that expand rapidly within 17 days of oncogenic activation with around 35% of mutant clones expanding throughout the entire gland in 33 days. However, mutations in the slowly dividing base region of the corpus gland produce clones that do not show rapid expansion even after 33 days of tracing. These results can be beneficial in developing methods to detect the initial stages of cancer transformation.

6 ALDH1A1 identifies cancer stem cells in ovarian cancer whose signaling is through the Hedgehog pathway

Nagare RP, Sneha VS, Krishnapriya S, Sidhanth C, Murhekar K, Ramachandran B, Shirley S, Ganesan TS

Cancer Institute (WIA), India

Keywords: cancer stem cells; composite approach; Hedgehog pathway

We have taken a composite approach for identifying Cancer stem cells (CSCs) in serous adenocarcinoma of the ovary (HGSOC). We identified 3 novel markers by an in silico approach (CD9, CD24 and EPHA1) in addition to those previously known (CD133, CD117, CD44). Primary malignant cells (PMCs) in ascites from patients with HGSOC (N=20) expressed all the 6 markers (range 9-60%) by flow cytometry. PMCs (n=6) expressed ALDH1A1 (2.5±0.2 %) and a fraction of these cells surface markers (2.09 ± 0.08 %). ALDH1A1 and side population assays identified non-overlapping putative CSCs in cell lines and PMCs. Spheroid formation using cell lines (n=7) was inhibited by the Hedgehog inhibitors (GDC0449, LDE225 and GANT61). Flow cytometry analysis of PMCs (N=25) pre-treated with inhibitors showed significant reduction ($p \leq 0.05$) in ALDH1A1 (2.5±0.9 %) and CD44 (18.6±5.5%) expression. GANT61 inhibited the quiescent (Hoechst33342low/PyroninYlow) cell population ($p \leq 0.05$) in PMCs. Neutralising antibody 5E1 against Gli1 abrogated spheroid formation in cell lines ($p \leq 0.05$). Sonic hedgehog (SHh) increased ALDH1A1 expression in cell lines. RNA sequencing showed significant reduction in expression of CSC markers in GANT61 treated cells. Increased expression of CD24 correlated adversely with survival in patients who underwent primary surgery for HGSOC ($p=0.012$, N=46). The expression of CD24 was reduced at interval cytoreduction as compared to initial biopsy ($p=0.0025$, n=16). Immunohistochemistry of HGSOC showed decreased expression ($p \leq 0.05$) of Gli1 in biopsies before and after chemotherapy that correlated inversely with recurrence. Murine experiments showed that either ALDH1A1, or SP alone or ALDH1A1+/CD24+ or ALDH1A1+/EPHA1+ cells formed tumours. GANT61 prevented tumour formation when ALDH1A1+ cells were injected in nude mice ($P \leq 0.001$). Pre-treatment of cells with GANT61 also abrogated tumorigenesis ($P \leq 0.001$). ALDH1A1 marks CSCs specifically in HGSOC. Components of Hedgehog pathway may be a target for treating HGSOC.

7 A human iPSC-based vascular model to study the HDAC9 genetic risk variant associated with large-vessel stroke

Granata A, Traylor M, Markus HS

University of Cambridge, UK

Keywords: iPSC; disease modelling; stroke; HDAC

A quarter of all strokes are caused by large-vessel atherosclerosis. Together with conventional risk factors, such as hypertension and diabetes, there is a strong heritable component contributing to risk of large artery stroke. Recently, a common genetic variant (rs2107595) in the Histone Deacetylase 9 (HDAC9) gene has been identified as the strongest genetic risk selectively associated with large-vessel atherosclerotic stroke to date. This offers a potential novel pathogenic pathway for stroke and a target for therapeutic modulation. HDAC9 is a member of the HDAC class IIa family of enzymes, which have a crucial role in many physiological and pathological processes, by interacting with tissue-specific transcription factors to repress/de-repress target genes in specific cell types. HDAC9 is expressed in a variety of cell types, including vascular cells of the large artery walls and appears to play a role in atherogenesis. Moreover, sodium valproate, a pan-HDAC inhibitor, was found to be associated with lower risk of stroke in human. However, the mechanisms linking HDAC9 variant with increased stroke risk are still poorly understood. We suggest that HDAC9 genetic risk variant increases expression of HDAC9 gene, which in turn promotes atherosclerosis by affecting the viability of SMC and promoting inflammation. To test this hypothesis, we have developed a vascular model by differentiating induced pluripotent stem cells (iPSC) carrying the HDAC9 stroke risk variant into vascular smooth muscle cells (SMC) and endothelial cells. We have used this iPSC-derived model to investigate how the risk variant affects vascular cells functions in response to inflammation as well as a platform for testing existing compounds to inhibit HDAC9 function.

8 Stress affects differentiation of bone marrow stromal cells

Husak Z, Dworzak M

St. Anna Kinderkrebsforschung, Austria

Keywords: stroma; stress; autophagy

Long-term survival of bone marrow-derived mesenchymal stromal cells (MSCs) due to autophagy is one of their most important characteristics. Under some conditions, MSC may develop tumorigenic properties. However, these transformation-induced conditions remain largely unknown. Many studies investigate MSCs tumorigenesis under hypoxia and serum starvation. Recently we identified association between Hsp70, a main participant in cellular stress response and tumorigenesis, and CD99, which is a widely expressed surface molecule on many cells but not MSCs. Preliminary observations revealed up-regulation of both proteins in stressed long-term cultured MSCs. We hypothesized that CD99 is implicated in stress-induced mechanisms of cellular transformation in MSC. Hence, we investigated the effects of prolonged stress on MSCs and the role of CD99 and autophagy in their survival. We found that chronic stress factors are able to change morphology of MSCs and to inhibit spontaneous differentiation into adipocyte lineage. Furthermore, CD99 elevation and disappearance of p53 and p21 accompanied defected autophagy, which is usually associated with tumor formation. Our data show that inhibition of autophagy promoted cell detachment and modulated CD99 expression level whereas CD99 overexpression suppressed autophagy. These results provide a model for chronic stress-induced transformation of MSCs via CD99 and thus are likely of relevance for mesenchymal tumorigenesis.

9 Functional Analysis of Crosstalk between Mesenchymal and Epithelial Cells during Alveolarization of the Developing Human Lung

Lim K, Sun D, Rawlins EL

Gurdon Institute, UK

Keywords: human lung development; crosstalk; tip organoids; fibroblasts; alveolarization

Development of the human lung alveoli requires highly complicated, systematic interactions between subsets of progenitor cells of multiple lineages including epithelial and mesenchymal cells. Extensive studies have been performed to reveal epithelial-mesenchymal crosstalk during the branching stages of mouse lung development, but little is known about the extent to which the mesenchyme contributes to lung alveolar epithelial differentiation. We are using self-renewing human embryonic lung tip epithelial organoids to functionally investigate how interactions with different mesenchymal fibroblast populations can influence tip progenitor cell self-renewal and alveolar differentiation capacity. To this end, we developed a double-layered in vitro co-culture system for mimicking the in vivo situation of functional cross-talk between mesenchymal fibroblasts and the epithelium. We found that the self-renewal capacity of the tip organoids is fully supported by fibroblasts during the co-culture, without alteration in their identity as a tip epithelium. Notably, during self-renewal the fibroblasts function as a major source of Wnt ligands to facilitate the LGR4/5-mediated Wnt signaling pathway in the growing tip organoids. Furthermore, the fibroblast co-culture suppresses alveolar differentiation of SFTPC-expressing tip organoids that are derived from later lung developmental stages. These data indicate a strong requirement for the investigation of the functional interactions between the mesenchymal and epithelial cells at a single-cell level. For this reason, we are investigating the spatiotemporal distribution of fibroblasts in the developing human lung using single-cell RNA sequencing and proximity ligation in situ hybridization analyses. Moreover, we are analyzing how different fibroblast populations functionally control human lung tip epithelial cell self-renewal versus alveolar differentiation. This study will provide mechanistic insight into human alveolarization.

10 Comparison of the effect of Wharton jelly-derived mesenchymal stem cells and their conditioned media in rat model of spinal cord injury

Chudickova M, Vackova I, Urdzikova-Machova L, Kubinova S

Institute of Experimental Medicine, Czech Republic

Keywords: spinal cord injury; mesenchymal stem cells; conditioned medium

Spinal cord injury (SCI) repair is a great challenge in tissue engineering and regenerative medicine. One of the treatment approaches represents transplantation of mesenchymal stem cells (MSCs), which possess anti-inflammatory, anti-apoptotic and generally supportive impact in wounded tissues by the paracrine effect. However, survival rate of transplanted MSCs is limited. Application of conditioned media (CM) derived of MSCs is an admitted approach how to bypass these limitations. Here we compared the effect of application of MSCs and their CM in the treatment of SCI in rats. We used MSCs derived from Wharton's jelly of umbilical cord (WJ-MSCs), which display high proliferative potential and produce large amounts of neurotrophic and growth factors. 1. 5M of MSCs and their CM, pooled from three different donors, were intrathecally transplanted in three doses 1st, 2nd and 3rd week after the induction of balloon compression lesion. The behavioural tests (Beam walk, BBB-test, plantar test) showed significant improvement after all three treatments (CM, WJ-MSCs and non-conditioned concentrated medium), in comparison with non-treated controls. We conclude that we found statistically significant improvement after CM application in comparison with WJ-MSCs and non-conditioned concentrated medium in Beam walk time and score measurements and plantar test and non-significant improvement after CM application in comparison to WJ-MSCs and non-conditioned concentrated medium in BBB-test.

11 Deriving an in vitro source of canine corneal stromal cells for future studies of corneal disease and therapeutic applications: a translation from human research towards veterinary science

Kafarnik C, Daniels JT and Guest JD

Institute of Ophthalmology, University College London, UK

Keywords: corneal stromal stem cells; dog; canine

Purpose: Corneal pathology as corneal crystalline dystrophy is described in eight different dog breeds. Cholesterol and phospholipids are deposited in the stroma, similar to Schnyder's dystrophy in humans. Chronic corneal fibrosis is one of the leading causes for visual impairment in veterinary ophthalmology. The purpose of the study is to determine whether canine corneal stromal stem cells (CSSC) can be derived from fresh, adult corneas and keratocyte-like cells can be derived from canine induced pluripotent stem cells (iPSC) to use for future disease modelling and assess potential therapeutic applications. Methods: Fifteen donated, healthy corneas were used to isolate CSSC in vitro. Keratocytes derived from CSSC (KDC) were obtained by differentiation of the CSSCs in serum free medium containing ascorbic acid for 21 days. Immunohistochemistry (IHC), Immunocytochemistry (ICC) and flow cytometry were performed to characterize and quantify marker expression in cultured CSSCs, and qPCR used to quantify gene expression of CSSC and KDC. Trilineage differentiation of CSSC into osteoblast, adipocytes and chondrocytes was performed. Canine iPSC were differentiated first into neural crest-like cells (NCC) using media containing TGF-beta and GSK3 inhibitor and secondly into keratocytes using serum free and ascorbic acid containing medium for 3 weeks. The marker expression was determined using ICC. Results: IHC demonstrated a distinct population of CD90 and N-cadherin expressing cells in the anterior stroma throughout the limbal and central cornea. Stem cell markers CD90, CD73, CD105, N-cadherin and Pax6 were expressed by cultured CSSCs, whereas CD34 was negative. Quantification determined a mean of 80.06% Pax6, 88.6% CD90, 96.6% CD73 and 1.2% CD34 positive gated CSSCs. KDCs expressed Keratocan, Lumican, and ALDH1A3 proteins. KDC gene expression of Lumican, Keratocan and ALDH1A3 were upregulated, whereas Pax6 and N-cadherin were downregulated. Limbal and central CSSC differentiated into osteoblasts, chondrocytes and adipocytes confirming their multipotency. Canine iPSCs differentiated into dendritic cells expressing p75 and Pax6 after NCC differentiation and keratocyte markers (Lumican, Keratocan, ALDH1A3) were expressed after keratocyte differentiation. Conclusion: This is the first characterization of canine corneal stromal cells with mesenchymal stem cell potential similar to corneal stromal stem cells in people. IPSC derived keratocytes could serve as a source of cells for tissue engineering and studying corneal diseases in the field of veterinary ophthalmology.

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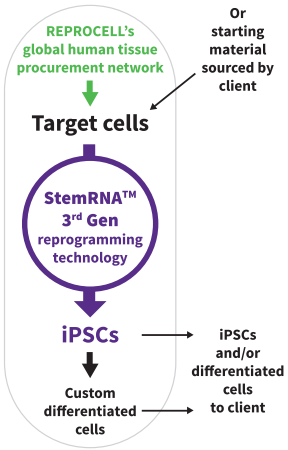

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12 Grafting CRISPR/CAS9 modified basal cells into the mouse airways

Kool HM, Rawlins EL

Gurdon Institute, UK

Keywords: airway; basal cells; regeneration

The airway epithelium has a low cell turnover rate and is in a quiescent state during homeostasis. The three main cell populations that make up the airway epithelium in the mouse are ciliated cells, secretory club cells and basal stem cells. After injury, the basal cells have the capacity to quickly respond and can either self-renew, or differentiate into one of the other two cell types. The Fibroblast growth factor (Fgf) signalling pathway plays an important role in both the process of self-renewal and the differentiation of basal cells. Moreover, our previous work shows that the conditional knockout of Fibroblast growth factor receptor 1 (Fgfr1) in basal cells results in impaired differentiation into ciliated cells while the conditional knockout of Fibroblast growth factor receptor 2 (Fgfr2) results in a self-renewal phenotype. Downstream targets of the Fgf pathway have been described in many cell types, but remain to be revealed in basal cells. Studying the cell type specific downstream targets of Fgf signaling using in vivo models has previously been challenging due to complex genetics required for the generation of lineage specific knockouts. Here, we take in vitro expanded mouse tracheal basal cells, which we can genetically-modify using Crispr/Cas9, and engraft them back into their in vivo tracheal niche to rapidly assay basal cell phenotypes. Using the Crispr/Cas9 technique we have created knockout cells for potential downstream targets of the Fgf pathway, the transcription factors Ets variant 4 (Etv4) and Ets variant 5 (Etv5). We are now able to study the role of Etv4 and Etv5 in self-renewal and differentiation processes of basal cells in vitro using organoid cultures and the well established Air-Liquid Interface (ALI) culture system. Furthermore, using the transplantation assay, we can study the role of Etv4 and Etv5 in basal cells in vivo after injury and any long term role during homeostasis. In conclusion, we have established an injury/engrafting model to study the self-renewal and differentiation of genetically-modified basal cells in vivo. The in vitro expansion of the basal cells, together with the engrafting method, will provide with us a platform for studying the activation of proteins downstream of Fgf signaling and upstream of Etv4/5 activity.

13 Reduced culture temperature attenuates oxidative stress and inflammatory response facilitating expansion and differentiation of adipose-derived stem cells

Shani N, Tirza G, Solodeev I, Sela M, Greenberg I, Pasmanik-Chor M, Gur E

Tel Aviv Sourasky Medical Center, Israel

Keywords: adipose derived stem cells (ASCs); culture temperature; reactive oxygen species (ROS)

Mesenchymal stem cells (MSCs) are multipotent and can be derived from most adult tissues. Culturing MSCs under atmospheric oxygen levels (21%) was previously shown to lead to increased reactive oxygen species (ROS) accumulation and DNA damage and to lower proliferation compared to cells cultured under physiological oxygen levels (2-8%). Despite its many advantages culture under physiological oxygen levels is costly and requires specified equipment. A simpler method to reduce ROS accumulation and improve culture conditions is desirable. Lowering culture temperature was previously shown to benefit the survival of bone marrow-derived cultures but the mechanism for this effect remains obscure. The current study was therefore aimed at determining the effect of reduced culture temperature on adipose derived stem cells (ASC) growth and phenotype. We found that culture of human ASCs isolated from routine liposuction procedures under 35OC lead to reduced ROS accumulation and apoptosis compared to ones cultured at 37OC. The finding in human ASCs was reaffirmed when we found that rat visceral ASCs (vASCs) that were cultured under 35OC, demonstrated also reduced ROS overproduction and apoptosis and enhanced expansion and adipogenic differentiation compared to that of vASCs cultured at 37°C. Comparison of gene expression profiles of 35OC versus 37OC vASCs revealed a significant reduction in the pro-inflammatory phenotype in correlation with reduced culture temperature and ROS production. The correlation between increased ROS levels and enhanced pro-inflammatory phenotype was further verified when subcutaneous human and rat ASCs were also found to display a pro-inflammatory phenotype in correlation with increased ROS production. This is first evidence for the effect of culture temperature on ASC growth and differentiation properties. We suggest that reduced temperatures may provide superior ASC cultures with enhanced expansion capacities in vitro and effectiveness in vivo.

14 TGR5 activation by taurocholic acid promotes cholangiocyte differentiation: An integral role for bile acid signaling in liver development

Ali R, Shakeri A, Rozycki M, Liu J, Parmar A, Kamath BM

The Hospital for Sick Children, University of Toronto, Canada

Keywords: TGR5; cholangiocytes; taurocholic acid

Background: Cholangiocytes are epithelial cells lining bile ducts in the liver and play a role in bile transport. Diseases of these cells (cholangiopathies) have no effective therapies and account for 70% of pediatric liver transplants. An impediment to therapeutic development is the lack of an in vitro human model of cholangiocytes. Our group developed a protocol for differentiating cholangiocyte-like cells (CLC) from human embryonic stem cells. CLC's recapitulate many functional characteristics of cholangiocytes however display mature markers (primary cilium, CFTR) sporadically. The goal of this study was to optimize the CLC differentiation protocol by incorporating bile acids (BA). BAs are signaling molecules which target receptors such as TGR5 to mediate multiple effects including cell proliferation via cAMP. Cholangiocytes are exposed to high levels of BAs in vivo and we hypothesized that the incorporation of BAs into the differentiation protocol would enhance maturation. Methods & Results: During differentiation, cholangiocyte precursors (CP) were exposed to the BAs (CA-cholic acid, CDCA-chenodeoxycholic acid, TCA-taurocholic acid). Differentiation efficiency was assessed by counting CK19+ (biliary marker) ciliated cells. TCA exposure led to a 3-fold increase in ciliated cells. The addition of a TGR5 (TCA's receptor) agonist (IN7) recapitulated the increase in ciliation seen with TCA. Both TCA and IN7 heightened cAMP levels. Relative to control, CPs exposed to either IN7 or TCA had increased nuclear accumulation of proliferation marker Ki67 and had a greater percentage of cells in the G2/M stage of the cell cycle. Finally, when CP's were inserted into Matrigel for 3D culturing, the addition of IN7 heightened cell survival and cholangiocyte cyst formation. Conclusion: TCA mediated activation of TGR5 in CLCs is a positive mitogenic and differentiation cue. This optimization of our protocol improves the system for the study of human development and disease .

15 Adult liver cholangiocytes require optimal levels of TET1 to be reprogrammed to a proliferative stem/progenitor state

Aloia L, McKie MA, Cordero-Espinoza L, Antonica F, Zernicka-Goetz M, Huch M

Gurdon Institute, UK

Keywords: epigenetics; regeneration

The adult liver is a slowly self-renewing organ. However, upon injury the liver has a remarkable regeneration potential. When the hepatocytes are severely harmed, cholangiocytes (or ductal cells) acquire proliferation and de-differentiate to bipotential progenitors, being capable to differentiate to hepatocytes and thus restore tissue architecture. Recently, organoid cultures have been established allowing long-term expansion of adult cholangiocytes in their proliferative state and retaining their physiological features. However, the molecular mechanisms behind the activation of cholangiocytes remain largely unknown. Here we describe that during the transition from a non-proliferative state to a de-differentiated proliferative state, ductal cells undergo epigenetic reprogramming, showing increased levels of the methylcytosine dioxygenase TET1 and its epigenetic mark 5-hydroxymethylcytosine (5hmC). TET1 and DNA 5hmC levels increase both in vivo upon liver injury and in vitro when cholangiocytes isolated from adult mouse liver are grown as organoids. TET1 binding and 5hmC significantly increase at stem-cell markers such as Lgr5 or Trop2 prior to their up-regulation, thus suggesting that TET1/5hmC facilitates transcription of the stem-cell signature. Following liver injury in vivo, TET1 hypomorphic mice (retaining ~35% of Tet1 expression) exhibit reduced numbers of proliferating cholangiocytes, affecting regeneration of the ductal compartment. In line with this, TET1 hypomorphic organoids are impaired in long-term expansion, while ectopic expression of catalytically active TET1 rescues this phenotype. Therefore, we demonstrate that TET1 is required for de-differentiation of adult cholangiocytes to a proliferative progenitor state, acting as a key player of liver regeneration.

16 Directed differentiation of porcine colonoids with culture medium

Barnett A, Mullaney J, Roy N, McNabb W

AgResearch/ Riddet Institute, New Zealand

Keywords: porcine colonoids

Colonoids are multicellular 3D models of the colon epithelium comprised of stem cells and terminally-differentiated cell lineages such as goblet and enteroendocrine cells, and enterocytes. Understanding how culture medium components influence the composition of colonoids is important to ensure a suitable and reproducible model of the colon epithelium. To do that, a conditioned medium (CM) containing the growth factors Wnt, noggin and R-spondin was combined with basal medium at a 50/50 ratio (50% CM). Inclusion of EGF, gastrin, nicotinamide, and inhibitors to p38 and ALK5 activity to the 50% CM was essential for maintaining the stem cell niche to ensure porcine colonoid longevity over multiple passages. This complete CM was used as the control medium (50% CCM). Exclusion of both inhibitors from the 50% CCM increased expression of the stem cell markers Sox9, Lgr5, and Olfm4. Atoh1 and Pcna expression also increased, indicating increased proliferation. Chga and Sglt1, markers for enteroendocrine cells and enterocytes respectively, also increased. When EGF and both inhibitors were excluded from the 50% CCM, expression of Sox9, Lgr5, Atoh1 and Pcna increased. Chga also increased relative to control. Reducing the concentration of CM to 5%, and excluding both inhibitors from the medium, resulted in a 10-fold increase of Pcna, no change to Sox9 and Olfm4, and decreased expression of Lgr5 relative to control. Expression of Chga and Muc2 was increased, but that of Sglt1 was decreased. The exclusion of EGF and both inhibitors from the 5% CCM increased the expression of Atoh1 and Pcna while Lgr5 expression decreased. Expression of Chga and Muc2 increased, whilst Sglt1 expression was unchanged. These results suggest the composition of porcine colonoids can be manipulated by culture medium components alone, enabling maintenance of the stem cell niche and development of differentiated epithelial cell lineages. This ensures an appropriate mix of cells to model the colon epithelium.

17 DNA repair and replication stress in Drosophila intestinal stem cells

Boumard B, Siudeja K, Bardin AJ

Institut Curie, France

Keywords: aging; replication stress; DNA repair

Genome integrity in long-lived tissue stem cells is essential to maintain tissue function and prevent cancer initiation. How stem cells cope with DNA lesions determines their mutation rate, susceptibility to cancer, and likely age-related functional decline. Here we aim to understand what are the DNA damage causing factors, and what mechanisms are acting in adult stem cells to prevent spontaneous mutation. Our previous work in Drosophila, demonstrated that in aging intestinal stem cells, frequent gene inactivation leads to neoplastic growth. Using whole-genome sequencing, we determined that the gene Notch becomes inactivated by small and large deletions. Deletions and more complex structural rearrangements reminiscent of Fork Stalling and Template Switching (FoSTeS) events are also found throughout the genome, suggesting a contribution of replication stress in stem cell genome instability. Using gene Knockdown in the Drosophila stem cells and hydroxyrurea feeding, we are currently exploring the consequences of replication stress on stem cell physiology and genome integrity. We also aim to unveil the genomic outcome of replication fork collapse in stem cells by inducing a site-specific fork stalling. Finally, we are investigating the role of DNA damage response and DNA repair pathways on genome instability and more specifically on the deletion and rearrangements we characterized.

18 Inflammatory niche during lung injury repair and regeneration

Choi J

Wellcome - MRC Cambridge Stem Cell Institute, UK

Keywords: inflammation; lung stem cells; injury repair

The lung epithelium is maintained by epithelial adult stem/progenitor populations in homeostasis and injury repair. In the alveoli, the site of gas exchange, the type II (AT2) cells can self-renew and generate type I (AT1) cells during homeostasis and after injury. However, it still remains unclear how this stem cell functions, such as how proliferation and lineage differentiation of AT2 cells are regulated. Despite the vulnerability of epithelial cells to inflammatory environment upon damage, the primary response of epithelial stem cells to an inflammation is not well understood. Here we identified alarmin IL-1β, which is secreted by interstitial macrophages, as an essential signal that enables AT2 cells to proliferate and differentiate into AT1 cells after tissue damage. We found that Il1r1, a receptor for IL-1β, is expressed in AT1 cells, specifically adjacent to AT2 cells which give rise to AT1 cells after injury. Coculture of lung interstitial macrophage as well as IL-1β treatment with AT2 cells showed the increase in size and forming efficiency of alveolar organoids with enhanced differentiation of AT1 cells. However, the absence of Il1r1 on AT1 cells dampened the effects of inflammation on AT2 cells in organoids and also in the lung tissue after injury. These findings identify acute inflammation as an alarm signal that allows stem cells to exit quiescence followed by rapid proliferation and lineage differentiation during injury repair.

19 Modelling genetic mechanisms of osteoarthritis using human induced pluripotent stem cells

Cibrian Uhalte E, Fernando R, Zeggini E, Vallier L

Wellcome Sanger Institute, UK

Keywords: hiPSC; osteoarthritis; disease modelling

Osteoarthritis is an age-related degenerative joint disease that poses a substantial health, social and economic burden. Despite significant advances in recent years, the molecular basis of its pathogenesis is still poorly understood. The overall aim of our work is to model genetic mechanisms of osteoarthritis (OA) using chondrocytes derived from human induced pluripotent stem cells (hiPSCs). Using this human in vitro model, we aim at studying how individual genetic variation contributes, at cellular and molecular level, to the susceptibility to develop OA and the role that may play in disease progression. For the generation of the chondrocytes we are using standard open access hiPSC lines derived from healthy donors and fully characterised by the HIPSCI project (www.hIPSCI.org). The differentiation protocol recapitulates developmental stages observed during chondrogenesis in vivo and relies on chemically defined culture media. We are using the data from the molecular phenotyping of primary human samples and the hiPSC-derived chondrocytes, to select OA susceptibility loci that represent functional regions (promoter/enhancers), with particular emphasis on those involved in chondrocyte development and function. To understand their contribution to the disease phenotype, we will knockout the expression of the associated candidate genes in hiPSC using the CRISPR-Cas9 system and phenotype, molecularly and functionally, the derived chondrocytes. Overall, this study will contribute to understand the architecture of transcriptional networks characterising cartilage cells and the mechanisms by which changes in the genome affect OA disease susceptibility and progression. It will also help identify potential pathways and targets for OA diagnosis or disease-modifying drug development. Finally, this work will provide a proof of principle for the use of hiPSC-derived chondrocytes as an in vitro model to study OA and as a drug discovery platform.

20 In vitro validation of human iPSC-derived atrial cardiomyocytes for cell-based assays and drug discovery

Broadbent S, Dark N, Paonessa M, Williams S, El-Haou S, Harper S, Shi Y

Axol Bioscience, UK

Keywords: atrial; cardiomyocytesiPSC-derived

The development of atrial cardiomyocytes from iPSCs offers the potential for disease models of atrial fibrillation to be established from patients which may provide information on therapeutics that modify the phenotypic markers of cardiovascular disease and atrial fibrillation. Atrial fibrillation is one of the most common arrhythmias to affect the heart, as such there is a need to develop drugs to target atrial arrhythmia. However, current mouse models fail to translate in vitro due to fundamental differences in the electrophysiology of cardiac action potentials. Here we present data on the molecular and electrophysiological characterization of Axol's Human iPSC-derived Atrial Cardiomyocytes. We determined the protein and gene expression, beat rate and action potential parameters, along with identifying the functionality of the core cardiac and atrial-specific ion channels. Molecular characterization of Axol's Human iPSC-derived Atrial Cardiomyocytes reveals the expression of cardiac and atrial-specific markers troponin T, atrial myosin light chain 2 (MLC2a) and atrial natriuretic peptide (ANP) and key ion channels, Kv1.5, and Kir3.1/3.4. Functionally, Axol's Human iPSC-derived Atrial Cardiomyocytes elicit spontaneous action potentials, express functional core cardiac ion channels, INa, ICa,L and IKr and exhibit a steady beat rate. Axol's Human iPSC-derived Atrial Cardiomyocytes are shown to be a highly validated, physiologically relevant model that offers the opportunity to study atrial-specific disorders, such as atrial fibrillation, and develop cell-based assays to identify disease-modifying treatments.

21 Modelling Monogenic Diabetes using human induced pluripotent stem cells

Cujba A-M, Watt F, Sancho R

King's College London, UK

Keywords: Monogenic Diabetes, Human Induced Pluripotent Stem Cells

Diabetes is characterised by the body's inability to regulate blood glucose. Pancreatic β-cells regulate glucose through insulin release. Monogenic Diabetes is a rare type of diabetes caused by single gene mutations that make β-cells dysfunctional. However, it is not understood how the mutations affect the development and function of β-cells, resulting in the occurrence of heterogenous diseases phenotypes among patients. In my study, I am using induced Pluripotent Stem Cells (iPSCs) derived from patients with Monogenic Diabetes banked through the Human Induced Pluripotent Stem Cells Initiative (HipSci). The effect of the mutations is investigated during pancreas development in-vitro. In this study, we show that iPSCs derived from Monogenic Diabetes patients have impaired capacity to reach progenitor and maturation stages compared to the iPSCs derived from heathy donors in 2D and 3D culture conditions. Future experiments will focus on delineating the mechanism of action of mutated genes associated with Monogenic Diabetes.

22 Human long-term deregulated circadian rhythm alters regenerative properties of skin and hair precursor cells

Deshayes N, Genty G, Berthelot F, Paris M

L'Oréal Research & Innovation, France

Keywords: circadian rhythm; clock pathway; epidermal precursors; dermal papilla; regenerative properties

Background: In mammals, desynchronized circadian rhythm leads to various biological symptoms. In skin and hair, human epidermal stem cell function in vitro is regulated by circadian oscillations, and thus contributes to tissue aging when deregulated. In mice, circadian arrhythmia of hair follicle stem cells contributes to age-related hair follicle cycling defects. Despite the well-described impact of circadian oscillations through a feedback loop involving the clock pathway on hair and skin stem cell function in vitro, little is known about the change in characteristics or regenerative properties of hHF (human hair follicle keratinocytes), hEpi (human interfollicular epidermal keratinocytes), and hHFDP (hair follicle dermal papilla stem cells) after long-term alteration of circadian rhythm in vivo. Objectives: The present study was designed to asses hHF, hEpi, and hHFDP precursors and stem cell properties in response to clock pathway alteration due to long-term deregulated circadian rhythm in vivo. A clinical study protocol involving two groups of women was designed: diurnal workers (control) and shift workers (deregulated). After informed consent, two 3-mm fresh punch biopsies were taken from the occipital region of each donor (10 donors/group). Cell culture characterization, measurement of colony area, culture medium analysis, and RT-qPCR analysis were carried out. Results: Long-term circadian rhythm deregulation affected clock pathway protein expression and correlated with alterations in hHF, hEpi, and hHFDP properties. Conclusion: This study provides, for the first time in humans, evidence that in vivo deregulation of the clock pathway affects regenerative properties of human skin and hair precursor cells.

23 The role of polycomb-group complex PRC2 in human pancreatic cell differentiation

Fabian C, Soares F, Jhaveri K, Rugg-Gunn R

Babraham Institute, UK

Keywords: epigenetics development

The ability to direct cell differentiation through epigenetic manipulation is an underexplored area in stem cell and developmental biology, with a shortage of mechanistic studies of epigenetic pathways in human tissue formation. The Polycomb Repressive Complex, PRC2, which catalyses the repressive H3K27me3 mark, is required for numerous cell fate decisions and is an attractive target for modulation. During pancreatic differentiation, the distribution of H3K27me3 changes and PRC2 inhibition modifies the pancreatic cell populations formed. In this project, we will use ChIP-Seq to monitor H3K27me3 as a readout of PRC2 activity, during human pluripotent stem cell to pancreatic beta-cell differentiation. We will investigate PRC2 loss of function using chemical inhibitors and CRISPR knockouts. We will also attempt to produce an auxin-inducible degron system for PRC2, which would allow precise temporal control of PRC2. These experiments will allow us to investigate the functional role of epigenetic mechanisms during long-term, human tissue development.

24 Investigating SOX2 and SOX9 Function in Human Fetal Lung Progenitor Cells

Sun D, Rawlins EL

Gurdon Institute, UK

Keywords: Human lung development, organoids, gene manipulation, SOX2, SOX9

The human lung is a complex organ that primarily carries out a gas exchange function. This is fulfilled by tree-like epithelium terminating at numerous of gas exchange units, known as alveoli. Current understanding of human lung development comes from recent intensive mouse research. Human fetal lung tip progenitors, an analogue to mouse tip progenitors, self-renew and differentiate into all lineages of pulmonary epithelial cells. However, recent evidence that SRY-related HMG-box (SOX) family proteins-SOX2 and SOX9 have a distinct expression pattern in developing human lung epithelium compared with mouse, suggests that the mouse model may not fully recapitulate human lung developmental processes. Here, we use a newly-developed human fetal lung tip organoid culture system to understand SOX2 and SOX9 functions in multipotent epithelial progenitor self-renewal and differentiation. We have developed suitable methods for genetic manipulation using the organoid platform. The SOX2 endogenous locus was correctly targeted with the auxin inducible degron (AID) system to down-regulate SOX2 protein. Inducible SOX2 and SOX9 overexpression were also achieved through use of the PiggyBac transposon system. Preliminary results suggest that SOX2 overexpression leads to self-renewal breakdown, whereas SOX9 overexpression doesn't appear to interfere with progenitor self-renewal.

25 Single-cell RNA Sequencing of Primary Human Liver for Analysis of Cellular Diversity and Improved hiPSC-Derived Hepatocyte Differentiation

Wesley B, Miau C, Tomaz R, Teichmann S, Vallier L

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: Liver, Differentiation, Single-Cell RNA Sequencing

The liver is an organ with a diversity of essential functions including xenobiotic removal, bile acid production, storage of iron and vitamins, and metabolism of glucose and fatty acids. Hepatocytes, which represent the most abundant cell type in the liver, fulfill the majority of these functions. As a result, disorders affecting these cells are life threatening, and end-stage treatment relies upon a limited supply of liver donations for transplant. Thus, the production of hepatocyte-like cells (HLCs) from human pluripotent stem cells (hPSCs) for clinical applications including cell-based therapies and toxicology screens has become a strong research focus. However, hPSC-derived hepatocytes lack the functional repertoire of their in vivo counterpart and are currently unable to satisfy this clinical need. Here, we propose to address this limitation by using single-cell RNA sequencing (scSeq) to compare HLCs with fetal and adult primary hepatocytes isolated from patient tissue. ScSeq offers the opportunity to explore cellular diversity by providing precise transcriptomic information at the single cell level and subsequent insight into molecular signatures that are lacking in current differentiation systems. Accordingly, we have developed a protocol to dissociate primary adult human liver tissue into single-cell suspensions. We applied this method to isolate fresh primary hepatocytes and non-parenchymal cells for scSeq using SmartSeq technology, with the aim to uncover cell-to-cell interactions and diversity within cell populations. ScSeq data of hPSC-derived hepatocytes at sequential differentiation stages was compared to fetal and adult liver scSeq profiles to uncover mechanisms of cellular diversity which may be important in understanding the immature nature of HLCs. We have identified putative hepatocyte subpopulations, including a progenitor population known to reside in adult tissue, with unique proliferation and zonation properties. Overall, our novel approach will identify important regulators involved in late-stage maturation of hPSC-derived hepatocytes and allow us to generate hepatocyte subpopulations in vitro for disease modelling and translational applications.

26 Regional differences in human biliary tissues and corresponding in vitro derived organoids

Rimland C, Tilson S, Morell C, Tomaz R, Lu WY, Adams S, Georgakopoulos N, Otaizo-Carrasquero F, Myers T, Sun HW, Gieseck III RL, Sampaziotis F, Tysoe O, Wesley B, Oniscu GC, Hannan NRF, Forbes S, Saeb-Parsy K, Wynn TA, Vallier L

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: Biliary Tree; Extrahepatic Bile Duct; Organoids

The biliary tree is a series of ductular tissues responsible for the drainage of bile produced by the liver and pancreatic juices from the pancreas. The biliary tree is affected by a diversity of life-threatening diseases collectively called cholangiopathies. Cholangiopathies show regionalization, with some diseases such as biliary atresia predominantly targeting extrahepatic bile ducts (EHBDs) outside of the liver. Despite this, little is known on whether anatomical location within the biliary tree contributes to differences in functionality of biliary epithelium, especially in the EHBD compartment. Additionally, reports have demonstrated the possibility for in vitro culture of bile duct stem/progenitor cell organoids from both intrahepatic (IHBD) and EHBD sources. The relation of these organoid systems to each other, and to their tissue of origin, is largely unknown. Here we address these major questions by combining transcriptional analyses and in vitro culture of human bile duct organoids derived from primary IHBD and EHBD epithelium. Primary tissue from human gallbladder, common bile duct, pancreatic duct, and liver were used to generate organoid cultures and obtain primary tissue RNA. Characterization of IHBD and EHBD organoids demonstrated expression of the stem cell markers LGR5 and PROM1 and ductal markers KRT19/7. IHBD organoids appeared distinct from EHBD organoids and required different conditions for sustained growth. Our results also suggest IHBD, but not EHBD organoids, are capable of low-level expression of hepatocyte markers when subjected to a previously published differentiation protocol. RNA-Seq analyses revealed that primary tissues from different regions of the biliary tree display unique expression profiles. Further, a limited number of these differences are maintained in the in vitro organoids. EHBD organoids appear transcriptionally similar to each other regardless of their tissue source, while IHBD organoids are distinct. Taken together, our results uncover regional specific markers for different anatomical regions of the biliary tree. Further, we demonstrate that major differences exist between IHBD organoids and EHBD organoids in vitro. Ultimately, these results may help to identify new targets for therapeutic development for cholangiopathies.

27 Epigenetics of adipogenic and osteogenic differentiation of MSCs isolated from amniotic fluid of healthy and fetus-affected pregnancies

Zentelytė A, Treigytė G, Baronaitė S, Krasovskaja N, Savickienė J, Borutinskaitė V, Navakauskienė R

Vilnius University Life Sciences Center, Lithuania

Keywords: amniotic fluid stem cells; differentiation; epigenetics

Human amniotic fluid derived mesenchymal stem cells (AF-MSCs) is a relatively new and very attractive alternative source of MSCs which have the potential to be used for therapeutic purposes because of their self-renewal and multi-differentiation capabilities. The goal of our study was to evaluate the efficiency of MSCs from AF of normal and fetus-affected gestations to differentiate towards adipogenic and osteogenic lineages and to elucidate epigenetic states responsible for these differentiations. AF-MSCs were obtained using a two-stage isolation protocol [3], expanded in a monolayer culture where the typical spindle shaped morphology was observed. AF-MSCs from two cell sources demonstrated quite similar expression of specific cell surface (CD44, CD90, CD105) and pluripotency (Oct4, Nanog, Sox2, Rex1) markers and under appropriate conditions underwent mesodermal lineage differentiation as determined by morphological changes and the expression of adipocyte-specific (PPAR-γ, adiponectin) and osteoblast-specific (ALP, osteopontin, osteocalcin) genes. Differentiation processes were linked with differential expression of miR-17, miR-21,miR34a and mirR-148a/b, decreased levels of acetylated H4 and H3K9, tri-methylated H3K4 and H3K9 and the retention of H3K27me3. The levels of HDAC1, DNMT1 and PRC1/2 proteins (BMI1/SUZ12) were reduced as well. There were no significant differences in the expression levels of all epigenetic marks between undifferentiated MSCs derived from AF of normal and fetus-affected gestations and between those differentiated toward adipocytes or osteoblasts. The expressional changes of histone marks and miRNAs that occurred during differentiation to mesodermal tissues indicate subtle epigenetic regulation, yet more detailed studies in epigenetic mechanisms are required for a better understanding of AF-MSCs differentiation in fetus-diseased conditions and their usage in autologous therapeutic application and prenatal disease research.

28 Nanoscale architecture of the unique focal adhesions and actin cytoskeleton of human pluripotent stem cells

Stubb A, Guzmán C, **Närvä E**, Aaron J, Miihkinen M, Ivaska J

Turku University, Finland

Keywords: hPSCs; adhesion; iPALM

Human pluripotent stem cells (hPSCs) require a supporting layer of extracellular matrix (ECM) which highlights the role of adhesion, high cytoskeletal contractility and integrin signalling in the maintenance and establishment of pluripotency (Närvä et al. 2017). To study the adhesion properties of hPSCs in detail, we performed super-resolution experiments using interferometric photoactivation and localization microscopy (iPALM), on hPSCs, to unveil the nanoscale architecture of the focal adhesions (FAs). iPALM experiments show three main structural differences in pluripotent cells: 1) Lateral organization of talin revealed lower densities of molecules in the centre of the FA, suggesting that the structure at the core of the individual FAs is different to their periphery; 2) Vinculin appears to be significantly higher with respect to the cell membrane, suggesting that accelerated FA maturation could be a feature of hiPSC; and 3) Actin nanoscale localization in FAs reveals two previously undescribed discrete layers. Our study also reveals the nanoscale localization of novel FA scaffold components Kank1 and Kank2. This study provides a detailed description of unique cytoskeletal organization and FA patterning as a part in regulating hPSC pluripotency and mechanotransduction. Our future interests lie in finding out how the FA signalling and mechanical forces interplay with pluripotency related transcriptional program.

29 From hiPSCs to cardiomyocytes: Mimicking cardiogenesis and unravelling the role of let-7 family of microRNAs

Paiva S, Kathirvelu P, Joanne P, Agbulut O

Institut de Biologie Paris-Seine, France

Keywords: let-7; cardiogenesis; microRNAs

Epigenetically in terms of human heart development, the exact role of one of the first discovered microRNAs, the let-7 family has not yet been fully elucidated. Its understanding however would have important therapeutic repercussions in the discovery of new pathophysiological mechanisms but also significant applications in the in vitro field of human cardiac tissue engineering. Appropriately, protocols to differentiate hiPSCs into cardiomyocytes (CMs) provide a good model to study let-7 family implications. A complete screening of all let-7 family members was conducted by qRT-PCR for 35 days. Results showed an early transient peak at the time of mesoderm formation, after which their expression extinguish to only gradually re-increase later in the course of CMs maturation. These profiles in accordance with literature data however do not explain the role played by let-7 family in each different cellular contexts. From there, the project involved modulations via transfections of let-7 mimics and/or family inhibitors at different stages. In the context of early cardiac progenitors (Pgs), let-7g mimic downregulated at the mRNA level a number of Pgs markers (Isl1, Mef2c, Tbx5) as well as contractile proteins (cTnT, Myh6, Myh7). In silico analysis came to reinforce some suggested direct targets. Oppositely to let-7, the cardiac specific microRNA miR-1 showed a propensity to induce those markers when transfected. On the context of late Pgs, on the other hand, transfection of let-7g mimic had no longer any effects whereas miR-1 continued to promote upregulation of CMs markers (Myh6, Myh7, PLN) and precociously stimulated the expression of some let-7s. All together results pointed out that let-7 family has to be repressed upon mesodermal induction in order to give rise to cardiac Pgs. Antagonistically, overexpression of miR-1 contributed to their progression into CMs and fast-tracked their maturation status mirrored by the accompanied let-7 family upregulation.

30 Identifying Gene Regulatory Networks during Neuroectodermal induction of human iPSC

Dutan Polit L, Perfect L, Havlicek S, Subramanian S, Srivastava D, Price J, Buckley N

King's College London, UK

Keywords: neuroectoderm; transcriptome; gene regulatory networks

Neural induction is the earliest step in the formation of the human nervous system. However, the regulatory signals underlying neural fate acquisition in humans are largely unknown due to limit access to live cells during this stage. Dual SMAD inhibition induces ESC/iPSCs cells to acquire neural fate, providing an exceptional in vitro system to identify of genes and pathways underlying iPSC transition from pluripotency to neural competence. Experimental approaches enabled us to capture the complete transcriptome data at different time points and unwrap the global expression patterns. Accordingly, it appears that inhibition of the pluripotency program occurs during the initial 2 days of 2i neural induction followed by activation of thousands of genes that orchestrate neural fate acquisition. Among these genes several neural precursors and forebrain markers such us PAX6, ZEB2 and SOX11 were identified, validating previous findings from animal and ESC studies. Additionally, we report activation of novel genes that have not been previously related with CNS development. The potential regulatory interactions between genes activated during neural induction were captured in a gene regulatory network, which enables clear visualization of the causal relations between genes at different stages of induction. We identified GRN modules and with similar expression profiles, which might regulate specific the biological processes required neural induction. This study provides an insight into mechanisms that may orchestrate fate specification of iPSC derived neuroectodermal stem cells and also cast light on the biological processes that might underwrite the initial stages of human neural development in vivo.

31 Using 3D single-molecule imaging to dissect the assembly and function of the NuRD complex on chromatin in mouse embryonic stem cells

Basu S, Shukron O, Parruto P, Carr A, Sober L, Magre A, Lando D, Cramard J, Floyd R, Garbi S, Boucher W, Zhang W, Balmer J, Brown GR, Aubert A, Communie G, Murison K, Leitner A, Abersold R, Berger I, Berger-Schaffitzel C, Lee SF, Klenerman D, Holcman D, Hendrich B, Laue ED

University of Cambridge, UK

Keywords: single-molecule, NuRD, enhancer

The multi-protein nucleosome remodelling and deacetylase complex (NuRD) is required during stem cell fate transitions and induced pluripotent stem cell formation (1,2). NuRD binds at enhancer/promoter regions to act as a 'fine-tuning' agent (3,4), but how it functions remains poorly understood. Here, we combine in vitro reconstitution of NuRD, live-cell 3D single-molecule imaging in mouse embryonic stem cells and stochastic analysis of a large ensemble of single trajectories to study how different NuRD sub-complexes assemble and function. We show that NuRD is pre-assembled before it binds to chromatin and that it can bind for long periods to chromatin. During these interactions, it combines nucleosome remodelling and histone deacetylation to modulate transcription factor binding and the movement of enhancers.

32 Chemical reprogramming of mouse embryonic and adult fibroblast into endoderm lineage

Cao S, Yu S, Chen J, Liu J, Pei D

Guangzhou Institute of Biomedicine and Health, China

Keywords: ciMET; ciEPCs; Sox17

Here we report an approach to redirect somatic cell fate under chemical defined conditions without transcription factors. Through high output chemical screening, we successfully convert mouse fibroblasts into endoderm progenitor cells (ciEPCs) at a frequency up to 1% using a combination of six small-molecules. Interestingly, we find that ciEPCs can self-renew in vitro and differentiate into functional hepatocytes and pancreatic progenitor cells in vitro. Afterwards, we demonstrated these ciEPCs derived hepatocytes can rescue acute liver failure mice in vivo. Our results may provide a alternative chemical approach to generate other functional cells which may be applied for disease therapy in the future.

33 Capturing formative pluripotency

Kinoshita M, Barber MA, Dietmann S, Smith A

Wellcome - MRC Cambridge Stem Cell Institute, UK

Keywords: cell state transition, embryonic stem cells, epiblast, pluripotency

ES cells and EpiSCs are pluripotent stem cell in culture and their characteristics are distinguished by two terms, naïve and primed. Pluripotency in mouse embryo is a continuum from pre-implantation until the end of gastrulation, however, naïve cells corresponds to pre-implantation epiblast (E3.4-4.5) and that of primed cells are gastrulating cells (around E7.0). There is an uncharacterised phase between E5.0 and E6.5. After implantation, naïve epiblast cells epithelialise and expand without expression of lineage makers until gastrulating primed stage. Epiblast at this stage are able to respond to germ cell induction signals, which is neither naïve or primed epiblast/stem cells not. We deconstruct the pluripotency phases and coin the term "Formative" to this state. Here we discuss features of formative pluripotency and how to capture formative pluripotency as a novel stem cell in culture.

34 Identification of a novel obstacle towards somatic reprogramming in malignant transformation and iPS generation

Furlan G, Huyghe A, Wajda P, Gasa R, Lavial F

Cancer Research Centre of Lyon, France

Keywords: Cellular identity, Reprogramming, Atoh8

Cellular identity and plasticity are key features of development and cancer. Differentiated cells can be reprogrammed into induced pluripotent stem cells (iPSCs) through pluripotent reprogramming (PR) (1), characterized by loss of somatic identity and reacquisition of cellular plasticity. Despite the major potential of these cells for regenerative medicine, their generation is still inefficient. Deciphering mechanisms underlying PR may lead to a fundamental improve for their derivation. In the first steps of tumorigenesis cells need to acquire new plasticity and epigenetic remodelling, in a process known as oncogenic reprogramming (OR) (2). This process emerged as a fundamental step in cancer initiation but molecular mechanisms triggering it remain unclear (3). Even if PR and OR drive the emergence of different cell types (iPSCs and cancer cells), the initial steps of both processes render somatic cells tolerant to profound changes. We propose to consider the early stages of OR and PR as novel models to identify factors safeguarding cellular identity and constraining PR and OR. We focused on the function of the basic Helix-Loop-Helix transcription factor (bHLH TF) family during PR and OR (4). We investigated the role of two bHLH TFs: c-Myc, a well-known facilitator of PR and OR, and Atoh8, a new reprogramming obstacle identified in the first part of my PhD. We showed the fundamental role of endogenous c-Myc in pluripotent and oncogenic reprogramming: its depletion abrogate drastically both PR and OR. We next wondered if there could exist one or more bHLH TFs competing with c-Myc to safeguard cellular identity. A systematic screening of BHLH TFs led to identify Atoh8 as a novel obstacle towards PR and OR. We reported that Atoh8 is rapidly downregulated during both processes and that c-Myc is responsible for Atoh8 decrease of expression. Altogether, my project will give new insights on the mechanisms triggering PR and OR, showing the role of a new obstacle towards iPS and cancer generation.

35 CRISPR/Cas9-mediated genome-wide knockout screening identifies novel regulators of reprogramming to pluripotency

Beniazza M, Kaemena D, Yusa K, Kaji K

University of Edinburgh, UK

Keywords: iPSC; CRISPR-Cas9; screen

Induced Pluripotent Stem Cell (iPSC) technology holds huge potential in the field of regenerative medicine, but iPSC reprogramming also offers a model to study the common regulators of all forced cell identity changes. Despite this, the mechanism of reprogramming remains poorly understood and the efficiency of induced pluripotent stem cell (iPSC) generation, inefficient. To identify genes detrimental or essential to successful iPSC reprogramming, we have performed CRISPR-Cas9 genome-wide knockout (KO) screening in the context of reprogramming from mouse embryonic fibroblasts (MEF). After transduction of a pooled gRNA library, MEF were reprogrammed and gRNAs enriched or depleted in iPSCs were identified by Illumina sequencing. A statistical analysis identified 23 genes acting as roadblocks against successful reprogramming, including p53, p21, Dotl1, c-Jun, Men1, Gtf2i as well as 18 novel candidate roadblock genes. In addition, we have identified candidate genes essential for reprogramming, but not for MEF proliferation or embryonic stem cell self-renewal. Overexpression of several of these essential genes alongside the Yamanaka factors leads to a marked enhancement in reprogramming efficiency. Investigation into how these candidates regulate the transition to pluripotency can lead to improved iPS technologies and a better understanding of forced cell identity conversions.

36 A phosphorylation-ubiquitylation module for the control of X-chromosome inactivation

Bustos F, Segarra-Fas A, Williams CAC, Cassidy A, Gourlay R, Vargesse J, Toth R, Macartney T, Zhang T, Brandenburg L, Nardocci G, Montecino M, Gray N, Shultz E, Findlay GM

University of Dundee, UK

Keywords: RNF12; embryonic stem cells; phosphorylation; ubiquitin; kinase

Embryonic Stem Cells (ESCs) are pluripotent, meaning they can give rise to all cell types in the adult organism. Fully exploiting the developmental capacity of ESCs requires complete knowledge of their biological regulatory systems. The E3 ubiquitin ligase RNF12/RLIM is a critical regulator of ESC differentiation and imprinted X-chromosome inactivation during embryogenesis. Furthermore, mutations in RNF12/RLIM cause the developmental syndrome X-linked intellectual disability. However, the mechanisms by which RNF12 is regulated remain poorly understood. Via a combination of proteomics, inhibitor screening, genome editing, transcriptomics and cell biology approaches we have studied RNF12 regulation by phosphorylation in ESCs. Here, we unravel a kinase signaling pathway that serves as a key regulator of RNF12 activity and developmental functions. Phosphorylation of a nuclear localisation signal (NLS) is required for RNF12 to co-localise and degrade its key substrate, the transcription factor REX1. Disruption of nuclear localisation impairs RNF12 dependent posttranslational and transcriptional responses, including regulation genes involved in X-chromosome inactivation and ESC lineage specification. Thus, this research sheds light on a new signaling pathway involving phosphorylation and ubiquitylation networks to control cell identity and X-chromosome inactivation, which we propose may be disrupted in patients with intellectual disability.

37 Stem cell size heterogeneities and cell fate

Chaigne A, Smith MB, Labouesse C, Chalut KJ, Paluch EW

University College London, UK

Keywords: division; cell size; cell fate

Embryonic stem cells are pluripotent cells that can give rise to the three germ layers and the germline. However, stem cell populations exhibit heterogeneities. For example, capacity for differentiation in pluripotent cells is heterogeneous in culture, and exit from pluripotency asynchronous. In particular, mouse embryonic stem cells display a vast range of sizes when they grow in 3D colonies, which could potentially influence how they exit pluripotency; indeed, some stem cells such as C.elegans neuroblasts produce daughter cells of unequal size that will in turn have different fate. However, how these size heterogeneities arise and the consequences of cell size in exit from naïve pluripotency is poorly understood. To investigate this question, we developed tools to study cell size in 3D colonies. Using 3D segmentation, we first show that cell size is highly heterogeneous in a mouse embryonic stem cell (mESC) colony. This does not depend on the size of the colony, and cell position does not influence cell size. Furthermore, we show that in contrast to isolated mESC, cells at the periphery of a multicellular mESC colony often divide asymmetrically in size. The extent of asymmetry depends on the orientation of the spindle with respect to the colony. Interestingly, division asymmetry is not controlled by the asymmetric localization of Myosin-II but is influenced by the asymmetric distribution of the adhesion protein E-Cadherin. We show that size heterogeneities arise from a lack of clear regulation of spindle positioning and a loose control of cell growth during the cell cycle. Finally, by sorting the cells by size or by cell cycle, we show that cell size and cell cycle seem to influence the dynamics of exit from pluripotency. We are currently exploring the relative contribution of cell size and cell cycle in the dynamics of exit from pluripotency, and the role of size heterogeneities in the first events of pluripotency exit and lineage specification.

38 NaïveCult™: A Defined Media System for Transgene-Free Induction and Expansion of Reset Naïve Human ES and iPS Cells

Chen Y, Hunter AL, Stacey AR, Guo G, Brys KM, Chang Y, Chen A, Merkulova Y, Chang WY, Smith AG, Thomas TE, Eaves AC, Louis SA

STEMCELL Technologies, UK

Keywords: naïve pluripotency

Human pluripotent stem cells (hPSCs) are traditionally captured in a primed pluripotent state. Recently, the laboratory of Austin Smith, University of Cambridge, developed a transgene-free method to chemically-induce primed hPSCs to a reset naïve hPSC state1. NaïveCult™ is a defined media system developed to support chemical-induction and robust expansion of reset naïve hPSCs. Chemical generation of reset naïve hPSCs using NaïveCult™ Induction Kit involves sequential steps in which hPSCs, maintained in mTeSR™1 or TeSR™-E8™, are first exposed to a histone deacetylase inhibitor and then transitioned stepwise to reach a naïve hPSC state. This process is carried out on inactivated murine fibroblasts and under 5% oxygen conditions. During the transition to reset naïve hPSCs, early passage colonies undergo robust morphological changes characterized by the acquisition of a domed phase-bright morphology. Using our optimized protocol and defined media, we generated multiple reset naïve hPSC lines (n=5) from primed human embryonic stem cell lines Shef6, H1 and H9 and human induced pluripotent stem cells WLC-1C and STiPS-F016. These reset naïve hPSC lines, which can be maintained and continuously expanded in NaïveCult™ Expansion Medium, exhibit domed colony morphology and demonstrate the expected signature gene expression profiles associated with naive pluripotency1-3. We also tested the ability of reset naïve hPSCs induced and maintained in NaïveCult™ to differentiate into endoderm, mesoderm and ectoderm by using the STEMdiff™ Definitive Endoderm Kit, STEMdiff™ Mesodermal Induction Medium and STEMdiff™ Neural Induction Medium kits, respectively. Our results demonstrate that these cells are capable of differentiation to all germ layer lineages with optimal results obtained following re-priming in TeSR™-E8™ or mTeSR™1. In summary, we have demonstrated robust establishment and expansion of chemically reset naive hPSCs using NaïveCult™ Induction Kit and Expansion Medium.

39 Identification of the Germ Stem Cells (GSC) in the Pacific oyster using a histological quantitative approach combining morphological criteria and molecular markers

Cherif--Feildel M, Kellner K, Goux D, Elie N, Adeline B, Lelong C, Heude Berthelin C

University of Caen, France

Keywords: crassostrea gigas, germ stem cells, germinal niche, quantitative histology, vasa

Crassostrea gigas (e.g. Magallana gigas) is an alternative hermaphrodite lophotrochozoan presenting an annual reproductive cycle. Each year, the Germ Stem Cells (GSC) in their microenvironment give rise to a new male or female germ line. Functioning of GSC in their germinal niche is poorly documented in lophotrochozoa and especially in molluscs. In order to identify the GSC and understand their functioning in the Pacific oyster C. gigas, we used an original approach combining morphological criteria and expression of the Vasa marker. Our strategy is based on a two step quantitative histology approach (Gunderson and Jensen, 1985). We first consider the aspect of chromatin in the nuclei of germinal cells to localise putative GSC in the gonad tubules. We then observe the Oyvlg (Oyster vasa-like gene) immunolabelling throughout the gametogenetic cycle in the gonad. The stereological approach allowed us to identify two types of early germ cells (early GC) in the germinal epithelium, with round or irregular nuclei and vasa-positive. The microenvironment of early GC in oyster involves a myoid cell and an associated somatic cell presenting an immunolabelling for BMP2/4. On the basis of bibliographical data (Sato et al., 2006; Schulz et al., 2010) and frequencies of both cell types, we propose that putative germ stem cells in C. gigas correspond to the early GC with irregular nucleus shape. Those with a round nucleus may consist in progenitors. In addition, our study assessed that in adult oyster, germinal niches are permanent and uniformly spread in the gonad without any specific location.

40 Characterising the reprogramming dynamics between human pluripotent states

Collier AJ, Chovanec P, von Meyenn F, Corcoran AE, Reik W, Rugg-Gunn PJ

Babraham Institute, UK

Keywords: human naive pluripotency; reprogramming dynamics

Human pluripotent stem cells (hPSCs) exist in multiple states of pluripotency, broadly categorised as naïve and primed states. Naïve cells can be obtained through primed-to-naïve reprogramming; however, current isolation strategies are incompatible for the enrichment of naïve hPSCs early during reprogramming. Consequently, we know very little about the temporal dynamics of transcriptional changes and remodelling of the epigenetic landscape that occurs during the reprogramming process. To address this knowledge gap, we sought to develop an isolation strategy capable of identifying nascent naïve hPSCs early during reprogramming. Comprehensive profiling of >400 cell-surface markers by flow cytometry in naïve and primed hPSCs identified pluripotent state-specific antibodies. We compiled state-specific markers into a multiplexed antibody panel that can track the dynamics of primed-to-naïve reprogramming, as the state-specific surface markers collectively reflect the change in pluripotent states. Using the newly identified surface markers, we can isolate nascent naïve hPSCs from a heterogeneous cell population early during reprogramming. Additionally, we can track naïve hPSC formation at frequent time points throughout reprogramming which provides an unprecedented insight into the early molecular events leading to naïve cell formation, and permits the direct comparison between different naïve reprogramming methods. Molecular characterisation of early naïve hPSCs revealed distinct transcriptional changes associated with early and late stage naïve cell formation, and that divergent routes are taken from primed to naïve pluripotency using different reprogramming methods. Analysis of the DNA methylation landscape showed that nascent naïve cells are globally hypomethylated, whilst imprint methylation is largely preserved. Moreover, the loss of DNA methylation precedes X-chromosome reactivation, which occurs primarily during the late-stage of primed-to-naïve reprogramming, and is therefore a hallmark of mature naïve hPSCs. Taken together, the identified state-specific surface markers provide a robust and straightforward method to isolate distinct human PSC types, which has revealed the order of transcriptional and epigenetic changes associated with primed to naïve reprogramming.

41 RNA methylation and pausing of mESCs

Collignon E, Bulut-Karslioglu A, Ross R, Ramalho-Santos M

Lunenfeld-Tanenbaum Research Institute, Canada

Keywords: embryonic stem cells; RNA methylation; pausing

Methylation of adenine (m6A), the most abundant mRNA modification, and its machinery have recently been highlighted as key regulators of development, controlling stem cell renewal, proliferation and differentiation1-3. Here, we explore the role of m6A in the context of developmental pausing. In a previous published work, our laboratory established that mTOR inhibition of mouse blastocysts induces reversible developmental pausing, suppressing cellular functions in a manner similar to hormonal diapause4. This phenomenon can be recapitulated in mouse embryonic stem cells (mESCs), which notably display low proliferation and reduced RNA output per cell. Mass spectrometry revealed increased global m6A levels in poly(A)+ RNA in paused mESCs. To investigate local m6A changes, we mapped the transcriptome-wide distribution of m6A by methylated RNA immunoprecipitation followed by sequencing (MeRIP-seq). Taking into consideration the global transcriptional reduction in paused mESCs, we developed a method for cell number normalization (CNN) in MeRIP-seq using spike-ins of human cells. The m6A signal is enriched around the stop codon and 3'UTR. We identified 867 and 771 differentially methylated regions (dmRs) between paused and control cell transcriptomes by traditional and CNN approaches, respectively. The two analyses largely overlap, with approximately 2/3 of target genes identified in common. However, specific targets highlight the interest of considering m6A relative to both RNA amount and cell number. Overall, changes in m6A are anticorrelated with changes in gene expression, and targets were associated with regulation of transcription and cell cycle, pathways that are differentially expressed in paused ESCs. Future work will explore the potential role of m6A in the establishment of a paused state.

42 Crosstalk between cell surface mechanics and fate decisions in embryonic stem cells

de Belly H, Chalut K, Paluch E

University College London, UK

Keywords: cytoskeleton; naive pluripotency; cell shape

Embryonic Stem Cells (ESCs) are able to generate any tissue in a given organism; this ability is called naïve pluripotency. When mouse ESCs differentiate and exit naïve pluripotency they undergo a striking shape change, going from a rounded to a spread morphology. Membrane mechanical properties, such as membrane tension, have been shown to influence cell shape and differentiation in many systems. However, very little is known about plasma membrane dynamics and mechanics in ESCs. Here, we investigate the link between the mechanical properties of the plasma membrane and the molecular factors that lead to exit from pluripotency. Using optical tweezers, we show that naïve mouse ESCs have a higher effective membrane tension compared to cells exiting pluripotency. This higher tension appears to be due to a higher expression and activity of proteins regulating membrane-to-cortex attachment, such as Ezrin-Radixin-Moesin in naïve ESCs compared to exiting cells. We show that preventing the membrane tension change prevents shape change and interferes with exit from naïve pluripotency. We also observe that physically preventing spreading using micropatterns also results in defects in exiting pluripotency. Together, our data uncover a cross-talk between membrane mechanics and cell shape, and exit from naïve pluripotency in mouse ESCs. We are currently investigating how cell mechanics and shape regulate the pluripotency signalling network.

43 Dppa2 and Dppa4 are master positive regulators of the zygotic transcriptional network

Eckersley-Maslin MA, Blotenburg M, Alda-Catalinas C, Kreibich E, Krueger C, Reik W

Babraham Institute, UK

Keywords: epigenetics; transcription; genome activatione

Little is known about the transcription factors and chromatin regulators of zygotic genome activation (ZGA) in mammals. Embryonic stem cells (ESCs) contain a rare "2C-like" cells that transiently express transcripts and epigenetic features of the 2-cell embryo, thus represent a useful in vitro model for ZGA. Through a candidate-based overexpression screen in 2C-like cells, we identified Developmental Pluripotency Associated 2 (Dppa2) and 4 (Dppa4) as positive regulators. These genes are expressed at the time of zygotic genome activation and transcriptional analyses confirmed a dramatic upregulation of early embryonic transcripts following their overexpression in ESCs. Furthermore, both knockdown and knockout experiments led to depletion or abolishment respectively of the 2C-like cell population and associated transcripts, indicating Dppa2 and Dppa4 act as direct regulators of the 2C-like state. Despite the complete absence of 2C-like cells in Dppa2/4 knockout ESCs, these cells retained expression of pluripotency markers and could be passaged for over 20 generations, indicating that 2C-like cells are dispensable for ESC pluripotency. Importantly, overexpression of Dppa2 and Dppa4 restored 2C-like cells in knockout ESCs, suggesting these proteins act together to regulate the ZGA transcriptional network. Additionally, while overexpression of the 2C-like marker Zscan4c was also able to increase the 2C-like cell population in wild type cells, this ability was lost in the Dppa2/4 DKO ESCs. Excitingly, ChIP-seq and transcriptional analysis revealed Dppa2 binds the promoter of and directly regulates Dux, a recently identified regulator of ZGA transcripts. We propose a model in which Dppa2/4 binding to the Dux promoter results in Dux upregulation and subsequent activation of the 2C-like transcriptional network, independently of Zscan4c. In summary, our results reveal Dppa2 and Dppa4 as the first master positive regulators of the zygotic transcriptional network in mammals.

44 Is differentiation robust to replication impediments?

Eldridge C, Crisp A, Sale J

MRC Laboratory of Molecular Biology, UK

Keywords: G-quadruplex; replication; definitive endoderm

Human induced pluripotent stem cells can undergo differentiation into each of the three germ layers with relatively high efficiency when induced to differentiate by the addition of external factors. The process of differentiation to definitive endoderm has been well studied and can be reproduced accurately in cell culture. During the course of differentiation there are dramatic changes in gene expression and chromatin remodelling. However, the impact of DNA replication impediments on this process has not been extensively explored and is examined in this work. During unperturbed differentiation to definitive endoderm, the DNA damage marker γH2Ax peaks during the time at which the cells go through the epithelial mesenchymal transition. Paradoxically, this upregulation of γH2Ax coincides with the reduction in total levels of p53 and checkpoint proteins. However, when differentiation takes place in the presence of the adduct-forming agent methyl methanesulphonate, the downregulation of p53 is prevented and the proportion of SOX17 positive, and therefore differentiated, cells decreases dramatically. This is accompanied by a higher percentage of cells in G2/M phase compared to the untreated cells. When the TP53 gene is knocked out, differentiation in the presence of damage results in the restoration of SOX17 upregulation despite continued accumulation of cells in G2/M phase. This suggests the existence of a p53-dependent 'differentiation checkpoint' that prevents damaged cells from transitioning to definitive endoderm. In contrast, when treated with G-quaduplex binding ligands the DNA damage response is not activated but a similar decrease in cells expressing definitive endoderm markers is observed. Using RNA sequencing we have shown that these ligands are causing the cells to differentiate down a different pathway.

45 Examining the DNA methylation age reset that occurs during iPSC reprogramming

Gill D, Stubbs T, Milagre I, Reik W

Babraham Institute, UK

Keywords: reprogramming; DNA methylation; ageing

In mammalian cells, DNA methylation occurs mostly on cytosine residues that are followed by guanine residues. DNA methylation plays many roles in cells from suppressing transposable elements to maintaining X chromosome inactivation. As individuals age, the pattern of DNA methylation changes with some sites gaining and some sites losing this mark. These changes are relatively consistent between individuals and as a result, the methylome can be used to predict age. These predictors (such as the Horvath epigenetic clock) produce a value called the DNA methylation age, which is thought to represent the biological age of an individual. As a result, lifestyle factors that affect the ageing rate also affect the DNA methylation age. During the process of iPSC reprogramming, DNA methylation age is drastically reduced to approximately 0 years old, regardless of the initial donor age. In this project, we are investigating how and when this DNA methylation age reset occurs during iPSC reprogramming. In addition, the project aims to determine if this age reset can occur without complete iPSC reprogramming: either by conducting partial reprogramming or by overexpressing specific pathways. So far, we have examined the DNA methylation dynamics during human iPSC reprogramming using whole genome bisulphite sequencing data of cells at various timepoints during the reprogramming process. As demonstrated previously, there is a transient decrease in DNA methylation at day 11 of the process and following this, the methylation levels rise to above somatic levels in iPSCs. In addition, the gain of histone modifications such as H3K27me3 is associated with methylation loss and the loss of histone modifications such as H3K4me3 is associated with methylation gain. Overall, this suggests there is crosstalk between the histone code and the methylome, which may be driving the methylation dynamics we observe during reprogramming.

46 On the edge of cell cycle and differentiation: deciphering Seh1 function in mESC

Gonzalez A, Souquet B, Reina R, Doye V

Institut Jacques Monod, France

Keywords: nuclear pore complex; mESC; Seh1

Nuclear pore complexes (NPCs) are huge macromolecular assemblies composed of around 30 different proteins called nucleoporins (Nups). One of the main structural complexes of the NPC is the Nup107-160 complex (Y-complex) that is composed of 9 different subunits. Besides its canonical roles in nucleo-cytoplasmic transport and pore assembly, this complex localizes at kinetochores where it contributes to chromosome congression, correct bipolar assembly and cell cycle progression. Among the subunits of the Y-complex, Seh1 appears to play a key function in chromosome alignment and segregation. However, Seh1 also belongs to another unrelated complex, the GATOR2 complex, which regulates mTORC1, a master regulator of cell growth. In addition, Seh1 has recently been described to recruit the GATOR2 complex to mitotic chromosomes. It is however unknown whether the mitotic functions of Seh1 rely on its localization at the pore, at kinetochores, within the Y- or the GATOR2-complex, or if it relies on other localizations and partners. Since several nucleoporins, including the Y-complex subunit Nup1336, are also involved in developmental processes, we decided to use mouse embryonic stem cells (mESCs) to revisit the functions of Seh1 in both cell division and cell differentiation. We used the CRISPR/Cas9 technology to generate Seh1 KO mESC. The cell lines obtained are viable at undifferentiated state, but show a reduced cell growth rate. In addition, Seh1-/- mESC viability is strongly impaired upon induction of differentiation. To understand whether the defects of Seh1 KO mESCs in cell growth and differentiation are due to the absence of Seh1 in the Y-complex (at the NPC or at kinetochores) or within the GATOR2 complex, we decided to impair the interaction of Seh1 with its direct partners within these two complexes, namely Nup85 and Mios respectively. In the case of Nup85 we used a CRISPR/Cas9 approach to delete a N-terminal blade that is directly involved in the interaction with Seh1. Functional characterization of the Nup85ΔN and Mios KO mESC lines is ongoing and will be presented.

47 Identification of naïve bovine iPS cells

Hyder I, Kumar D, Kues WA

Institute of Farm Animal Genetics, Germany

Keywords: Induced pluripotent stem cells, Naïve cells, LTR7

Induced pluripotent stem cells (iPSCs) are a seminal breakthrough in stem cell research and are a promising tool for advanced reproductive biotechnological approaches in farm animals. Previously, we generated bovine iPSCs by a transposon-mediated approach, using a polycistronic six factors reprogramming cassette in a piggyBac (PB) transposon and a PB transposase gene on a helper plasmid. Studies in murine pluripotent revealed the existence of two distinct stages of pluripotent stem cells (PSCs), i.e., primed and naïve (ground state) cells. Primed PSCs show a certain bias towards lineage-specific differentiation, whereas naïve PSCs can differentiate into the three embryonic germ layers in an unbiased fashion. The naïve stem cells have their own advantage in terms of easy genetic modification, improved clonogenicity and faster expansion with lesser possibilities of unwanted spontaneous differentiation, while maintaining a normal karyotype. Recently a correlation between naïve potency and expression of endogenous retroviruses has been revealed in murine and human embryonic stem cells. Here, we assessed the suitability of a reporter construct driven by the long terminal repeat (LTR) of an endogenous retrovirus for the identification of genuine bovine iPS cells. Hence the objective our study is to apply the LTR7-GFP reporter along with six reprogramming factors (6F) in bovine embryonic fibroblasts (BEFs) to enrich and later identify naïve bovine iPS cells The BEFs are co-electroporated with fixed concentrations of PB-6F and LTR7-GFP reporter. The cells are maintained in DMEM medium containing 10% FCS for 4 days, which is then replaced with iPS medium supplemented with LIF and bFGF. The naïve like cell populations are characterized by robust LTR-GFP expression. By using the reporter, the clones can be picked to propagate by re-plating steps and sorted to obtain homogeneous bovine iPS cells. Cells of different GFP fluorescent intensities can be collected to study different stages of pluripotency.

48 Chromatin Accessibility Dynamics During Somatic Cell Reprogramming

Jing L, Cao S, Li D, Wang B, Yu S, Guo J, Chen J, Pei D

Guangzhou Insititutes of Biomedicine and Health, China

Keywords: Reprogramming, Chromatin Dynamics, Cell fate determination

Chromatin dramatically rearranges during cell fate transitions, and is both an important consequence and a potential driving force in cell fate changes. However, the mechanisms underlying these rearrangements remain unclear. Taking advantage of the factors induced reprogramming (FIP) and chemicals induced reprogramming (CIP) systems, we here describe the dynamic logic for chromatin remodeling during somatic cell reprogramming. Global mapping of open and closed chromatin loci by ATAC-seq reveals dynamic changes from open to closed (OC) and closed to open (CO), with a large burst of OC at the beginning, and a climactic burst of CO at the end in both FIP and CIP. The OC loci are largely composed of genes associated with somatic fate, and enriched for motifs such as AP-1, ETS, RUNX, TEAD and MAD family of transcription factors. While the CO loci are enriched for OKS motif. These results reveal the chromatin accessibility logic during reprogramming that may apply for other cell-fate decisions.

49 EGA-associated genes in cellular reprogramming

Jouhilahti E-M, Weltner J, Katayama S, Balboa D, Trokovic R, Kere J, Otonkoski T

University College London, UK

Keywords: CRISPRa, reprogramming, PRDL homeobox genes

Human embryo development begins in relative transcriptional silence. Activation of transcription within the embryonic genome (EGA) after fertilization occurs stepwise starting at 4 –cell stage in human. In the study by Kere and colleagues, 32 and 129 genes were shown to be transcribed during the transition from oocyte to 4-cell and from 4- to 8-cell stage, respectively (Töhönen et al. 2015). A regulatory motif overlapping with the Alu elements was discovered to be enriched in the promoters of EGA associated genes (EEA-motif). This motif included binding site for evolutionary conserved PRD-like (PRDL) homeobox genes. The genes and control elements involved in human genome activation are potential candidates to reprogram cells into earlier stages than the iPSCs today. Recent advancements in CRISPR/Cas9 technology have allowed the use of modified versions of the Cas9 protein as a mediator of target gene activation. By providing guideRNAs, the Cas9 can be directed to specific genomic locus or multiple loci simultaneously. The complex can be used to target endogenous genes for activation (CRISPRa). In our recent study, we showed that CRISPRa can be applied to reprogram somatic cells to iPSCs and it is an attractive tool for cellular reprogramming due to its multiplexing capacity and direct targeting of genomic loci (Weltner et al. 2018). The reprogramming of human fibroblasts into iPSCs is enhanced by targeting the EEA-motif. The basal CRISPRa reprogramming system without EEA-motif targeting is at low efficiency and thus provides a useful system to study other factors that might enhance reprogramming efficiency. PRDL factors being among the first genes activated in human are potential candidates as powerful reprogramming factors. Our results suggest that the PRDL factors may have a positive effect on reprogramming efficiency.

50 Genomic organization of UHRF1 in mouse embryonic stem cells

Kurowski A, Andino BE, Walsh MJ

Icahn School of Medicine at Mount Sinai, USA

Keywords: Epigenetic regulation, mouse embryonic stem cells, UHRF1

UHRF1/ NP95 is a multi-domain, chromatin binding protein required for stem cell self-renewal and cell fate determination and is found to be overexpressed in many human cancers. The protein plays an essential role in propagating DNA methylation patterns during DNA replication through binding 5mC and recruiting DNMT1. It can also bind to nucleosomal histone modifications, DNA modifications, interact with chromatin modulators and directly regulate proteins by its E3 ligase activity. UHRF1 itself is subject to post-translational modifications which can alter its function. URHF1 has been implicated in multiple cellular processes, however, the regulation of its different binding modules is not well characterized. The binding of UHRF1 to H3K9me3 is known to be necessary for DNA methylation. Surprisingly, our ChIP-seq in ESCs showed that UHRF1 does not co-localize with H3K9me3 but is primarily enriched in euchromatin, especially at enhancers and promoters including these of pluripotency genes. This suggests that UHRF1 can exist in different conformations that allow modular use of its domains. The functional relevance of UHRF1 binding at euchromatic regions is not known. Interestingly, UHRF1 is enriched at sites that contain TET1 and 5hmC, and its binding at Nanog and Nodal is decreased upon TET1 depletion. Whether UHRF1 recruitment is mediated by 5hmC or through an interacting protein remains to be characterized. Using a conditional ablation of Uhrf1 in ESCs derived from embryos bearing a conditional Uhrf1floxed/floxed model, we aim to illustrate the importance of UHRF1 to mark loci for occupation.

51 De novo DNA methylation suppresses extraembryonic lineage potential during transition from naïve to formative pluripotency

Li MA, Kinoshita M, Barber M, Dietmann S, Smith A

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: Dnmt3a/3b, de novo DNA methylation, pluripotency transition, extraembryonic lineage induction

The major wave of global DNA methylation in mammalian development occurs around implantation. While DNA methylation acquisition is essential to development, its cellular function is unclear. We investigated progression from naïve embryonic stem cells (ESCs) to lineage specification using mouse ESCs lacking de novo DNA methyltransferases, Dnmt3a/3b. Dnmt3a/3b double knockout (dKO) ESCs are unable to specify all three germ layer lineages. Instead, dKO cells aberrantly initiate trophectoderm lineage program. In defined conditions, dKO cells convert more efficiently to both trophoblast and primitive endoderm fates. Single cell expression revealed transitory activation of extra-embryonic lineage gene expression in the developmental trajectory at the stage of formative pluripotency. Dnmt3a/3b dKO cells failed to repress these extra-embryonic lineage genes and transit through aberrant states with gene expression signatures of E6.5 extra-embryonic ectoderm. Moreover, dKO cells also aberrantly co-express markers of post-implantation, trophectorderm, and naïve pluripotency. When introduced into wild type host blastocysts, although contributing highly to the inner cell mass, Dnmt3a/3b dKO cells are excluded from the epiblast post-implantation from E6.5 onwards with diminished contribution at E12.5 in the embryo. Instead, ectopic contribution to E6.5 ectoplacenta cone was observed with the dKO cells but rarely with wild type cells. Taken together, we conclude that the cellular function of de novo DNA methylation is to supresses extra-embryonic lineage potential so as to ensure the embryonic lineage competency.

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Role of NF-YA isoforms in mouse Embryonic Stem Cells

Libetti D, Mantovani R, Dolfini D

University of Milan, Italy

Keywords: Mouse ESCs, CCAAT box, NF-Y, CRISPR

Embryonic stem cells (ESCs) maintain pluripotency thanks to a handful of transcription factors which prevent cell differentiation by transcriptional control. Analysis of regulatory regions of genes responsible for stemness maintenance found that the CCAAT box is one of the most enriched DNA elements. It is specifically recognized by the subunit A of the trimeric transcription factor (TF) NF-Y. NF-YA exists in two isoforms, the long and the short one that originates from an exon skipping event. The latter seems to have a crucial role in maintenance of stemness by activating directly key stem cell genes. To shed light on the function of NF-YAs and NF-YAl in controlling cell differentiation, we generated mouse ESC (mESC) line with a genomic deletion of exon 3, coding for the long isoform, by using the Cas9 Nickase and 4 different single guide RNAs, in order to have low off-target effects. The CRISPR modified clones induced for cardiac differentiation by embryoid bodies formation show high expression levels of key stemness genes and a low expression levels of ectodermal and endodermal markers, compared to the wild type. Moreover, CRISPR modified clones induced to differentiate into epiblast-like cells (EpiLCs) maintain the typical stem cell morphology and high expression level of Alkaline Phosphatase (ALP), one of the key markers in the identification of pluripotent embryonic stem cells. Further characterization of these CRISPR modified clones will lead to better understand the role of the NF-YA in the differentiation process and stemness control.

53 Mesenchymal stem cells support leukaemia stem cells in the bone marrow and endow them with antioxidant defence to survive chemotherapy

Forte D, García-Fernández M, Sánchez-Aguilera A, Stavropoulou V, Fielding C, Martín-Pérez D, López JA, Vázquez J, Barber M, Dietmann S, Bernardo-Castiñeira C, Sommerschield A, Gallipoli P, Marando L, Arranz L, Tzankov A, Attrot K, Frey-Wagner I, Catani L, Curti A, Huntly BJ, Schwaller J, Méndez-Ferrer S

University of Cambridge, UK

Keywords: AML, microenvironment, BMSCs

Introduction: Acute Myeloid Leukaemia (AML) is a heterogeneous disease characterised by the proliferation of clonal stem-cell-like blasts in the bone marrow (BM).^1 The BM microenvironment (niche) contributes to AML development and chemoresistance.^2 However, most BM stromal cells are reduced in AML and the mechanisms by which the surviving stromal cells promote leukaemogenesis remain incompletely understood. Results: Unlike the bulk of stromal cells, BM mesenchymal stem cells (BMSCs) identified by the expression of the intermediate filament protein nestin^3 are not diminished in AML patients or an inducible MLL-AF9 leukaemic mouse model.^4 In vivo, nestin+ cell depletion decreases leukaemia burden and significantly improves mouse survival and chemosensitivity to Ara-C. In vitro, leukaemic blast survival after Ara-C doubles in co-culture with BMSCs. Increased blast survival is partially explained by mitochondrial transfer from BMSCs, which increases the bioenergetic capacity of AML cells. However, BMSCs also decrease AML cells excessive reactive oxygen species (ROS) and toxic lipid peroxidation, which correlate with increased glutathione (GSH), a key antioxidant molecule. In vivo, Ara-C treatment increases GSH in the leukaemic blasts, but this is conditional to the presence of nestin+ cells. Genomics and proteomics analyses confirmed the mitochondrial, antioxidant and GSH-related pathways in the leukaemic support by Nestin+ BMSCs. We focused especially in GPX1, which uses GSH for ROS detoxification and has been involved in AML chemoresistance.^5 Importantly, AraC increased GPX1 in the leukaemic blasts mostly in the presence of nestin+ BMSCs, both in vivo and in vitro, where GPx inhibition decreased the protection of AML cells by BMSCs during chemotherapy. Conclusions: Nestin+ BMSCs provide leukaemic blasts with increased bioenergetics and essential ROS detoxifying tools for AML development and chemoresistance.

54 Targeting MEK in vemurafenib-resistant hairy cell leukemia

Hodson D, Caeser R, Collord G

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: Leukaemia, Therapy, Resistance

UHRF1/ NP95 is a multi-domain, chromatin binding protein required for stem cell self-renewal and cell fate determination and is found to be overexpressed in many human cancers. The protein plays an essential role in propagating DNA methylation patterns during DNA replication through binding 5mC and recruiting DNMT1. It can also bind to nucleosomal histone modifications, DNA modifications, interact with chromatin modulators and directly regulate proteins by its E3 ligase activity. UHRF1 itself is subject to post-translational modifications which can alter its function. URHF1 has been implicated in multiple cellular processes, however, the regulation of its different binding modules is not well characterized. The binding of UHRF1 to H3K9me3 is known to be necessary for DNA methylation. Surprisingly, our ChIP-seq in ESCs showed that UHRF1 does not co-localize with H3K9me3 but is primarily enriched in euchromatin, especially at enhancers and promoters including these of pluripotency genes. This suggests that UHRF1 can exist in different conformations that allow modular use of its domains. The functional relevance of UHRF1 binding at euchromatic regions is not known. Interestingly, UHRF1 is enriched at sites that contain TET1 and 5hmC, and its binding at Nanog and Nodal is decreased upon TET1 depletion. Whether UHRF1 recruitment is mediated by 5hmC or through an interacting protein remains to be characterized. Using a conditional ablation of Uhrf1 in ESCs derived from embryos bearing a conditional Uhrf1floxed/floxed model, we aim to illustrate the importance of UHRF1 to mark loci for occupation.

55 Lymphoma Cell Cycle Regulation by the RNA-Binding ZFP36 Proteins

Runge HFP, Hodson DJ

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: Lymphoma, Cell Cycle, RNA-Binding Proteins

The RNA-binding zinc finger proteins ZFP36, ZFP36L1 and ZFP36L2 promote quiescence during defined stages of B cell development in a partly redundant fashion. They bind AU-rich elements (AREs) in the 3' untranslated region (3' UTR) of cell cycle-inducing mRNAs to trigger deadenylation and degradation. Further targets regulate cytokine production, cell adhesion and migration. ZFP36L1 loss of function mutations occur frequently in diffuse large B cell lymphoma (DLBCL) and Zfp36 overexpression impairs MYC-induced lymphomagenesis in a mouse model. Hence, we suspect a tumour suppressor role of the ZFP36 family in lymphoma with knockouts leading to de-repression of the cell cycle and a proliferative advantage. Here, we tested the effect of ZFP36 family knockouts on fitness in competitive growth assays and investigated the influence on cell cycle progression and apoptosis.

56 Niche heterogeneity impacts evolution of myeloproliferative neoplasms driven by the same oncogenic pathway

Korn C*, Rak J*, García-García A, Fielding C, Khorshed RA, González-Antón S, Li J, Norfo R, Baxter J, McKerrell T, Roberts T, Eldaly H, Godfrey AL, Castillo-Venzor A, Kusumbe A, Mead A, Green AR, Kent D, Lo Celso C, Mendez-Ferrer S

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: Haematopoietic stem cell, Myeloproliferative neoplasms, Bone marrow niche

Different myeloproliferative neoplasm (MPN) entities such as polycythemia vera (PV) and essential thrombocytosis (ET) can originate from hematopoietic stem and progenitor cells (HSPCs) carrying the same driver mutation. However, the transformation rate into secondary myelofibrosis and leukemia is higher for PV than for ET 1. Whereas secondary mutations might cause transformation, it remains unknown whether distinct bone marrow (BM) niches influence MPN progression 2. BM niches close to bone (endosteal) have been suggested to promote HSPC quiescence, whereas non-endosteal vessels permit transmigration of activated HSPCs 3-6. To study the contribution of different BM niches to MPN evolution we performed intravital imaging of the skull BM of mice injected with HSPCs from mouse models of ET (VavCre;JAK2V617F) and PV (Mx1Cre;JAK2V617F). Interestingly, ET HSPCs home and expand close to the bone surface, whereas PV HSPCs localize further from bone. Asymmetric expansion of mutant CD34+ HSPCs in ET and PV was also confirmed in human BM trephines. Fast migration of murine ET HSPCs close to the bone surface suggests an exploratory strategy of ET HSPCs to find endosteal niches. Integrin β3+ might trigger endosteal lodging of ET HSPCs, as integrin β3+ HSPCs are enriched in endosteal BM of ET mice and ET HSPCs show strong adhesion to extracellular matrix substrates of integrin β3. Asymmetric HSPC expansion caused differential microenvironment remodeling. Non-endosteal sinusoids are dilated in PV mouse models, whereas CD31hiSca1hi arterioles and aberrant bone-forming integrin β1+ and laminin alpha 4+ blood vessels increase in ET mice. Consequently, increased bone, osteoblasts and osteoclasts were found in ET but not PV mice. Increased expression of vascular-derived bone-forming factors downstream of endothelial laminin alpha 4/integrin β1 signaling might trigger osteosclerosis in ET mice. In conclusion, mutant HSPCs preferentially occupy and remodel different BM niches in ET and PV.

57 Deciphering the role of DNMT3A pre-leukemic mutations in human clonal haematopoiesis and AML development using genome editing

Piganeau M, Ariza-McNaughton L, Batsivari A, Rouault-Pierre K, Bonnet D

The Francis Crick Institute, UK

Keywords: clonal haematopoiesis, CRISPR, DNMT3A

Acute myeloid leukaemia (AML) is the most frequent haematological myeloid malignancy. Recently, deep sequencing of AML samples has identified mutations in various parts of the cellular machinery, including epigenetic regulators (e.g. DNMT3A, TET2, ASXL1). Notably, those mutations have been shown to be initiating events in AML development. In addition, recent studies have highlighted the presence of identical mutations in the blood of haematologically healthy adults, giving rise to the concept of clonal haematopoiesis of in-determinant prognosis (ChIP). Although those individuals do not display clinical manifestation of the disease, they show increased risk of developing leukaemia and atherosclerosis, suggesting that mutations in epigenetic modifiers occurring in hematopoietic stem cells (HSCs) may lead to a latent pre-leukemic stage preceding AML. If the characterization of genomic events in AML is well described, the stage of clonal haematopoiesis remains poorly understood. To address this question, we used the CRISPR/Cas9 system to introduce de novo pre-leukemic mutations in human cord blood HSCs. We specially focused on the example of DNMT3A, the most frequent gene associated with clonal haematopoiesis, also mutated in 30% of AML patients. By adopting an HDR-based targeted mutagenesis approach, we set out a strategy to generate mutations at DNMT3A R882 residue. Transplantation of edited HSCs in immunodeficient mice allowed us to follow the long-term and multi-lineage reconstitution capacity of mutant cells. In addition, we were able to show that DNMT3A mutant cells are maintained over serial transplantation, and to study their evolution at a clonal level. Specially, cells following xenotransplantation were able to give rise to both granulo-macrophagic and erythroid lineages, with mutations detected in both lineages. These new models will provide new insights into the consequence of DNMT3A mutations on human haematopoiesis, and help deciphering the path from clonal haematopoiesis to overt leukaemia.

58 Chronic Lymphocytic Leukaemia remodels the bone marrow haematopoietic niche

Santoro A, Mangolini M, Moore A, Park E, Korn C, BelmonteM, Ringshausen I

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: CLL; haematopoiesis; stromal cells; endothelial vessels

Chronic Lymphocytic Leukaemia (CLL) is a non-curable neoplastic disease characterised by the accumulation of CD5-expressing mature B-cells in the circulation, bone marrow (BM) and lymphoid organs. Normal haematopoiesis is impaired in CLL, and patients often present with different types of cytopenias. It has been extensively reported that CLL requires the support from the microenvironment to receive survival signals. In particular, crosstalk of CLL cells with mesenchymal stromal cells (MSCs) contributes to the generation of an inflammatory environment, supporting survival and development of the leukaemia. MSCs are self-renewing precursor cells that can differentiate into bone, fat, cartilage and stromal cells of the BM. Evidence indicates that MSCs are a key component of the haematopoietic stem cells (HSC) niche in the BM, where these two distinct stem cell populations arrange closely, providing a specialised microenvironment for controlling the processes of normal and malignant haematopoiesis. Moreover, the composition of this niche is highly complex, containing also more differentiated specific cell types, such as different subtypes of endothelial cells, which display different functions with regards to haematopoietic cells maintenance. In this study we seek to understand how the BM haematopoietic niche is remodelled in response to CLL infiltration, and to reveal the biological mechanisms underlying the remodelling of stromal cells in the haematopoietic niche in a CLL mouse model. Preliminary experiments provided the evidence that murine CLL cells preferentially home the BM niche in close proximity to endothelial cells. In addition, we found that transplantation of murine CLL into syngeneic NestinGFP recipient mice remodelled the BM haematopoietic niche. In particular, up-regulation of SCA-1 occurs in CD45-CD31+ endothelial cells, in response to accumulation of leukaemic cells in the BM. Current work is focusing on identifying the gene expression profiles of stromal cells, and endothelial SCA1+ cells of disease-bearing mice with the perspective of identifying novel disease-driven biological functions. Future work will also focus on investigating whether the transformation of the BM niche contributes to the disruption of normal haematopoiesis. Overall, this study will help to identify specific mechanisms adopted by the tumour to communicate with the surrounding microenvironment. This will not only increase our knowledge on the biology of CLL, but will importantly provide us with novel putative targets that can be used to develop new therapies

59 Regulation of human stem and progenitor cells by acute myeloid leukaemia in the human niche

Waclawiczek A, Hamilton A, Rouault-Pierre K, Bonnet D

The Francis Crick Institute, UK

Keywords: Microenvironment, acute myeloid leukemia, hematopoietic stem cells

In acute myeloid leukemia (AML) the suppression of hematopoietic stem and progenitor cell (HSPC) differentiation and proliferation is a major factor contributing to treatment complications and mortality rates (1,2). In this study we generated humanized in vivo and ex vivo models to simultaneously investigate the crosstalk between AML, normal human HSPCs and the bone marrow microenvironment (BM). By co-transplanting AML cells and normal HSPCs into NSG mice and humanized 3D BM models, we observed a negative impact of AML engraftment on the proliferation and myelopoietic potential of hHSPCs. Interestingly, normal hematopoietic cells after exposure to AML were enriched for quiescent HSPCs suggesting an inhibition of HSPC maturation through reduced proliferation. Also in a simplified BM ex vivo system AML but not healthy hematopoietic cells rapidly suppress the proliferation and differentiation of normal HSPCs. Moreover, simple preconditioning of MSCs with AML for few days reduced hHSPCs proliferation, suggesting that AML modifies the microenvironment to suppress normal hematopoiesis. Transcriptomic interrogation of the MSCs after AML exposure identified an upregulation of Hypoxia inducible factor -1 alpha (HIF-1a) target genes and increased stabilization of (HIF-1a). In MSC cocultures and 3D scaffolds knockdown of (HIF-1a) helped to restore HSPC proliferation and differentiation. On the other hand artificial stabilization of HIF-1a in MSCs reduced HSPC expansion and kept them in an immature state. Downstream of HIF-1a we identified the secreted glycoprotein Stanniocalcin-1 (STC1) whose concentration significantly correlate with normal HSPC suppression. Inhibition of STC1 ex vivo and in vivo improves the proliferation, differentiation and colony forming ability of HSPCs in the presence of AML. STC1 supplemented media and overexpression reduced had an anti-proliferative effect on HSPCs. We are currently investigating the precise mechanism of STC1 on normal HSPCs and validating STC1 as novel target to improve survival.

60 Translation stress induced by inactivation of Elongator activates a p53-dependent antitumor checkpoint in hematopoietic stem and progenitor cells

Desmet CJ, Rosu A, Rouault-Pierre K, Ramery E, Rapino F, Somja J, Bai Q, Nguyen L, Cools J, Bonnet D, Chariot A, Close P, Bureau F

Liege University, Belgium

Keywords: Elongator, p53, transfer RNA, hematopoietic stem cell, translation

Tightly controlled messenger RNA translation is emerging as an essential requirement for the normal function, differentiation and fate decisions of hematopoietic stem and progenitor cells (HSPCs) (Guzzi et al., 2018; Khajuria et al., 2018; Liu et al., 2017; Signer et al., 2014). Here, to reveal translational determinants of HSPC fate decisions, we used mouse models of conditional inactivation of Elongator, an enzymatic complex that optimizes speed of translation elongation by catalyzing modifications of the wobble uridine of specific transfer RNAs. We report that loss of Elongator activity blocked HSPC differentiation and caused bone marrow failure through the activation of translation stress responses orchestrated by the transcription factors Atf4 and p53. In contrast, the maintenance, but not the reconstitution activity of Elongator-deficient hematopoietic stem cells, was spared. While deletion of p53 rescued Elongator-deficient HSPC function, simultaneous inactivation of p53 and Elongator synergistically promoted tumorigenesis. Loss of Elongator thereby reveals a p53-dependent quality control of translation that conditions antitumor fate decisions in HSPCs.

61 Advancing bone marrow toxicity assessment by temporal resolution of hematopoietic stem cell differentiation in a 2D liquid culture model

Komen J, Khan K, David R, Mead A

AstraZeneca, UK

Keywords: bone marrow toxicity, hematopoietic stem cells, liquid culture

Oncology drugs often give rise to bone marrow (BM) toxicity. In vitro assessment of BM toxicity in the drug discovery phase contributes to selecting the most promising drug candidates for further development. In the 2D BM assay BM-derived CD34+ human hematopoietic stem cells (HSCs) are differentiated in specialist medium towards either erythroid, myeloid or megakaryocytic cell-lineages over 5 days during which drug effect (viability) is tested. In the current study lineage-specific cell markers for flow cytometry (FC) have been optimised to monitor defined cell populations over time in the 2D BM assay. In erythroid medium CD34+ cells disappear completely after 5 days and cell populations expressing erythroid markers (CD71 & CD235a) reach a maximum at 10 days (>95%) after which viability decreases. Day 3 represents a mixed pool of stem and differentiated cells. In contrast, CD34+ cells are detected after 21 days in culture (~10%) in myeloid medium, with no changes in overall cell viability being observed. Myeloid marker expression (CD33 & CD15) increased to >75% on day 10, representing a mixed cell pool. In megakaryocyte medium, CD34+ cells are present after 21 days (~5%), CD41 is expressed after 5 days (40%), while CD42, expressed later in differentiation, was observed only after day 10 (52%). Therefore, the population on day 10-15 represents a mixed pool of cells, although overall cell viability does decrease 10% at day 15 compared to day 10. These data give us essential information about the differentiation kinetics of HSCs over time in lineage-specific medium, and the time to generate mixed-cell populations has been determined. This can be used to more precisely assess drug-induced toxicity on progenitors, differentiated cells, or a mixed population over a specific timeframe, which will significantly contribute to a more precise preliminary assessment of drug-driven BM-toxicity.

62 Molecular regulation of exit from quiescence in human Haematopoietic Stem Cells

Belluschi S, Drakopoulos M, Sham K, Lau W, Diamanti E, Wang X, Göttgens B, Laurenti E

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: Activation, quiescence, Human haematopoietic stem cells

Haematopoiesis in humans is maintained by haematopoietic stem cells (HSCs), which are characterised by the ability to self-renew and to differentiate into all mature blood cell types. To maintain their stem cell properties, HSCs divide infrequently and reside in a specific state outside the cell cycle called "quiescence" (or G0 phase). However, upon sensing mitogenic signals, they exit quiescence and enter the cell cycle in a process called "activation". While past studies have principally focused on studying the molecular networks that maintain HSC quiescence, very little is known about how these networks change during quiescence exit and activation. This is of importance, as it is known that upon activation in culture, HSCs partially lose their self-renewal capacity by yet unclear mechanisms. To investigate this, I used an in-vitro model system of human HSC quiescence exit and activation which takes advantage of the inhibition of CDK6, a master regulator of quiescence exit, which blocks HSC division in G0/early G1 phase of the cell cycle. Time course single cell RNA-sequencing of HSCs progressing from quiescence to activation demonstrated that the transcriptome changes gradually with time but 2/3 of the transcriptional changes occurring during G0 exit are independent from cell cycle progression. Fast up-regulation of cell growth and metabolic pathways are temporally the first responses associated with mitogenic stimulation and exit from quiescence and they do not depend on cell cycle progression. Moreover, the activity of most transcription factors known to maintain HSC quiescence is also abrogated during G0 exit independently of cell cycle progression. Our data collectively indicate that dismantlement of the molecular network maintaining HSC quiescence is achieved before progression of the cells past early G1, and highlights that early molecular events triggered during ex vivo culture may be crucial to modulate HSC function.

63 Mesenchymal stem cells cardiac regenerative properties can be boosted by Human Platelet lysate

Bordin A, Milan M, Scaccia E, Pagano F, Mangino G, Totta P, De Falco E

Sapienza University of Rome, Italy

Keywords: Mesenchymal stem cells, platelet lysate, cardiovascular, regeneration

Mesenhymal stem cell (MSC)-based therapies have imposed as a powerful strategy to enhance the intrinsic regenerative capacity of heart. Recently, the combination of MSCs and growth factors has gained attention as greater regenerative benefits can be achieved. Yet, clinical formulations of soluble mediators and MSCs suitable for cardiac repair are still to be verified. Platelet Lysate (PL) is a hemoderivate enriched of soluble mediators employed in several regenerative-based clinical applications and MSCs expansion ex vivo. We have patented an optimized PL preparation (Mesengen®, N. WO2013042095), based on standardized concentration of growth factors/ cytokines and with reduced interference of residual proteins, providing high in vitro performance combined to low immunogenic/ inflammatory potential. We have demonstrated that Mesengen® is able to increase in vitro MSC clonogenic potential, proliferation and cardiac-like commitment. We aim here to unravel the regenerative potential of human PL as a boost for MSC-mediated cardiac/ endothelial regenerative properties. Our results highlight that PL significantly increases the in vitro angiogenic capacity of cardiac MSCs and endothelial cells. We showed that PL contains miRNAs, such as miR-126, coherently with its derivation from platelets, and extracellular vescicles displaying subpopulations of different size (0.8/0.45/ 022µm). Cultures supplementated with the sole fraction containing particles below 0.22µm abolishes both angiogenic and migratory capacity of PL. The in vivo injection of PL in a mouse model of myocardial infarct within the ischemic area enhances functional recovery at day 3 with a parallel reduction of cardiac fibrotic area and increased number of α-SMA+ vessels and collagen3/1 ratio, hallmarks of cardiac regeneration. The biological activity of PL is not associated to phenomena of platelets aggregation. This study supports the regenerative proficiency of PL in the ischemic context.

64 Restriction of myelo-lymphoid lineage potential occurs within the human haematopoietic stem cell pool

Calderbank EF, Belluschi S, Ciaurro V, Pijuan-Sala B, Santoro A, Mende N, Diamanti E, Wang X, Lau W, Jawaid W, Göttgens B, Laurenti E

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: Haematopoiesis, lineage restriction

Blood production is coordinated by a functionally heterogeneous pool of multipotent haematopoietic stem cells (HSCs), downstream of which lineage-restricted progenitors are generated. However, how loss of multipotency is orchestrated at the cellular and molecular levels remains unclear. Here we combined index sorting, single cell functional assays in vitro, RNA-seq and in vivo assays to show that, in human cord blood, lineage restriction events occur within the CD34+CD38-CD45RA-CD49f+CD90+ (49f+) HSC compartment to generate myelo-lymphoid committed cells with no erythroid differentiation capacity. In in vitro functional assays, less than 5% of HSC/MPP pool cells were multipotent, and single cells with myelo-erythroid potential could be distinguished from single cells with myelo-lymphoid potential based on gradual changes in cell surface expression of CD34 and novel HSC marker CLEC9A. This was mirrored in 49f+ HSCs. In addition, at single cell resolution, we observed a continuous but polarised organisation of the 49f+ compartment, where transcriptional programmes progressively change along a gradient of opposing cell-surface expression of CLEC9A and CD34. We established novel prospective purification strategies, in both HSC/MPP pool and 49f+ HSCs, that maximise enrichment of cells with myelo-erythroid (CD34lo CLEC9Ahi; Subset1 and 49f+ Subset1) or myelo-lymphoid (CD34hi CLEC9Alo; Subset2 and 49f+ Subset2) potential in vitro. In vivo, we used an optimised NSG xenograft model for detection of erythroid potential, to show that Subset2 and 49f+ Subset2 cells were restricted to myelo-lymphoid differentiation and displayed infrequent long-term repopulation capacity. In conclusion, we demonstrate that the first lineage restriction step in human haematopoiesis occurs in highly purified human HSCs and generates myelo-lymphoid committed cells with no erythroid differentiation capacity.

65 Dual cholinergic signals regulate circadian hematopoietic stem cell traffic by modulating β-adrenergic signaling

García-García A, Korn C, García-Fernández M, Domingues O, Zimmer J, Michel T, Airaksinen MS, Méndez-Ferrer S

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: Hematopoietic stem cell, traffic, leukocyte, circadian, sympathetic, parasympathetic

Hematopoietic stem and progenitor cells (HSPCs) and leukocytes circulate between the bone marrow (BM) and peripheral blood following circadian oscillations. Autonomic sympathetic noradrenergic signals have been shown to regulate HSPC and leukocyte trafficking, but the role of the cholinergic branch remains unexplored. Here we show that the autonomic cholinergic nervous system (both parasympathetic and sympathetic) dually regulates daily migration of HSPCs and leukocytes. Central parasympathetic signals dampen sympathetic noradrenergic tone at night, when local sympathetic cholinergic fibers repress nocturnal HSPC and leukocytes egress by inhibiting β3-AR signaling. Increased (nor)epinephrine concentration in blood at night predominantly signals through β2-AR to increase vascular cell adhesion and BM homing. Light-triggered sympathetic cholinergic activity inhibits vascular cell adhesion locally in the BM at daytime, when de-repressed sympathetic noradrenergic activity causes predominant HSPC and leukocytes egress via β3-AR. Therefore, both branches of the autonomic nervous system cooperate to orchestrate circadian traffic of HSPCs and leukocytes.

Theme 4: Neural Stem Cells

66 Clonal analysis defines the cellular dynamics of medulloblastoma

Azzarelli R, Selvadurai HJ, Dirks PB, Philpott A, Simons BD

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: neural stem cells, brain tumors, clonal analysis

Brain tumour stem cells have been identified in different neurological cancers and are thought to sustain tumour growth and generate the differentiated cell types present within the tumor (Azzarelli et al., Development 2018). The paediatric brain tumour medulloblastoma (MB) contains a rare population of Sox2+ quiescent stem cells that fuel tumour growth and are selectively enriched following anti-mitotic treatments (Vanner et al., Cancer Cell 2014). These findings suggest Sox2+ cells are a likely source of post-treatment disease relapse and therapeutic benefit could be achieved by targeting these cells. Here we hypothesized that these cells drive tumour progression through hierarchical growth. To test this, we performed both in vivo clonal-level lineage trace from the Sox2+ fraction with a multi-coloured fluorescent reporter and in vitro clonal analysis by looking at the growth of single MB cells isolated from a mouse model of Sonic Hedgehog MB and expanded in vitro in neural stem cell conditions. Quantitative modelling of clonal fate data conformed to a hierarchical mode of growth, with Sox2 expression defining a population of long-lived, self-renewing cells. Our modelling also predicted that Sox2+ stem cells give rise to a rapidly dividing progeny of intermediate progenitors that will then terminally differentiate. Thus our data are consistent with the presence of a lineage hierarchy reminiscent of normal neural development and could provide novel insights into selective vulnerabilities that could be exploited therapeutically.

67 Role of CXCR4/R7-CXCL12 axis in putative stem cells from human meningiomas

Solari A, Rogna L, Fiaschi P, Zona G, Barbieri F, Florio T

University of Genoa, Italy

Keywords: Meningioma, CXCL12, putative stem cells

Meningiomas, the most common primitive intracranial tumor, are divided into three grades of malignancy, showing progressive increased risk of recurrence. Generally, surgical resection is the definitive therapy; however, about 12% of meningiomas recur, representing a serious therapeutic challenge, although no specific geno/phenotypic characteristics have been identified as predictor factors. In malignant tumors recurrence is due to cancer stem cells (CSCs) that are also involved in development, metastasis and drug resistance of neoplasia. CSC are characterized by stem marker expression, self-renewal, and ability to differentiate into tumor-specific cells type. Recently, CSCs and their functional role have been also studied in benign tumors. In fact, some biological features of CSCs, spherogenesis, or expression of putative stemness markers (SOX2, NANOG and OCT4), have been also identified in different benign tumor cells. An inverse correlation between the expression of endoglin (CD105) and survival without recurrence has been reported in meningiomas, and CD105+ cells were proposed as meningioma CSCs. To study the role of putative CSCs in meningiomas as determinants of tumor aggressiveness, CD105+ stem-like cells were selected by immunomagnetic sorting from 30 primary cell cultures from human meningioma. Compared to bulk meningioma cells, CD105+ subpopulation is composed by smaller cells expressing stemness markers, showing higher proliferation rate, ability to form meningospheres and to differentiate in adipocytes and osteocytes. Moreover, the activation of chemokine receptors by CXCL11/CXCL12 induced proangiogenic activity (as for tube formation assay) and cell migration only in CD105+ meningioma cells. In conclusion, human meningiomas contain a small CD105+ subpopulation, which, at odd to the CD105- counterpart, shows stem-like features, including stem marker expression, self-renew ability, invasiveness, and proangiogenic activity.

68 Editing of IDH1 R132H mutation in iPS cells using CRISPR/Cas9 to investigate tumour genesis in glioma

Köpp A, Lehmann M, Gawehn L, Richter S, Mircetic J, Marrone L, Heide M, Sterneckert J, Kunz-Schughart L, Temme A, Schröck E, Klink B

TU Dresden, Germany

Keywords: Glioma, CRISPR/Cas, IDH1

Gliomas are the most frequent primary brain tumours in adults and are currently not curable. Low grade gliomas and secondary glioblastomas are characterised by a hot-spot mutation in the Isocitrate dehydrogenase 1 (IDH1). The mutation causes a new catalytic function of the enzyme resulting in the production of 2-Hydroxyglutarat (2-HG) which is known as an oncometabolite. To this day, the pathomechanism by which IDH1 mutation promotes tumorigenesis is not completely understood and the tumour cell of origin for glioma is not known. Moreover, reliable cell models reflecting the patient´s situation are not available. Thus, we aim at creating human induced pluripotent stem cells (hiPSC) carrying the IDH1 R132H mutation by CRISPR/Cas9-mediated genome editing. We will utilize this cell model to investigate how the mutation influences stem cell properties and cell differentiation in neuronal progenitor cells. For designing the cell model, we applied the novel CRISPR/Cas9 based genome editing tool Base Editor 3 . We transiently transfected the cells with two plasmids containing the Cas9 enzyme and sgRNA respectively. To determine editing efficiency, cells were investigated using the T7 Endonuclease 1 assay and Next Generation Sequencing (NGS). Single cell clones were picked to analyse allele status using allele-specific PCR with subsequent Sanger Sequencing. NGS of the transfected cells revealed a base change at the expected position in 1,11% of the reads. Out of 96 picked clones one clone has the IDH1 mutation. Thus, the overall editing efficiency is about 1%. The presence of the mutation was confirmed by Sanger Sequencing and Western Blot. In addition we measured the tricarboxylic acid cycle metabolites and found a 40-fold increased concentration of 2-HG compared to the wildtype cell. We successfully established a heterozygous IDH1 R132H mutated hiPSC cell line which represents a promising cell model for future studies.

69 Mechanism of neural stem cell transformation by fusion onco-proteins

Kupp R, Gilbertson RJ

University of Cambridge, UK

Keywords: neural stem cell, brain tumor, epigenetics

Ependymomas are tumors of the central nervous system, arising within the ependymal lining at the ventricle-parenchyma interface. Molecular profiling studies suggests ependymomas in different anatomical compartments are distinct and disparate diseases, with unique cells of origin and genetic drivers. We have recently described a highly recurrent 11q structural variant, producing a fusion translocation between the C11orf95 gene of unknown function and RELA, the principal effector of NF-kB signaling. C11orf95-RELA Fusion proteins, when introduced into neural stem cells, rapidly transform to form ependymoma. Furthermore, recent studies analyzing the genomes and transcriptomes of 500 primary ependymomas have reinforced these findings, showing that C11orf95-RELA fusion proteins are found within ~70% of forebrain (supratentorial) ependymomas and correlated with negative overall survival. However, the molecular events preceding and following Fusion transformation remain largely unknown. In this study we will present our recent efforts integrating transcriptome, proteome, interactome, and genome wide mapping of Fusion proteins (as well as their individual components) to understand the mechanisms by which neural stem cells transform to form ependymomas.

70 Improving Models of Microglia: The Development of Physiologically Functional Human iPSC-derived Microglia

Rock D, Paonessa M, **Nilsson Z**, Harper S, Shi Y

Axol Bioscience, UK

Keywords: Microglia, iPSC-derived, CNS

Microglia are commonly described as the immune cells of the brain, they constantly survey their surroundings to seek out invading pathogens and clear up cell debris, apoptotic cells, and misfolded proteins; all to retain a homeostatic balance in the CNS. To date, much of microglia research has been conducted using animal models, immortalized microglia-like cell lines or primary human microglia. Each of these models has many caveats ranging from limited cell number, variability in homogeneity and unrepresentative inflammatory responses. Axol's method for generating iPSC-derived microglia mimics the in vivo pathway of development for brain resident macrophages and produces microglia that are homogenous and representative of primary human microglia in vitro. Here we demonstrate that Human iPSC-derived Microglia provide a model which exhibit a highly dynamic ramified morphology and express expected myeloid and microglia-specific protein markers; TREM2, IBA-1, and TMEM119. Our data shows that once activated, Human iPSC-derived Microglia change morphology from elongated and ramified to rounded and amoeboid, they become highly phagocytic and produce microglia-relevant cytokines. Additionally, we have shown that Human iPSC-derived Microglia can be co-cultured with Human iPSC-derived Cortical Neurons from the same donor, to study cell-cell interactions. These phenotypes make Axol's Human iPSC-derived Microglia suitable models for investigating neuroinflammation in Alzheimer's disease, multiple sclerosis, and Parkinson's disease. This research shows that Axol's Human iPSC-derived Microglia provide an assay-ready, reliable and consistent model that is able to phenotypically recapitulate human microglia response in vitro.

71 Induced pluripotent stem cell (iPSC) from autistic individuals undergo atypical neurogenesis and show disrupted excitatory-inhibitory neural differentiation

Adhya D, Swarup S, Nagy R, Shum C, Jozwik K, Mendez MA, Horder J, Nowosiad P, Lee I, Skuse D, Murphy D, McAlonan G, Geschwind DH, Price J, Carroll J, Srivastava DP, Baron-Cohen S

University of Cambridge, UK

Keywords: Autism, iPSC, neurogenesis

Non-syndromic autism are a set of neurodevelopmental conditions with a complex genetic basis involving low penetrant mutations. Functional genomics analyses of post mortem brain tissue from individuals with non-syndromic autism have identified convergent atypical gene correlation networks. However, it has not been possible to study early prenatal neurogenesis in autism using post mortem brain tissue. To address this, we used an in-vitro model of prenatal human neurogenesis by generating neural cells from induced pluripotent stem cells (iPSCs) derived using keratinocytes from autistic individuals. We found significant delay in appearance of cortical neural precursors during neural differentiation of autism iPSCs compared to healthy neural differentiation. RNA-sequencing and functional genomics analysis identified altered gene co-expression networks in autism neural cells highly correlated with gene networks in autism post-mortem brains, which included neuronal maturation, synaptic maturation, immune response and inflammation, and gene regulatory mechanisms, and also suggested altered excitatory-inhibitory receptor expression in autism. This excitatory-inhibitory alterations were not restricted to the RNA-seq sample set, and we found divergence of excitatory and inhibitory neuronal markers in a larger cohort. We also found greater prevalence of CD44 in autism neural cells, a highly connected gene in our RNA-seq analysis. In conclusion, our study demonstrates significant differences in neurogenesis between autism and control iPSCs, which might reflect the enrichment of altered autism-associated gene co-expression networks.

72 Resolving white matter dysfunction in Alzheimer's Disease

Varga B, Kronenberg-Versteeg D, Wong JJY, Steindel M, Charlesworth P, Evans K, Horrocks M, Pankotai E, Lee ZWC, Spillantini MG, Coleman P, Brown G, Graff C, Ginhoux F, Lee S, Hall E, Káradóttir RT

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: Alzheimer's disease, oligodendrocyte, white matter

Recent evidence suggests a close correlation of changes in the brain's blood vessel conditions and dementia including Alzheimer's disease. The cerebral white matter is highly vulnerable to ischemic events and high-power imaging studies revealed that small white matter lesions correlate strongly with cognitive decline and begin to accumulate before first AD symptoms occur. In order to investigate the role of white matter infarcts in AD in a clinically relevant system, we generated a series of new tools: (1) to recreate human cerebral grey and white matter of clinically diagnosed AD patients in vitro; (2) to induce focal hypoxia and hypoglycemia in the white matter; and (3) a super-resolution method to detect the spreading of AD associated risk factors ßAMYLOID and TAU between cells. We differentiated cortical neurons, astrocytes, oligodendrocytes and microglia/macrophages from iPSCs of clinically diagnosed AD patients and their relatives. The neural and immune cell types can be then assembled in microfluidic devices in such to establish a cortical 'grey' and 'white' matter. We created a small oxygen scavenging electrode to induce focal hypoxia in the white matter compartment and combined this with super-resolution imaging method to detect different AMYLOID species in and around the cortical neurons and trace the spreading between cells in the cultures. This method allows now to directly address whether there is an underlying susceptibility of neural cells derived from AD patients to focal white matter ischemia and whether there are alterations in AMYLOID species, between patient lines and control. Understanding the role of hypoxia and hypoglycemia in the cerebral white matter requires focal control of oxygen and glucose levels within the subcortical white matter, which is vastly challenging in the rodent brain. Alzheimer's disease (AD) is pathophysiologically characterized by the accumulation of extracellular amyloid-beta plaques and intracellular tau tangles.

73 Rapid and efficient generation of neurons and astrocytes from human induced pluripotent stem cells

Baranes K, Abdul-Karim MKB, Kotter MRN

University of Cambridge, UK

Keywords: Reprogramming; Neurons; Astrocytes

Transcription factor-based reprogramming of human pluripotent stem cells (hPSCs) is about to revolutionise the way mature cells are generated. Direct conversion of hPSCs has the potential of being faster, more efficient and more reproducible than conventional directed differentiation. Recently, our lab developed OPTi-OX (optimised inducible overexpression system), which has overcome gene silencing of transgenes in hiPSCs by targeting an efficient overexpression system into two separate genomic safe-harbour sites. Using OPTi-OX, we overexpressed the transcription factor NGN2 to generate iNeurons and the transcription factors NFIA, NFIB and SOX9 to generate iAstrocyes. This results in the generation of cells resembling mature neurons with strong expression of pan-neuronal and glutamatergic markers and in a high proportion of cells with a strong expression of astrocytic markers such as GFAP and S100B, after 1 week of induction. In addition, the iNeurons demonstrated spontaneous electrophysiological activity within two weeks' post-induction. This activity was blocked with tetrodotoxin, confirming that the recorded activity relies on the activity of voltage-gated sodium channels. By around day 20, iNeurons developed synchronous burst firing patterns across multiple electrodes, which demonstrate that iNeurons develop functional networks within three weeks. Thus, these cells can be used as an in vitro model to study neuronal networks. Ongoing work are aimed at evaluating the functionality of our iAstrocytes. One of our future goals with the present work is to develop an entirely human in-vitro co-culture system for the study of neurons and astrocytes and potentially a simplistic and powerful platform for disease modelling and drug discovery.

74 Interneuron deficits in Down Syndrome

Bhattacharyya A, Giffin-Rao Y, Tao Y, Medo M, Strand B, Dantinne S, Keshan A, Daley R, Levesque B, Amundson L, Huang L, Reese R, Zhang SC

University of Wisconsin, USA

Keywords: interneuron, Down syndrome, iPSCs, patterning, WNT signaling

Many human neuropsychiatric disorders and disorders characterized by intellectual disability are linked to defects in cortical interneuron development and function. Maldevelopment of interneurons can lead to abnormal numbers, subtypes and/or placement of neurons that significantly affect the functioning of the cortex, leading to cognitive impairment. Understanding how mistakes in interneuron development result in cortical dysfunction provides opportunities to identify novel targets and develop novel therapies for human disorders. Down syndrome (DS, trisomy 21, Ts21) is a complex human disorder and the most common genetic cause of intellectual disability. Surprisingly little is known about the underlying mechanisms that lead to the characteristic cognitive impairment in DS, in part because phenotypes in mouse models of DS have not been validated in humans or are contradictory to human data. Neuropathology of the DS cerebral cortex includes fewer interneurons in upper cortical layers. Using Ts21 iPSCs to model cortical interneuron development in DS, we investigate missteps in their development that may lead to lack of interneurons in the adult DS brain. Importantly, we have established isogenic pairs of Ts21 and control iPSCs to distinguish phenotypes that are due to trisomy 21 rather than human variation. Our results show that Ts21 interneuron progenitors have defects in patterning that alters interneuron subpopulations. These results link to transcriptomic data indicating that Ts21 iPSC-derived interneuron progenitors have altered WNT signaling. Taken together, these data point to patterning defects in DS neural development that lead to altered interneuron generation. Understanding how mistakes in interneuron development in DS result in cognitive disability may enable us to intelligently design therapies to positively impact individuals and families living with DS.

75 Effective improvement of Spinal Muscular Atrophy with Respiratory Distress Type 1 in mice, by transplantation of a specific iPSC-derived neural stem cell subpopulation

Bono S, Forotti G, Nizzardo M, Bucchia M, Bresolin N, Comi G, Corti S

University of Milan, Italy

Keywords: SMARD1, iPSCs-derived neural stem cells, NSC transplantation

Spinal muscular atrophy with respiratory distress type I (SMARD1) is a severe infantile autosomal recessive disease caused by mutations in the IGHMBP2 gene and characterized by dysfunction and progressive loss of α-motor neurons, leading to muscle atrophy in the distal limbs and respiratory complications1. Currently, there are no effective therapies able to cure or even prolong SMARD1 patients' lives. A viable therapeutic strategy could be the transplantation of neural stem cells (NSCs) derived from induced pluripotent stem cells (iPSCs), that ameliorate the phenotype of a SMARD1 mouse model (Nmd2J mouse2), by protecting their endogenous motor neurons (MNs)3. We leveraged the technologies previously employed in an ALS4 to assess the therapeutic potential of the specific NSC subpopulation expressing LewisX (LeX), CXCR4 and β1-integrin in the context of SMARD1. Wild-type iPSCs have been differentiated into NSCs and selected for the expression of the specific stem cell markers LeX, CXCR4, and β1-integrin, essential for cell adhesion and migration. To investigate their therapeutic potential, we intrathecally transplanted the NSC selected subpopulation into Nmd2J mice at postnatal day 1 and we found that after injection LeX+CXCR4+β1+ NSCs properly migrated from the central nervous system and engrafted in the spinal cord parenchyma, particularly in the anterior horns. Treated Nmd2J mice exhibited a significant improvement of neurological phenotype and increased survival. In addiction, these NSCs protected SMARD1 endogenous MNs from degeneration and, besides exerted a positive effect on the cell soma, they also had a beneficial effect at the periphery, promoting neural muscular junction maintenance and collateral axonal sprouting. Moreover, LeX+CXCR4+β1+ NSC treatment preserved muscle fiber morphology and organization and reduced its replacement by adipose tissue. Overall, our results demonstrate that transplantation of a specific NSC subpopulation, selected to enhance cell migration, survival and engraftment, lead to a significant level of rescue in treated SMARD1 animals, supporting NSC-mediated therapy as a promising potential treatment option for SMARD1.

76 Developmental emergence of adult SVZ neural stem cells as revealed by single cell transcriptional profiling

Borrett MJ, Yuzwa SA, Innes BT, Voronova A, Ketela T, Storer M, Kaplan DR, Bader GD, Miller FD

University of Toronto, Canada

Keywords: Neural Stem Cells, Cortex, single cell RNA-Seq

Radial glial cortical precursors, also known as radial precursors (RPs), are tasked with producing the major cell types of the cerebral cortex as well as establishing a population of adult neural stem cells (NSCs) that reside in the subventricular zone (SVZ) of the lateral ventricles in the mature brain. Although a significant portion of adult neural stem cells is known to derive from embryonic RPs, the manner in which this occurs remains poorly understood. Here, we have employed a droplet-based microfluidic single cell transcriptomic technology combined with a lineage tracing approach to further characterize the transcriptional identity of cortical RPs during embryogenesis when they produce primarily excitatory neurons and during postnatal life when they generate inhibitory neurons, glia and adult neural stem cells. This analysis shows that RPs become fully established in the developing cortex by E13.5 and express a transcriptional identity that becomes upregulated throughout the course of neurogenesis. We have identified novel marker genes that define this core identity and show that the strengthening of this identity coincides with a transition from a proliferative state to a non-proliferative state near the end of neurogenesis by E17.5. Comparative analysis shows that this transcriptional identity is maintained in slowly-proliferating postnatal SVZ NSCs, and in adult quiescent NSCs in the mature SVZ. Together, these findings support a model where a population of embryonic cortical RPs expressing a defined core transcriptional identity persists into the postnatal brain and maintains expression of this transcriptional identity through to adulthood as a quiescent adult NSC population.

77 Glypican4 as a target for innovative cell based replacement therapy in Parkinson's Disease

Corti S, Rattier D, Sorce M, Salin P, Melon C, TosoE , Kyba M, Kerkerian - Le Goff L, MainaF, Dono R

Developmental Biology Institute of Marseille, France

Keywords: Differentiation, Tumorigenesis, Parkinson's Disease

Human induced pluripotent stem cells (hiPSCs) are a major breakthrough for biomedical science. One of their most promising applications is Parkinson's Disease (PD) therapy with the aim is to restore the dopamine (DA) deficit through transplantation of hiPSCs-derived ventral midbrain (Vm) DA neurons. However, hiPSCs clinical application still requires overcoming two major issues: their intrinsic ability to generate tumors in vivo and their low differentiation efficiency. In embryos, the signalling proteins SHH, WNT and FGF8 act in a temporal/spatial/concentration manner to guide VmDA neuron development and prevent differentiation of other cells. These findings have let to an extensive research of the medium formulation triggering the highest yield of VmDA progenitors in vitro. We are developing an unconventional approach. Our challenge is to confer to hiPSCs the propensity to become VmDA neurons at the expense of other cell types. This can be achieved through downregulation of Glypican4 (GPC4) in hiPSCs, a SHH, WNT, FGF regulator. Although GPC4mutant (GPC4mut) cells maintain self-renewal/pluripotency in stemness conditions, they undergo: 1) efficient commitment into VmDA fate at the expense of self-renewal and serotonergic identity in differentiation conditions, 2) a 3-fold VmDA neuron increase in comparison to control cells. With transplantation experiments in rat brains we have also tested the tendency of GPC4mut hiPSCs for VmDA neuron differentiation in vivo. Cells were transplanted at neural committed stage prior to expression of Vm markers. Preliminary analyses of dissected brains at two-month post-transplantation show that GPC4mut cell grafts become enriched into Foxa2+/Lmx1a+ VmDA precursors. Interestingly, tumour growth is impaired in GPC4mut hiPSCs as shown by flank xenografts in nude mice. Thus, we anticipate that GPC4 downregulation is a promising strategy for hiPSCs-based PD therapy as it enables enhanced VmDA neuron generation and reduced tumorigenesis.

78 A quick and efficient method to differentiate iPSC-astrocytes for the analysis of APOE gene effects in Alzheimer's disease

de Leeuw S, Schoeffmann S, Wanner D, Nitsch R M, Tackenberg C

Institute for Regenerative Medicine UZH, Switzerland

Keywords: Alzheimer's disease, iPSC-derived astrocytes

Alzheimer's disease (AD) is the most common neurodegenerative disease, affecting memory and cognitive functioning. Sporadic AD (SAD) is the major form, with the APOE4 allele as strongest genetic risk factor. ApoE is mainly expressed by astrocytes in the brain, which play a prominent role in AD, through an exacerbated inflammatory response, lack of synaptic support, or aberrant Aβ-peptide metabolism. Even though the role of APOE4 as major genetic risk factor has been confirmed, the molecular pathogenic contributions of APOE4 in AD and its function in human astrocytes are unclear. To investigate the role of APOE4 in astrocytes, with a physiologically and genetically relevant background, we will use human isogenic ApoE2/2, ApoE3/3 and ApoE4/4 iPSC lines, differentiated to astrocytes (hiPSCAs). Conventional astrocyte differentiation methods are elaborate and laborious; therefore, we have adapted a recently established protocol to successfully direct iPSCs to a neural progenitor stage, and subsequently deriving functional astrocytes within 30 days. This protocol does not require forced genetic overexpression, complicated combinations of small molecules, or FACS sorting. NPC populations were characterized as CD184+/CD133+/CD271- using flow cytometry, confirm homogeneity of the cultures. After astrocyte differentiation, cell phenotype was assessed by positivity for GFAP/S100β/GLAST-1 using flow cytometry and immunocytochemical analysis. Astrocyte functionality was confirmed by the ability of cells to take up glutamate, and RNA expression levels of astrocytic genes by qPCR. In conclusion, this differentiation technique yields functional astrocytes in 30 days, which will allow us to characterize cell specific pathomechanisms involving APOE4 relevant to AD.

79 The role of elevated FOXG1 in glioblastoma stem cells

Ferguson KM, Bulstrode HJ, Marques-Torrejon MA, Gangoso E, Dewari P, von Kriegsheim A, Bressan RB, Garcia-Diaz C, Blin C, Grant V, Pollard SM

MRC Centre for Regenerative Medicine
University of Edinburgh, UK

Keywords: RFOXG1; neural stem cells; glioblastoma stem cells

Glioblastoma multiforme (GBM) is the most common and aggressive primary brain cancer in adults. Relapse after conventional chemo/radiotherapy is universal, thought to be driven by glioblastoma stem cells (GSCs) with neural stem (NS) cell characteristics. GSCs consistently overexpress many key neurodevelopmental transcription factors, including FOXG1 – a key forebrain transcription factor with reprogramming activity. Our recent work shows that increased expression of FOXG1 restricts astrocyte differentiation and can trigger de-differentiation to a highly proliferative NS cell state. However, transcription factors are difficult to 'drug'. Our goal is to elucidate how elevated FOXG1 functions through its transcriptional targets and protein partners to fuel the unconstrained self-renewal of GSCs. To identify the downstream transcriptional targets of FOXG1, changes in mRNA expression were monitored by mRNA-seq and qRT-PCR during FOXG1-induced de-differentiation of NS cell-derived astrocytes. Transcriptional targets of FOXG1 identified included many key cell cycle and epigenetic regulators (e.g. Foxo3, Foxo6, Mycn, Tet3). Foxo6 was further validated as a clear downstream transcriptional target of FOXG1. To identify FOXG1's protein partners, FOXG1 was epitope-tagged in human glioblastoma-derived NS (GNS) cells using CRISPR-Cas9 technology. Immunoprecipitation coupled to mass spectrometry (IP-MS) was used to reveal potential FOXG1-interacting proteins. Here, we present recent progress further defining the key transcriptional targets and protein partners of FOXG1. Further understanding the mechanism of FOXG1 function will open up new opportunities for finding dependencies of GSCs and hence new targets of therapeutic value.

80 Functional heterogeneity of oligodendrocytes in the central nervous system

Förster S, Floriddia EM, Crawford AH, van Bruggen D, Tripathi RB, Richardson WD, Castelo-Branco G, Franklin RJM

University of Cambridge, UK

Keywords: Heterogeneity, Oligodendrocyte, Locomotor

In the mouse embryonic forebrain, oligodendrocyte progenitor cells (OPCs) are generated in consecutive waves from distinct brain regions along a spatiotemporal gradient, with ventral OPCs emerging before dorsal OPCs. The developmentally distinct OPCs, and their progeny, persist in the brain throughout life. Additionally, the oligodendrocyte lineage is transcriptionally heterogeneous, forming twelve distinct subpopulations, raising the question whether oligodendrocyte lineage cells are functionally heterogeneous based on their developmental origin. Transcriptional profile analysis by Chromium Single-Cell (sc) RNA-Sequencing (10X Genomics) revealed a heterogeneity of oligodendrocyte (OL) subpopulations. Whilst some OL subpopulations are of mixed developmental origin, others are exclusively formed by either ventrally or dorsally derived OLs. To investigate whether ventrally and dorsally derived OLs fulfil distinct functions in the adult brain, dorsally derived OPCs were ablated using a Sox10-driven diphtheria toxin A (DTA) mouse model. scRNA-sequencing of the OL populations after ablation revealed that ventral OPCs are not capable of forming the exclusively dorsally-derived OL subpopulation of the control animals. In compensation, newly-formed subpopulations appear, resulting in comparable OL numbers between control and ablated animals. As dorsally derived oligodendrocyte lineage cells mainly populate the cortex, motor functional coordination abilities after the ablation of dorsally derived OPCs were assessed. Mice ablated of dorsally derived OPCs presented an impaired locomotor coordination, while general vigilance, gait, balance and sensation are comparable to control groups. The locomotor coordination disabilities are a result of alterations in brain, not spinal cord homeostasis, as only a very minor number of oligodendrocyte lineage cells are affected by the ablation model in the spinal cord. Furthermore, no signs of neuronal cell death or chronic inflammatory response was detected in response to the ablation. In conclusion, our results unveil the influence of the developmental origin of OPC on their differentiation potential and demonstrate a functional heterogeneity of oligodendrocyte subpopulations in homeostatic brain function.

81 Neuroligin-3 and Neuroligin-4X Promote Nanoscopic Growth Cone Actin Reorganization in Early Neurodevelopment with Implications for Autism Spectrum Disorder

Gatford NJF, Michael Deans PJ, Duarte RRR, Chennell G, Raval P, Tegtmeyer M, Creeney, Price J, Gordon-Weeks P, Srivastava DP

King's College London, UK

Keywords: Autism Spectrum Disorder, super-resolution, neurites, growth cones

Background: Autism spectrum disorders (ASD) are heterogenous neurodevelopmental disorders exhibiting complex genetic and neurobiological aetiology. Despite this complexity, genetic risk-factors often converge to influence the development of neuronal morphology e.g. neuritogenesis. Cell adhesion proteins are key regulators of neuritogenesis, particularly at the growth cone leading edge. Gene mutations in the neurexin-neuroligin cell adhesion complex are frequently associated with ASD pathogenesis, particularly, in neuroligin-3 and neuroligin-4X (NLGN3/4X). However, the role these proteins and their ASD-associated counterparts play during neuritogenesis remains enigmatic. Methods: NLGN3/4X wild-type (NLGN3/4X-WT) or NLGN3-R451C/NLGN4X-D396 mutant constructs were overexpressed in immature neurons derived from human neural progenitor cells. Neuritogenesis, molecular nanodomains, and growth cone actin organisation were examined by confocal and super-resolution microscopy. Molecular mechanisms were revealed by immunoblotting, pharmacological intervention, and confocal/super-resolution microscopy. Results Here we show novel roles for NLGN3/4X in neuritogenesis and growth cone structure in early human neurodevelopment. NLGN3/4X-WT was shown to significantly promote neuritogenesis in immature human neurons while the ASD-associated mutant variants did not. Super-resolution microscopy revealed NLGN3/4X-WT cluster at the leading edge of growth cones in nanodomains. Significant differences in growth cone enlargement and actin filament organization were also observed between NLGN3/4X-WT and their ASD-associated counterparts. Immunoblotting revealed actin regulators, such as p21-activated kinase 1 (PAK1) and cofilin, were involved in the molecular mechanism. Pharmacological manipulation of PAK1 combined with NLGN3/4X-WT overexpression attenuated all previously observed cellular phenotypes. Conclusions: NLGN3/4X nanodomains are required for growth cone enlargement and neuritogenesis in human neurodevelopment. These novel roles may have implications for early ASD pathogenesis.

82 Thalamic organoids from mESCs

Griffiths RS, Chan WK, Barbato M, Price DJ, Mason JO

University of Edinburgh, UK

Keywords: Stem cell differentiation, CRISPR, Thalamus, Autism spectrum disorder

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder affecting 1.1% of the UK population. It is characterized by impaired social interaction and behavioural inflexibility. Despite the thalamus being the 'gateway' for sensory and motor information to the cortex, its possible role in ASD is not well understood. We aim to develop an in vitro model of thalamus development to aid investigation of ASD. There is currently only one published protocol generating thalamic neurons (Shiraishi et al., 2017). This protocol differentiates mESC towards a thalamic fate using an organoid model. However only a small percentage of cells within the organoid express the thalamic marker Gbx2 and signaling molecules used are not consistent with the main pathways involved in embryonic thalamus development. We aim to make a more efficient and accurate model of thalamic differentiation by using two different methods. Firstly, by manipulating key signaling pathways involved in thalamus development (Wnt and Shh) we aim to recapitulate normal thalamus development. Secondly, by transient CRISPR activation (CRISPRa) of transcriptional regulators of thalamic genes with the aim of directing ESCs towards a thalamic fate. This study will provide insights into normal development of the thalamus along with providing an invaluable model for studying neurodevelopmental disorders such as ASD.

83 ALS associated FUS mutation results in impaired acetylcholine receptor clustering in hiPSC-derived motor unit components

Higelin J, Demestre M, Boeckers T

Institute for Anatomy and Cell Biology, Germany

Keywords: ALS, AchR, neuromuscular junction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the selective death of upper and lower motoneurons (MN), which leads to muscle denervation and death by respiratory failure 1. Mutations within FUS (Fused in Sarcoma) are known to be associated with the disease. FUS codes for a multifunctional RNA/DNA-binding protein and is involved in cellular processes such as splicing, translation, mRNA transport and DNA damage response 2–6. It could be shown that FUS is also present at synaptic sites (Aoki et al., 2012; Belly et al., 2010; Schoen et al., 2016). In FUS-ALS patients, the protein is partially or totally excluded from the nucleus, forming cytoplasmic inclusions in neurons of the brain and spinal cord 10–12. In addition to MNs, the pathophysiology in ALS may also involve the development or function of skeletal muscle in patients. During development MNs are potent inducers of acetylcholine receptors (AchR) clustering to form mature neuromuscular junctions (NMJs). Here, we hypothesised that ALS related FUS mutations affect AchR clustering in vitro. To that end, we examined peripheral synaptic contacts of motor units in neuron-muscle co-cultures derived from human induced pluripotent stem cell (hiPSC) lines from control and FUS-ALS patient. MNs expressing mutated protein variant were less potent in inducing mature AchR clusters on myotubes from healthy control. In FUS-ALS derived muscle cells, cultured with healthy MNs or without neuronal part, AChR clustering was even more impaired, indicated by higher proportions of immature AChR clusters. When FUS mutated MNs and mutant myotubes were co-cultured, as in a patient context, only immature or aberrant AchR clusters were seen. As a conclusion, the study provide evidence that ALS associated FUS mutations impair maturation of NMJs in single cultured myotubes, as well as when co-cultured with diseased MNs.

84 Imaging Remyelination in the CNS

Hill MFE, Guy J, Wright A, Neves A, Brindle K, Franklin RJM

University of Cambridge, UK

Keywords: Oligodendrocyte Precursor Cells (OPC), Myelin, Magnetic Resonance Imaging (MRI)

Demyelination is seen in many common neurological diseases including multiple sclerosis. Although usually robust, where the regenerative response fails, chronic denudation of the axon leads to axonal loss and neurological impairment. There is therefore a need for therapies that enhance remyelination, and outcome measures to assess their efficacy. Currently there are no non-invasive methods for specifically assessing remyelination. Here we present evidence of a novel non-invasive technique for imaging remyelination in the central nervous system using T1 weighted Magnetic Resonance Imaging and Magnetic Resonance Microscopy. Using a lentiviral system to infect progenitor cells, we are able to demonstrate T1 weighted contrast enhancement of cells expressing an organic anionic transporter channel (OATP), which takes up gadolinium contrast agent. We are able to control the expression of this transporter under myelination specific promoters, thus making contrast agent uptake specific to myelin synthesis by OPCs which have differentiated into myelinating oligodendrocytes. We demonstrate OATP expression in vitro and ex vivo; showing expression in both endogenously infected cells, and in transplanted cells. By applying this technique to models of demyelination we aim to develop a non-invasive method for imaging remyelination, applicable as an outcome measure, for both the development of transplant, and pharmacological therapies.

85 Epigenetic control of neurodevelopmental gene regulatory networks linked to neurodegeneration

Hnatova S, Paonessa F, Livesey FJ

Gurdon Institute, UK

Keywords: neurodegeneration, epigenetics, gene regulatory networks, neural development

Autosomal dominant mutations linked to familial forms of Alzheimer's disease (AD) and frontotemporal dementia (FTD) are expressed during neuronal development and may lead to changes in cell state. We observe cellular changes related to the processing of key proteins involved in AD and FTD during neuronal development of human induced pluripotent stem cell-derived (iPSC) cortical neurons from patients with familial dementia due to changes in gene dosage of APP and MAPT. The cellular changes may lead to coping strategies that would be reflected in gene regulatory networks (GRNs). We aim to identify pathways linked to autosomal dominant mutations underlying adult-onset dementia by investigating mechanisms of GRN control, with a specific interest in the role of small RNAs as fine-tuners of the networks. In order to investigate candidate GRNs linked to dementia, we are using in vitro iPSC-derived neurons and in vivo fetal and post-mortem cortical tissue from familial dementia and control patients. The GRNs are identified by whole genome bisulfite sequencing, total RNA-Seq and small RNA sequencing and subsequently computationally modelled. Preliminary small RNA sequencing data suggest differential expression of several miRNAs in the developing FTD compared to control neurons. These miRNAs target proteins relevant to the disease. Combining existing data from in vivo sporadic and in vitro familial AD neurons suggests shared differentially expressed genes, that partially overlap with the differentially expressed miRNA candidates. Preliminary data suggest that miRNAs differentially expressed in developing FTD neurons and their target genes differentially expressed in AD neurons may be part of GRNs linked to dementia. Understanding the early neurodevelopmental networks in familial dementia may shed light on the role of network regulation in degeneration decades later.

86 Novel approach to model Non-Alcoholic Fatty Liver Disease using human Induced Pluripotent Stem Cells

Morell CM, Tilson SG, Perrin M, Gieseck RL, Geti I, Rimland CA, Sampaziotis F, Vallier L

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: hIPSC, disease modeling, NAFLD

The incidence of Non-Alcoholic Fatty Liver Disease (NAFLD) has strongly increased in recent years, while therapeutic interventions remain limited to liver transplant for end stage disease. NAFLD pathogenesis remains elusive due to the lack of a suitable tool to faithfully recapitulate the human pathology. Indeed, growing primary hepatic cells remains difficult while currently available culture systems do not capture tissue complexity. human Induced Pluripotent Stem Cells (hIPSC) provide an advantageous opportunity, since they retain the ability to self-renew and differentiate into liver cells1. We aimed to take advantage of this unique system by developing an in vitro platform for NAFLD modeling that mimics the hepatic architecture by reproducing its multicellularity. hIPSC-derived hepatocytes, cholangiocytes, macrophages and stellate LX2 cells were co-cultured in a 3D system to recreate the hepatic microenvironment. Hepatocyte like cells (HLC) retained the expression of typical hepatocyte markers and functions, and their maturation improved with time in culture. Cholangiocytes preserved their biliary identity, while LX2 cells did not show an activated phenotype. Similarly, macrophage polarization was not influenced by the 3D environment. Interestingly, HLC spontaneously arranged around biliary structures without losing their functionality, while LX2 established contacts with HLC only after TGFβ treatment. Fatty acids treatment led to steatosis and lipotoxicity in HLC, and also interfered with CYP3A4 activity. The presence of non-parenchymal cells sustained fatty acid-induced hepatocyte damage. In conclusion, various hepatic cell types can be co-cultured in our novel 3D platform to model pathophysiological cellular interactions. We were able to mimic the mechanisms of hepatocytes steatosis and lipotoxicity, including the inflammatory and fibrotic response associated with NAFLD progression. Thus, our system represents an important step forward to model NAFLD in vitro.

87 Development of a 3D Bone Marrow Model to Investigate Leukaemic Stem Cell Invasion, Remodelling and Potential Combinational Chemotherapy

Busch C, Tuck B, Dalby M J, Busby G, Wheadon H, Berry CC

University of Glasgow, UK

Keywords: 3D model, bone marrow niche, leukaemia

Chronic myeloid leukaemia (CML) is characterised by the excessive proliferation of leukaemic stem cells (LSCs) in the bone marrow (BM). LSCs achieve this via modification of the BM microenvironment (niche) to their advantage, whilst impairing normal haematopoiesis. To date it has proved difficult to both understand how LSCs dominate and alter the niche and to target LSCs with current therapies. In the healthy BM, mesenchymal stem cells (MSCs) and haematopoietic stem cells (HSCs) reside together in the niche, where they interact closely, maintaining their stem cell properties via self-renewal. Most in vitro systems representing the BM niche are basic, relying on 2D models consisting of a stromal monolayer in co-culture with HSCs. However, such systems overlook many niche factors, including the BM 3D architecture. A 3D culture system provides a more realistic reflection of the BM microenvironment in vitro and can better predict in vivo responses of chemotherapy in disease modelling. We have established a 3D BM nice model, comprises MSC spheroids embedded in medical-grade collagen type I, mimicking the BM biological and mechanical microenvironment1,2. We have extended this model to include HSCs and aim to use our model to study MSC-LSC interactions within a niche-like environment and LSC-mediated remodelling. Initially, we investigated LSC invasion using a collagen type I coated Transwell/MSC culture, demonstrating migration into the collagen network and homing to the MSCs beneath. Subsequently, we introduced the LSCs to our 3D BM model and noted migration into the gel scaffold. Ultimately, we will use our MSC/LSC co-culture model to assess potential small molecules and drug treatments in combination, such as the bone morphogenic protein (BMP) ligands 2 and 4, which are abnormally abundant in the BM microenvironment of CML patients compared to normal donors. Chronic myeloid leukaemia (CML) is characterised by the excessive proliferation of leukaemic stem cells (LSCs) in the bone marrow (BM). LSCs achieve this via modification of the BM microenvironment (niche) to their advantage, whilst impairing normal haematopoiesis. To date it has proved difficult to both understand how LSCs dominate and alter the niche and to target LSCs with current therapies. In the healthy BM, mesenchymal stem cells (MSCs) and haematopoietic stem cells (HSCs) reside together in the niche, where they interact closely, maintaining their stem cell properties via self-renewal. Most in vitro systems representing the BM niche are basic, relying on 2D models consisting of a stromal monolayer in co-culture with HSCs. However, such systems overlook many niche factors, including the BM 3D architecture. A 3D culture system provides a more realistic reflection of the BM microenvironment in vitro and can better predict in vivo responses of chemotherapy in disease modelling. We have established a 3D BM nice model, comprises MSC spheroids embedded in medical-grade collagen type I, mimicking the BM biological and mechanical microenvironment1,2. We have extended this model to include HSCs and aim to use our model to study MSC-LSC interactions within a niche-like environment and LSC-mediated remodelling. Initially, we investigated LSC invasion using a collagen type I coated Transwell/MSC culture, demonstrating migration into the collagen network and homing to the MSCs beneath. Subsequently, we introduced the LSCs to our 3D BM model and noted migration into the gel scaffold. Ultimately, we will use our MSC/LSC co-culture model to assess potential small molecules and drug treatments in combination, such as the bone morphogenic protein (BMP) ligands 2 and 4, which are abnormally abundant in the BM microenvironment of CML patients compared to normal donors.

88 Unravelling epigenetics in the implanting primate embryo: SHOT-seq for spatially defined transcriptome and methylome analysis

Bergmann S, Madrigal P, Clark S, Reik W, Boroviak T

University of Cambridge, UK

Keywords: Nonhuman primate development, DNA methylation, Implantation

DNA methylation is one of the predominant epigenetic modifications in mammals with crucial functions in gene expression regulation, X-inactivation or genomic imprinting. The most dramatic changes in DNA methylation occur during preimplantation development, when the embryo undergoes epigenetic resetting and establishes a globally hypomethylated state. This permissive epigenetic environment subsequently allows progressive lineage-specific acquisition of methylation marks during germ layer specification, thus enabling maintenance of cellular identity and genomic integrity 3-6. Human embryos complete demethylation by the 2-cell stage and sustain this hypomethylated status throughout preimplantation development 3,7. Unfortunately, the dynamics of DNA re-methylation in our own species have remained enigmatic, due to the inaccessibility of early human postimplantation stages. In this project, we aim to delineate acquisition of DNA-methylation using the marmoset as a model for primate embryogenesis 1. We have developed a new protocol for Spatial metHylOme and Transcriptome sequencing (SHOT-seq) in the implanting primate embryo. SHOT-seq combines laser capture microdissection with joint single-cell methylome and transcriptome profiling 2. Carnegie stage 5 (postimplantation embryo prior to gastrulation) and 6 (early gastrulating embryo) marmoset embryos are cryosectioned and subjected to SHOT-seq followed by 4-colour immunofluorescence labelling. We are currently working on an image reconstruction pipeline to render high-resolution, 3D models of the early postimplantation primate embryo. Importantly, the finalised SHOT-seq protocol will preserve the spatial identity of each multi-omics sample within a reconstructed embryo. Profiling of the implanting primate embryo with SHOT-seq will be a powerful approach to faithfully track the acquisition of lineage-specific methylation patterns and to identify regulatory associations between transcriptome and methylome.

89 Molecular mechanisms underlying human pluripotent stem cell forward programming to megakaryocytes: from biology to transfusion medicine

Moreau T, Lawrence M, Jimenez Gomez P, Baye J, Dalby A, Mueller A, Evans A, Wernisch L, Reid J, Gottgens B, Ghevaert C

University of Cambridge, UK

Keywords: Pluripotent Stem Cells, Platelets, Transfusion Medicine

We have published a novel method for the large-scale in vitro generation of megakaryocytes (MKs), the blood platelet precursors, by applying a transcription factor (TF) driven forward programming strategy to human pluripotent stem cells (hPSCs). We have demonstrated that the concurrent expression of GATA1, FLI1 and TAL1 in hPSCs and chemically defined culture conditions with minimal supportive cytokines produce highly pure MK cultures with long-term growth and release of functional platelets. Unravelling the molecular mechanisms underlying MK forward programming will bring biological insights that shall lead to improvements of the MK programming technology. Particularly, we have been focusing on the characterisation of the MK progenitor that allows long-term expansion of pure MK cultures, which is a key asset of the method. Using single cell RNA sequencing of long-term cultures, we have started to identify and functionally confirmed surface markers of the MK progenitor. Particularly, we demonstrated that MICB (MHC Class I Polypeptide-Related Sequence B) allowed a 5-fold MK progenitor enrichment from whole cultures (1% vs. 0.2% clonogenicity). In addition, we have developed bespoke rainbow-reporter lentiviral vectors allowing live monitoring for expression of the three transgenes through the course of MK programming. By coupling index flow cytometry sorting with single cell colony assays on the early days of programming, we can assess the optimal TF combination and dose leading to the generation of the MK progenitor. Interestingly, we showed at day 10 that the cells with the highest FLI1-transgene expression comprise >80% of the MK progenitors and allowed a 10-fold enrichment from the MK committed CD41-positive cell population (18.8% vs. 1.9% clonogenicity). Our growing capacity to select for MK progenitors through the MK forward programming stages is key to succeed in their molecular characterisation. This deeper biological understanding of the MK progenitor's molecular identity and route of programming will guide enhancements of the initial and long-term culture conditions to achieve the best MK quality and yield, which will ultimately benefit in vitro platelet manufacture for transfusion.

90 Developing an hiPSC-derived 3D in vitro model of the blood-brain barrier to study COL4A1/2 cerebral small vessel disease

Goodwin M, Granata A, Davaapil H, Van Agtmael T, Sinha S, Markus H

University of Cambridge, UK

Keywords: Cerebral small vessel disease; COL4A1/2; blood-brain barrier

Cerebral Small Vessel Disease (SVD), which affects the small blood vessels in the brain, is a major contributor to stroke and dementia worldwide (Pantoni, 2010). Despite this, little is known about the mechanisms that cause or propagate the disease, but the blood-brain barrier (BBB) is thought to be affected. Gaining a better understanding of the underlying mechanisms could enable the development of new treatments. To address this, we are focusing on monogenic Collagen IV alpha 1 and 2 (COL4A1/2) mutations to model SVD in vitro. We hypothesise that COL4A1/2 SVD pathophysiology is caused by neurovascular unit (NVU) dysfunction, which leads to increased permeability of the BBB. Cells of the NVU include the brain microvascular endothelial cells (BMECs) of the small vessels, the surrounding mural cells (smooth muscle cells (SMCs)/pericytes) and astrocytes. These cells are embedded in the extracellular matrix (ECM) and collectively form the BBB, serving to restrict unwanted factors from entering the brain. To investigate our hypothesis, we are developing a 3D triple co-culture in vitro model of the BBB to study monogenic COL4A1/2 SVD, using wild type control (WT) and COL4A2G702D (Murray et al., 2014) patient-derived human induced pluripotent stem cells (hiPSCs). We have successfully established in the lab, differentiation of hiPSCs into the three cell types of the NVU, including BMECs, SMCs/pericytes and astrocytes, using previously published protocols (Hollmann et al., 2017; Cheung et al., 2014; Shaltouki et al., 2013). Ultimately, the 3D triple co-culture model will offer a platform to assess the functional capacity of the control and diseased cell types, enabling us to elucidate the disease-causing mechanism in COL4A1/2 SVD.

91 Geometrically defined 2D substrates and 3D scaffolds differentially affect YAP-localization and cell division rate in mouse embryonic stem cells

Bertels S, Jaggy M, Richter B, Scheiwe A C, Thiel M, Wegener M, Greiner A M, Autenrieth T J, Bastmeyer M

Karlsruhe Institute of Technology, Germany

Keywords: Embryonic stem cells, 3D scaffolds, YAP

Pluripotency is a delicate balance of simultaneously expressed lineage specifiers [1]. In culture this balance is mainly supported by adding biochemical factors. Interestingly, the mechanotransducer yes-associated protein (YAP) is highly expressed in mouse embryonic stem cells (mESCs) [2] and tightly linked to the pluripotency machinery [3], suggesting that biophysical cues might also influence mESC behavior. Routinely, mESCs are cultured on 2D surfaces whereas in the blastocyst they experience a complex 3D environment. Here, we asked whether single mESC behavior differs between 2D and 3D cultivation under geometrically defined conditions. Therefore we produced 2D micro-islands by micro-contact printing and 3D micro-wells by direct laser writing [4]. The structures had a defined geometry with varying size (15 x 15 up to 35 x 35 µm base area, and 20 µm high walls for 3D) and were fibronectin-coated. We found that the cell division rate (CDR) of mESCs increased with increasing base area for 2D culture whereas in 3D the highest CDR was observed in the smallest micro-wells. Interestingly, we found that during the first hours of cultivation most mESCs in small micro-wells were attached to the four walls but not to the base of the wells. They exhibited a cross-shaped morphology with prominent actin arcs which was also adopted by the nucleus. Inhibitors for actomyosin contractility abrogated the cross-shaped morphology and decreased the CDR to levels comparable to the large micro-wells. This indicates that intracellular contractility plays a prominent role, which is supported by the finding that mESCs in small micro-wells had a significantly higher amount of YAP in the nucleus than mESCs in large micro-wells. Again, this effect was abrogated by actomyosin inhibitors. Thus, we demonstrate that mESC behavior differs between 2D and 3D cultivation. In addition, our findings indicate that the CDR is influenced by a confined environment and intracellular contractility.

92 A Feeder-Independent Culture System to Convert and Maintain Human Pluripotent Stem Cells in a Naïve-Like State

Hunter AL, Snyder KA, Thomas TE, Eaves AC, Louis SA, Lee VM

STEMCELL Technologies, UK

Keywords: Pluripotent, Naive, feeder-independent

Human pluripotent stem cells (hPSCs) within the developing embryo progress through a spectrum of pluripotent states as they transition from naïve to a more lineage-restricted, primed state. Capturing these states in vitro requires specialized culture media and protocols. To date, conditions that maintain hPSCs in naïve-like states have depended on the use of feeder cells for robust long-term expansion. RSeT™ Feeder-Free (RSeT™-FF) is a defined medium that supports the reversion of primed hPSCs to a naïve-like state and supports their long-term feeder-independent maintenance. To revert primed hPSCs cultured in mTeSR™1 or TeSR™-E8™, colonies were seeded as small clumps on Corning Matrigel® coated plates. After 24 hours, the medium was changed to RSeT™-FF supplemented with 0.2% Matrigel® and hPSCs were cultured for 5 days with medium changes every other day. hPSCs maintained in RSeT™-FF + 0.2% Matrigel were dissociated to single cells and re-seeded at a density of 2.1 × 104 cells/cm2 on Matrigel®-coated plates every 4-6 days. Colony morphology and cell expansion was assessed at each passage (P). Transition to a naïve-like colony morphology was observed in cultures as early as P1, with colonies possessing a highly homogeneous naïve-like morphology with >90% of colonies displaying a domed morphology, and extremely low levels of background differentiation (n=5 hPSC lines). On average, hPSCs cultured in RSeT™-FF expand by 3.0 ± 0.9-fold per passage (n=4); comparable to hPSCs cultured in RSeT™ Medium on feeders. Cells from naïve-like colonies (>P5) expressed markers associated with undifferentiated hPSC such as OCT4, SSEA4, TRA-1-60 and ALP. Importantly, as in RSeT™ Medium on feeders, naïve-like hPSCs cultured in RSeT™-FF Medium showed upregulation of genes commonly enriched in the naïve-state (KLF2, KLF4, KLF17, TFCP2L1, STELLA, and DNMT3L1) compared to mTeSR™1-cultured primed hPSCs. Additionally, naïve-like hPSCs maintained in RSeT™-FF Medium are capable of direct differentiation to all somatic lineages using the STEMdiff™ Definitive Endoderm, Mesoderm Induction, and SMADi Neural Induction Kits. In summary, we have developed RSeT™-FF, a defined medium that promotes robust conversion of primed hPSCs to a naïve-like state and the continuous maintenance of these cells in a feeder-independent culture system.

93 Induction of multiple fates by a single transcription factor in human tissue organoids through dose-dependent GATA6 activity

Fortuna PRJ, Briggs JA, Wolvetang EJ, Klein A, Weiss R

University of Queensland, Australia

Keywords: organoids, development, Gata6

Experimental manipulation of transcription factor expression has emerged as a powerful way to convert cell states between distinct fates. Here we show that the activation of a single transcription factor, GATA6, is capable of reproducibly inducing a stereotyped mixture of multiple fates in human tissue organoids and monolayer cultures. We used single cell RNA sequencing to show that heterogeneity in GATA6 expression level correlates with downstream fate outcomes. Low GATA6 expression levels correlated with differentiation towards endothelial cell fates, moderate induction correlated with cardiac and hematopoietic fates, while high expression was correlated with hepatic liver formation. Experimental manipulation of GATA6 expression levels confirmed that fate choices can be controlled directly by the dose of GATA6 in differentiating cells. These results reveal how gene dosages can control the types and proportion of cell states in tissue organoids produced by transcription factor driven cell reprogramming.

94 Nanotopographies induce changes to mesenchymal stem cell metabolism and promote multipotency – linking adhesion and metabolism to multipotency

Ross EA, Turner LA, Saeed A, Reynolds PM, Mountford J, Burgess KEV, Meek RD, Gadegaard N, Oreffo ROC, Dalby MJ

University of Glasgow, UK

Keywords: MSC, nanotopography, multipotency, glycolysis

Naïve multipotent mesenchymal stem cells (MSCs) are a key cell product for patient therapy; their ability to regulate immune responses, haematopoiesis as well as driving tissue regeneration mean they have become a fundamental reagent for cell-based therapies. However, quality of the naïve MSC is crucial to its therapeutic potential; in culture MSCs spontaneously differentiate into mature stromal populations, losing their immunomodulatory properties. Previously we reported that culturing MSCs on nanotopographical surfaces with a square pattern of nanopits (SQ) can maintain their naïve phenotype, with increased expression of multipotency markers and delayed differentiation1. In this study we reveal a new link between adhesion and cellular respiration which promotes naïve MSC functionality. MSCs were cultured in normoxia on SQ or non-square (NSQ) nanotopographies, or flat control (Flat). Binding of MSCs to the SQ nanotopography resulted in an increase in aerobic glycolysis as demonstrated by tracing metabolomic conversion of heavy labelled glucose, revealing significantly increased glucose uptake and generation of pyruvate and lactate metabolites compared to Flat and NSQ surfaces. These observations were also confirmed at the protein level. SQ educated MSCs maintained their immunomodulatory capacity in culture and had higher expression of naïve multipotency markers compared to cells grown on Flat or NSQ surfaces. Using mitochondrial inhibitors, we modelled this change in respiration, and found that promoting aerobic glycolysis recapitulated the SQ surface phenotype, maintaining MSCs immunosuppressive activity and upregulating multipotency markers. Interaction of MSCs with specific nanotopographical surfaces alters cellular respiration to promote a naïve, immunosuppressive phenotype. This model reveals the importance of both metabolism and mechano-transduction signals in order to generate quality naïve, multipotent immunosuppressive MSCs for cell therapy.

95 Comparison of mesenchymal stem cell-conditioned media derived from human bone marrow, adipose tissue and Wharton's jelly

Vackova I, Turnovcova K, Petrenko Y, Chudickova M, Koci Z, Zaviskova K, Kubinova S

Institute of Experimental Medicine, Czech Republic

Keywords: conditioned medium, proteomics, MSCs

Mesenchymal stem cells (MSCs) used for cell therapies secrete a broad spectrum of cytokines, chemokines and growth factors that accelerate regeneration in various pathologies through paracrine-mediated effects. Therefore, using stem cell-derived secretomes represent a promising cell-free therapy, avoiding the disadvantages related to direct stem cells application. In this study, we focused on derivation of MSC-conditioned media (CMs) from human bone marrow (BM-MSCs), adipose tissue (AT-MSCs) and Wharton´s jelly MSCs (WJ-MSCs) and characterization of their protein expression profiles, immunomodulatory and neurotrophic potential, in response to different culture conditions. All three MSC types produced constitutively E-selectin, MCP1, BDNF, LIF, SCF, VEGF A, SDF1 and HGF. Normoxic and hypoxic cultures did not affect the production of monitored factors instead of higher production of ICAM1 in hypoxic cultures. After induction with pro-inflammatory cytokines (IFNγ and TNFα), all MSC types produced pro-inflammatory cytokines IFNγ, IL6, IL12 and IL8, as well as anti-inflammatory cytokines IL1RA, IL10 and IFNα. Moreover, stimulation with pro-inflammatory cytokines positively affected the production of RANTES, IP10, MCP1, MCP1, ICAM1, VCAM1, PGE2 and IDO. In addition, the influence of CMs on inhibition of peripheral blood lymphocyte proliferation and axonal sprouting of cultured sensory neurons isolated from dorsal root ganglions of adult rats were evaluated to confirm the immunomodulatory properties and neurotrophic potential. In conclusion, we confirmed comparable therapeutic potential of CMs from different MSC tissue sources. When compared with BM- and AT-MSC, WJ-MSCs was found as the most preferable CM source with the benefit of high cell yield and proliferation as well as non-invasive collection procedure of source material. This project was supported by GACR 17-03765S.

96 Novel functional applications of human intestinal organoids established and maintained in IntestiCult™ organoid growth medium (human)

Conder RK, Elstone F, Lankhorst M, **Micsik R**, West M, Stahl M, Brown T, Riedel MJ, Vallance BA, Stingl J, Thomas TE, Eaves AC, Louis SA

STEMCELL Technologies, UK

Keywords: human intestinal organoids, Functional application

We recently developed IntestiCult™ Organoid Growth Medium (OGM; Human) for long-term growth and maintenance of organoids derived from human small intestinal and colonic epithelia. We investigated whether the functionality of the cystic fibrosis transmembrane conductance regulator (CFTR) protein is maintained in organoids cultured in IntestiCult™ OGM, and whether the cells comprising the organoids form a functional barrier epithelium after being seeded as a monolayer onto 2-dimensional Corning® Transwell® inserts. In CFTR experiments, 10 day-old organoids cultured in IntestiCult™ OGM were treated with either the adenylate cyclase activator forskolin (5 µM) or DMSO, and organoid swelling was measured as an indicator of CFTR function. Forskolin-treated organoids increased in size by 34.3±4% (mean±SD; n>100 organoids measured) within 120 minutes, while DMSO-treated organoids exhibited no pronounced size increase, indicating that a functional CFTR protein was maintained by organoids cultured in IntestiCult™ OGM. To investigate barrier epithelium function, cultured intestinal organoids were dissociated with 0.05% trypsin-EDTA for 5 minutes and the resulting single-cell suspensions were seeded in IntestiCult™ OGM onto Corning® Matrigel®-coated Transwell® inserts. After 5–8 days, the cells had formed a confluent polarized monolayer containing the junctional proteins E-cadherin and claudin4. Goblet and M cells were also present, as revealed through staining of MUC2 and KRT18. When assayed for changes in short-circuit current (ΔISC, µA/cm2), monolayers cultured in IntestiCult™ OGM displayed a 15-fold higher response to CFTR activation (IBMX/forskolin treatment) and a 5-fold higher level of inhibition by CFTR inhibitor 172 compared to widely used Caco-2 cell monolayers ($p<0.05$, $n=4$). In summary, these results demonstrate that IntestiCult™ OGM can be used to maintain human intestinal epithelial cells in the form of a physiologically-relevant polarized barrier epithelium ex-vivo.

97 Mesenchymal stem cells labelling using magnetic particles for in vitro applications

ALKharji R, Sottile V

Prince Sultan Military Medical City, Saudi Arabia

Keywords: Mesenchymal stem cells, SPIONs

Cell-based therapy is not a new concept; it is considered as one of the most promising approaches for treating diseases and for regenerative medicine. In addition, effective cell therapy can greatly benefit from the ability to monitor transplanted stem cells post-intervention. Mesenchymal stem cells (MSCs) represent one of the leading candidate population for regenerative medicine. These cells, which are present in adult tissues, are non-hematopoietic stem cells with multipotent capacity toward a range of mesodermal lineages. Superparamagnetic iron oxide nanoparticles (SPIONs) represent contrast agents offering a possible way to track labelled cells after administration using MRI. Moreover, we have demonstrated that these magnetic particles (MPs) do not affect cell viability, proliferation, differentiation or migration. The aim of the present study was to determine the ability to use these iron particles to label mesenchymal stem cells (MSCs) and test their potential to control cell migration when exposed to a magnet. This aim was achieved by culturing labelled and unlabelled cells in 2D and 3D models, in presence or absence of magnet. Significant response to magnet exposure was observed in 2D culture where is 76% of labelled cells moved to the magnet side when compared to unlabelled cells. There was only 45% of unlabelled cells found to have moved to the magnet side. In addition, 64% of labelled cells moved to the magnet side in a 3D culture model, while the unlabelled cells showed around 50% cells moving to the magnet side. In summary, we have shown that MSCs can be labelled with MPs in vitro, and this strategy can contribute to improving the spatial tracking of transplanted stem cell, and therefore improve their efficiency for therapeutic applications.

98 Generation of an induced pluripotent stem cell macrophage platform for early discovery applications

Armesilla-Diaz A, Ashby C, Escudero-Ibarz L, Santiváñez-Pérez J, Maifoshie E, Bagri J, Mohamet L

GlaxoSmithKline (GSK), UK

Keywords: iPSC, macrophages, drug discovery

After decades of research there are still persistent failures to translate preclinical drug candidates into clinical success, highlighting the limited effectiveness of disease models currently used in drug discovery. iPSC technology has provided new tools to improve drug discovery efforts from efficacy and toxicity testing to novel target identification and understanding disease mechanisms. Macrophages perform key functions in regulating homeostasis, immune response and tissue repair. Equally, their dysfunction may drive pathogenesis of inflammatory and degenerative diseases, making them a key therapeutic target. Currently, macrophages are differentiated from peripheral blood monocytes (PBMCs), which is costly at scale and results in poor reproducibility due to donor variability. We have developed a robust cellular platform to generate human macrophages using innovative iPSC technology. This provides a cost-effective, reproducible and biologically relevant large-scale source of human macrophages for use in drug discovery. We have identified a simple three-step method which enabled the generation of a continuous source of human macrophages within twenty days of initial differentiation. Careful biochemical, functional and cellular assays were performed for comparative analysis with blood-derived macrophages and other commercially available iPSC-derived macrophages. The platform was further adapted to industry standards by miniaturisation into 384 well plates with automation. This ambitious methodology demonstrates comparable results to current primary cell based models and adds value to existing strategies by reducing donor variability, reducing timelines and ultimately decreasing costs.

99 Comparative analysis of chemically-defined green media for expansion of adipose-derived mesenchymal stem cells

Kong WH, Choi J-Y, Lee E-J, Kim N-R, Kim D-Y, Kim Y-D, **Choi B-H**

Advanced Bio Convergence Center, Republic of Korea

Keywords: mesenchymal stem cell; chemically-defined green medium; proliferation; differentiation

Adipose-derived mesenchymal stem cells (AMSCs) are multipotent cells that are attractive in clinical trials related to cell-based regenerative therapies. Although most of the current media for AMSCs use fetal bovine serum (FBS), animal-derived products show limitations and safety concerns in clinical applications. This study aims to determine whether chemically defined green (CG) media containing plant-derived biomaterials such as EGF, FGF and human serum albumin are suitable for efficiently expanding AMSCs. AMSCs were cultured in both FBS-based and CG media. AMSCs showed dramatic proliferation rate in CG medium compared to FBS-based medium. Moreover, AMSCs cultured in CG media showed a reduced doubling time, delayed cell senescence and maintenance of small cell size. The expression of AMSC surface markers, cell viability, and analysis of tri-lineage differentiation showed great similarities between FBS-based and CG medium. These results suggest that CG medium may provide an efficient condition for the expansion of AMSCs in clinical applications.

100 Innovative Tools to study Epigenetic Marks including DNA Methylation

Creppe C, Veillard AC, Laczik M, Schwartzman S, Werding R, Sabatel C

Diagenode, UK

Epigenetics is crucial for the regulation of gene expression and has broad relevance in biological processes like development, disease and response to the environment. For more than 10 years Diagenode has been developing innovative tools to study epigenetic marks such as post-translational modifications of histones and DNA methylation. We are now utilizing our expertise by offering custom services. Our services include full workflows for ChIP-sequencing as well as reduced representation bisulfite sequencing (RRBS) with our new optimized "Premium RRBS™ technology. In addition, we also offer bioinformatic analysis of your results, both standard and customized. The Diagenode Epigenetics Custom Services helps you to complete your epigenetics workflow from your starting biological material to your final results.

101 Elasticity of Human Mesenchymal Stem Cells by Scanning Force Microscopy in Combination with Fluorescence Microscopy

Davoudi N, Charif N, Roeder E, de Isla N, Müller-Renno C, Ziegler Ch

University of Kaiserslautern , Germany

Keywords: Elasticity, Young's modulus, Adhesion

Stem cells have the unique capability to differentiate into specialized cell types if appropriate growth conditions are provided. Human mesenchymal stem cells (hMSCs) are thought to have a great therapeutic and biotechnological potential. However, their use in cell therapy (1) and regenerative medicine (2) is still limited by cell source quantity. The hMSCs are only available in small numbers in the human body and still, their isolation and expansion are very limited. Therefore, an effective expansion process is crucial for achieving a therapeutic dose which is 100,000 to 200,000 times of the initial patient sample. The main goal of this Interreg project, Improve STEM, is the enhancement of the cell number of hMSCs by using microcarrier cultivation and by a correlation of the hMSCs properties (morphology, elasticity and adhesion) and properties of microcarriers (zeta potential, wettability, functionalization). Here, the focus lies on the elasticity of the hMSCs isolated from the Wharton's jelly of umbilical cords. The Young's modulus of the hMSCs is determined by scanning force spectroscopy. Employing this method, we are able to take a precise, point elasticity measurement on a single cell to identify the local variation due to the cell structure. A combination of scanning force spectroscopy and fluorescence microscopy provides accurate Young's modulus analysis of cell regions around the nucleus, with cytoplasm and on the substrate. In addition, the Young's modulus is compared of cells cultured in four different conditions: varying hypoxia and normoxia as well as the human platelet lysate and fetal bovine serum. We found a considerable difference in the elasticity of the analyzed hMSCs, reflected by a range of the Young's modulus (2-19 kPa). The different elasticity on a single cell or from one cell to the other suggests that the elasticity of the cell could serve as the basis of understanding the cell functions. Further measurements will help to understand it in detail.

102 Assessment of Drug Effects on Cardiomyocyte Function: Bridging HTS Ion Channel and Myocyte Results

Dragicevic E, Stoelzle-Feix S, Juhasz K, Bot C, Strassmair T, Okeyo G, Becker N, Thomas U, Doerr L, Rapedius M, Brinkwirth N, Haarmann C, Goetze T, Haeusermann F, Polonchuk L, Traebert M, Beckler M, George M, Brüggemann A, Fertig N

Nanion Technologies, Germany

Keywords: CiPA, Cardiomyocytes, Impedance, EFP, automated patch clamp

Human induced pluripotent stem cells (hiPSCs) are relevant for cardiac safety testing due to their validated predictivity as described in recent publications derived from the comprehensive in vitro proarrhythmia assay (CiPA) study. We combined automated patch clamp (APC), impedance and extracellular field potential (EFP) measurements to study cardiac ion channels in cell lines and hiPSC-CMs. Cell lines expressing different cardiac ion channels were recorded on two different APC instruments (8 or 384 wells simultaneously) at room and physiological temperature, at 4 different sites. Within the myocyte validation study of the CiPA initiative, hiPSC-CMs from different providers were used on a device combining impedance-based contractility and extracellular field potential (EFP) recordings. Within the ion channels working group of the CiPA initiative, ion channel data on 7 cardiac ion channel currents were measured. The effects of >20 CiPA reference compounds deemed low, intermediate and high risk by the FDA were investigated. For example, the NaV channel was blocked by the high-risk compound Vandetanib with an IC50 of 9.9 µM, a value which is consistent with the cardiac NaV1.5 channel. Vandetanib also blocked the hERG current with an IC50 of 0.23 µM. In parallel to APC investigations, arrhythmogenicity of this and other compounds were investigated via contractility and field potential recordings in 2D monolayers of iPSC-CM. Our results show that high risk compounds such as dofetilide prolong field potential duration which resulted in the detection of arrhythmic events in both impedance and EFP recordings. Datasets from multiple sites and cell types will be shown and compared. Cross-site/cell comparisons of APC ion channel data and myocyte repolarization data was combined in order to investigate the mode of action of reference compounds. This promotes a good understanding of drug induced arrhythmia and thus represents a valuable approach in drug development efforts.

103 Microscale tissue engineering for the treatment of Type I diabetes

Ungrin M, Yu Y, Gamble A, Pawlick R, Pepper AR, Salama B, Toms D, Razian G, Ellis C, Bruni A, Gala-Lopez B, Lu JL, Vovko H, Chiu C, Abdo S, Kin T, Korbutt G, Shapiro AMJ

University of Calgary , Canada

Keywords: diabetes; islet; tissue engineering

Islet transplantation is a promising approach for the treatment of type 1 diabetes. Current protocols using donated tissue(1–3) provide proof-of-principle of effectiveness,(4–6) and stem cell derived sources are poised to revolutionize this established cell-based therapy. Regardless of source however, the capacity to package the material for efficient transplant and durable engraftment will be a critical component of future therapies.(7, 8) Immediately following transplant, ischemia and an instant blood mediated inflammatory response (IBMIR) coupled with slow revascularization (even more so in the human system than the mouse) cause substantial loss of the transplanted material.(9) Reliance on diffusion for oxygen and nutrients likely leads to further cell loss in the interior,(10, 11) and natural levels of vascularization do not appear to develop.(12–14) This would be expected to lead to reduced function(15) and further islet loss in the longer term,(16) consistent with results from modelling,(11, 17) culture(18) and transplant(18) studies. It is important to note also that if encapsulation is employed as an immunoisolation strategy, engrafted islets will be completely dependent on diffusive delivery of oxygen and nutrients for the duration of the graft. We present here a centrifugal forced-aggregation approach for the rapid assembly of uniform, spherical, size-controlled pseudoislets from islet cell suspensions. In vitro, our pseudoislets exhibit improved size consistency, glucose-stimulated insulin secretion, and hypoxia tolerance. In a marginal-mass transplant model in STZ-induced diabetic mice, compared to native islet controls they show improved glucose clearance; and function at least as well – and potentially better – in reversion of hyperglycemia. We anticipate this work will provide an essential foundation for future cell based therapies for type 1 diabetes.

Poster Session 2
Thursday 20 September, 17:40 - 19:00

Theme 1: Adult Stem Cells

104 BCL11A interacts with SOX2 to control the expression of epigenetic regulators in lung squamous cell carcinoma

Lazarus KA, Hadi F, Zambon E, Bach K, Santolla MF, Watson JK, Correia LL, Das M, Ugur R, Pensa S, Becker L, Campos LS, Ladds G, Liu P, Evan G, McCaughan F, LeQuesne J, Lee JH, Calado D, Khaled WT

University of Cambridge, UK

Keywords: lung cancer, novel targets, cancer therapeutics

Introduction: Lung cancer accounts for the highest rate of cancer related diagnosis and mortality worldwide. Broadly there are two major types of lung cancer; small cell lung cancer and non-small cell lung cancer (NSCLC) which accounts for 85% of cases. NSCLC patients have a poor clinical outcome with only a 15% five-year survival rate. The major subtypes of NSCLC are lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). LUSC accounts for more than 400,000 deaths worldwide each year and unlike LUAD there are limited targeted therapeutic options. Therefore, a great deal of work still needs to be done to understand the drivers for this cancer. Results and discussions: Analysis of TCGA dataset has revealed BCL11A to be upregulated in LUSC but not LUAD. Subsequently reducing BCL11A levels in LUSC cell lines results in drastically diminished xenograft tumour growth. Inversely, its overexpression in vivo led to lung airway hyperplasia and the development of reserve cell hyperplastic lesions which is a precursor to squamous metaplasia. Moreover, deleting Bcl11a in mouse tracheal basal cells abolished the development of tracheosphere organoids while its overexpression led to solid tracheospheres expressing markers of squamous cells. At the molecular level we found BCL11A to be a target of SOX2 and we show that it is required for the oncogenic role of SOX2 in LUSC. Furthermore, we showed that BCL11A and SOX2 interact at the protein level and that together they co-regulated the expression of several transcription factors. We demonstrate that pharmacological inhibition of SETD8, a gene co-regulated by BCL11A and SOX2, alone or in combination with cisplatin treatment, shows significant selectivity to LUSC in comparison to LUAD cells. Conclusions: Collectively, these results indicate that the disruption of the BCL11A-SOX2 transcriptional program provides a future framework for the development of targeted therapeutic intervention for LUSC patients.

105 Gβ5 p.S81L causes bradycardia by increasing the IK,ACh and augmenting cholinergic response

Mengarelli I, Veerman CC, Koopman CD, van Amersfoorth SC, Bakker D, Wolswinkel R, Hababa M, de Boer TP, Guan K, Milnes J, Lodder EM, Bakkers J, Verkerk AO, Bezzina CR

Amsterdam University Medical Centers, Netherlands

Keywords: G protein β5 subunit, hiPSC-cardiomyocytes, bradycardia

Mutations in GNB5, encoding the G-protein β5 subunit (Gβ5), have been linked to a multisystem disorder that includes severe bradycardia (1). We here investigated the mechanism underlying bradycardia caused by the recessive p.S81L Gβ5 variant identified in families of Latin and North African descent (1). Using the CRISPR/Cas9-based genome editing technique we generated an isogenic series of human induced pluripotent stem cell (hiPSC) lines that were respectively wild-type, heterozygous and homozygous for the GNB5 c.242C>T p.S81L variant. These cells were differentiated into cardiomyocytes (hiPSC-CMs) that robustly expressed the acetylcholine-activated potassium current (IKACh). Baseline electrophysiological properties of the hiPSC-CMs from the three isogenic lines did not differ. Upon application of carbachol (CCh), homozygous p.S81L hiPSC-CMs displayed an increased IKACh density, a more pronounced membrane hyperpolarization and decrease of spontaneous activity as compared to wild-type and heterozygous p.S81L hiPSC-CMs, in line with the bradycardia in homozygous carriers. Application of XEN-R0703 (2), a specific IKACh blocker, resulted in near-complete reversal of the phenotype. Additionally, in vivo studies in zebrafish gnb5 knockout confirmed the effect of XEN-R0703 in rescuing the CCh-induced bradycardia. Our results provide mechanistic insights in the Gβ5 p.S81L-associated bradycardia and proof of principle for potential therapy in patients carrying GNB5 mutations.

106 Differential regulation of BMP4, DKK2, DLL1, FGF11 and HIF2A expression by consecutive hypoxia and intermittent hypoxia-reoxygenation are respectively associated with differential expression of the stem/progenitor cell markers CC10, OCT4, NKX2-1 and TP63 in healthy and diseased human small airway epithelial cells

Chen ST, Chen YH

National Defense Medical Center, Taiwan

Keywords: Human small airway epithelial cells, Hypoxia, Reoxygenation, Stem/progenitor cell markers

Background: Previous studies have shown that in airway epithelial cells consecutive hypoxia increases proliferation and hypoxia-reoxygenation (H/R) increases production of ROS and inflammatory cytokines whereas decreased levels of ATP and surfactant proteins [ref.1, 2]. However, it has not been reported whether consecutive hypoxia and intermittent H/R affected expression of stem/progenitor cell markers in human small airway epithelial cells. Methods: The human small airway epithelial cells (SAECs) derived from the same-race and age-matched healthy subject (i.e., N-SAECs) and subject with chronic obstructive pulmonary disease (COPD) (i.e., D-SAECs) were obtained from the Lonza Biotechnology Company. The cells were cultured in the PromoCell SAEC growth medium for 6~7 days followed by subculturing under consecutive hypoxia (1% O2) for 3 days and then returning to normoxia, or under 24/24-hour cycles of H/R (i.e., 24 hours of 1% O2 followed by 24 hours of 20% O2, repetitively) for a total duration of 6 days. Results: In both N-SAECs and D-SAECs, compared to normoxia, intermittent H/R significantly decreased FGFR3, FZD4, SOX2, HIF1A, and TP53AIP1 expression, while consecutive hypoxia significantly increased BMP4, DKK2, CC10, and AQP5 expression whereas decreased FGF10/11, FGFR3, NOTCH1/3, WNT10A, HIF1A, and TP53AIP1 expression. In addition, the differential changes of expression levels of the following genes were positively correlated with each other under intermittent H/R and consecutive hypoxia: CC10 correlated with BMP4 and DKK2, NKX2-1 correlated with FGF11, OCT4 correlated with DLL1, TP63 correlated with HIF2A. Conclusions: Our study shows for the first time that differential regulation of human small airway epithelial stem/progenitor cell marker gene expression by intermittent H/R and consecutive hypoxia is associated with differential expression levels of components of the BMP, FGF and WNT signaling pathways.

107 Evaluation of Cardiac Differentiation of Human Induced Pluripotent Stem Cells (hiPSCs) under Acidic pH

Ferrini A, Zwi-Dantsis L, Harrison M, Rosenthal N, Stevens MM

Imperial College London, UK

Keywords: hiPSC, cardiomyocytes, differentiation

Background: one of major events occurring after a myocardial infarction is an acidification of the tissue, with the pH dropping to 6.5-6.8, due to the switch from aerobic metabolism to anaerobic glycolysis (1). It has been reported that cardiac differentiation of murine embryonic stem cells is strongly inhibited by lowering the pH (2); however, the effects of pH on cardiac differentiation of human induced pluripotent stem cells (hiPSCs) remain to be investigated. Objectives. The aim of this study was to assess the effects of low pH on cardiac differentiation of hiPSCs in terms of cell viability, cardiomyocytes (CMs) yield and gene expression profile. Four pH values were tested: control (pH 7.7), pH 6.8 (value of the ischemic myocardium), pH 7.1 (intermediate between acidic and physiologic) and pH 7.4 (standard recommendation for cell culture). Results and methodology: results from MTT assay and LIVE/DEAD® staining showed that pH 6.8 significantly reduces hiPSC-CMs metabolic activity and viability during differentiation, respectively. The CMs yield quantified by flow cytometry analysis at day 21 was 83.8 ± 1.3% in the control, while it was halved at pH 6.8. Next, gene expression analysis showed a significant down-regulation of cardiac-specific markers in the cells cultured in acidic pH. Finally, we demonstrated that the pro-survival factor Insulin like Growth Factor-1 (IGF-1) is able to rescue the low pH phenotype, and by adding IGF-1 at concentrations of 10 and 50 ng/m L the yield and viability of the CMs cultured in pH 6.8 were increased back to the control values (pH 7.4). Conclusion and future work. Taken together, these findings demonstrate that acidic pH has a significant effect on cardiac differentiation of hiPSCs and that this could be rescued by IGF-1. A better understanding of the pH sensitivity of cardiac cells could then be used for the design of novel pH-responsive biomaterials for cardiac repair purposes.

108 IL-1β significantly impairs equine adult and fetal tenocyte function but ESC-derived tenocytes are resistant

Guest D, McClellan A, Evans R, Sze C, Kan S, Atkinson, F, Holland C

Animal Health Trust, UK

Keywords: Embryonic stem cells; tendon; IL-1β

Tendon injuries occur commonly in equine and human athletes and are associated with high re-injury rates. This is due to poor regeneration and the formation of mechanically inferior scar tissue. The use of cell based therapies to improve regeneration and reduce re-injuries is therefore being investigated. Following a tendon injury there is a local upregulation of inflammatory cytokines, including IL-1β, which may have a negative effect on the function of endogenous tendon cells and exogenous cells applied during cell-based therapies. Here we demonstrate that IL-1β upregulates the expression of matrix metalloproteinases (MMP) and changes the expression of tendon and cartilage-associated genes in adult and fetal tenocytes. IL-1β also inhibits the ability of adult and fetal tenocytes to remodel a collagen matrix and the resulting tendon-like constructs have a lower storage modulus. When used at a high concentration IL-1Ra (IL-1β receptor antagonist protein) can protect adult and fetal tenocytes from these effects. We have previously demonstrated that equine ESCs survive (1) and differentiate into tenocytes (2) in the injured horse tendon. In contrast to adult and fetal tenocytes, equine ESC-derived tenocytes undergo no changes in gene expression or matrix remodelling when exposed to IL-1β. We have found that ESC-derived tenocytes express significantly less of the signalling receptor IL1R1 and significantly more of the decoy receptor IL1R2 and the gene encoding IL1Ra, IL1RN. Furthermore, ESC-derived tenocytes are resistant to a combination of inflammatory cytokines consisting of IL-1β, TNFα and IFNγ. Understanding the mechanisms by which ESC-derived cells are protected from inflammation may enable the future development of novel pharmaceutical interventions to protect endogenous adult tenocytes and produce better tendon regeneration to reduce re-injury rates.

109 Generation of functional liver sinusoidal endothelial cells from human pluripotent stem cells

Gage BK, MacParland SA, Lui JC, Bader GD, McGilvray ID, Keller GM

McEwen Centre for Regenerative Medicine, Canada

Keywords: hPSC, Liver, LSEC, Endothelial

The liver contains many functional cell types, all of which are perfused by an anatomically unique vasculature where both hepatic arterial and portal venous blood mix in a sinusoidal endothelium that then drains to a central vein. In addition to scavenging functions, the liver sinusoidal endothelial cell (LSEC) plays a key role in liver homeostasis by tuning regeneration and fibrosis responses by dynamic and responsive cytokine production. To facilitate future tissue engineering and to illuminate human hepatic vasculature development, we have generated angioblasts from human pluripotent stem cells (hPSCs) and examined their in vitro and in vivo developmental ability to form functional LSECs. First, hPSCs were differentiated through a common vasculogenic mesoderm then specified to angioblasts by modulation of VEGF, bFGF, and NOTCH signaling. In vitro, purified CD34+ angioblasts adopted a proliferative endothelial cell (EC) fate upon adherent culture and responded to TGFß inhibition, cAMP signaling, and oxygen tension by upregulation of LSEC markers. To deliver physiological cues associated with functional LSEC development, a neonatal intrahepatic transplantation model was applied. Recovery of transplanted hPSC-derived cells revealed expression of mature LSEC markers at levels seen in isolated primary human LSECs including F8 which when mutated is clinically associated with Hemophilia A. Furthermore, scRNA-seq of recovered cells showed a close transcriptional profile to primary LSECs including LSEC zonation. Functional analysis of recovered hPSC-derived LSECs revealed a high degree of correctly sized fenestrations that were dynamically responsive to actin depolymerization by latrunculin A treatment. Taken together, this work describes the in vitro differentiation of hPSC-derived venous vasculature progenitors capable of in vivo maturation to functional LSECs with future uses in toxicology with human hepatic vascularized mice or as a cellular therapy for Hemophilia A.

110 Defining the identity and dynamics of adult gastric isthmus stem cells

Han S, Fink J, Jörg DJ, Merker S, Lee E, Yum M, Josserand M, Trendafilova T, Andersson-Rolf A, Dabrowska C, Kim H, Mort RL, Jackson IJ, Basak O, Clevers H, Marioni J, Stange D, Kim J-K, Simons BD, Koo B-K

Wellcome - MRC Cambridge Stem Cell Institute, UK

Keywords: Stomach isthmus stem cell, random labeling lineage tracing, mathematical modeling

The gastric corpus epithelium is the thickest part of the gastro-intestinal tract and is characterized by rapid tissue turnover. Several markers have been proposed for gastric corpus stem cells in both isthmus and base regions. However, the identity of isthmus stem cells (IsthSCs) and the interaction between distinct stem cell populations is still under debate. Here, based on unbiased genetic labeling and biophysical modeling, we show that corpus glands are compartmentalized into two independent zones, with actively-cycling stem cells maintaining the pit-isthmus-neck region and slow-cycling reserve stem cells maintaining the base. Independent lineage tracing based on Stmn1 and Ki67 expression confirmed that rapidly-cycling IsthSCs maintain the pit-isthmus-neck of corpus glands. Finally, single cell RNA-seq analysis is used to define the molecular identity and lineage relationship of a single, cycling IsthSC population. These observations define the identity and functional behavior of a single population of actively-cycling IsthSCs.

111 Towards the fully mature hepatocytes by genome wide CRISPR/Cas9 screening

Huynh LM, Stuart F, Kaji K

MRC Center for Regenerative Medicine, UK

Keywords: iPS, hepatocyte, regenerative

Generation of fully functional mature cell types, including hepatocytes, are a bottleneck in the field of regenerative medicine even after human iPS cells become widely available. In collaboration with the Forbes lab, we recently established the mouse Chemical Liver induced Progenitors (CLiPs) from adult hepatocytes as Katsuda et al. reported (1). These CLiPs can self-renew at least 5 passages without losing their differentiation capacity into fully mature hepatocytes in vitro and in vivo. In vitro, 20% of redifferentiated CLiPs express Asgrp1/2, an asialoglycoprotein, which specifically present in fully mature hepatocytes. Since human ES/iPS cell-derived hepatocyte-like cells do not become mature hepatocytes, human CLiP cell line could be a unique, unlimited source of human mature hepatocytes. However, the same culture condition failed to generate human CLiP lines. Thus, it is important to understand genes essential for mouse CLiP self-renewal and differentiation capacity. Our lab has previously performed genome-wide knockout screen during induced pluripotent stem cell (iPSC) reprogramming using a gRNA library containing 90,000 gRNAs. It successfully identified >10 novel genes detrimental and essential for the iPS cell generation and we managed improve reprogramming efficiency 5-10 fold. We will apply the same technology to uncover the key players in maturation of hepatocytes.

112 Neuregulin1 / ErbB signalling regulates intestinal stem cells during tissue homeostasis and regeneration

Jardé T, Rossello F, Kurian Arackal T, Flores T, Giraud M, Prasko M, Nefzger CM, Polo JM, Abe S, Abud HE

Monash University, Australia

Keywords: Intestinal stem cells, homeostasis, tissue regeneration

Defining signals that regulate intestinal stem cells (ISCs) may enable stem cell pools to be manipulated in degenerative diseases and intestinal pathologies. The Neuregulin1/ErbB signalling pathway plays a pivotal role in regulating aspects of tissue homeostasis and regeneration in the nervous system. However, the function of this pathway in the intestinal epithelium is currently unknown. We examined the expression of Nrg1 and its receptors in the small intestine using immunofluorescence and q-RT-PCR. We observed that supporting niche cells were expressing Nrg1, in contrast to ISCs that expressed high levels of its receptors, suggesting that Nrg1/ErbB signalling directly regulates ISCs. To investigate the functional activity of Nrg1 in the intestine, 12 week-old mice were injected with 15ug Nrg1 for 5 days. Activation of Nrg1/ErbB signalling increased cell proliferation in the intestinal crypts by 43% and was associated with alteration of cellular differentiation. Similarly, inducible loss of Nrg1 in mice significantly decreased cell proliferation in ISCs and progenitor cells. To characterise the molecular signature of Nrg1 in ISCs, RNA sequencing was performed on ISC and progenitor cell populations isolated from Nrg1-treated and control animals. This analysis revealed activation by Nrg1 of a molecular proliferative signature in both cell populations. Importantly, the ISC population became more homogeneous and expressed higher levels of stem cell markers. To define the role of Nrg1/ErbB signalling during tissue regeneration, two mouse models of injury/regeneration (irradiation and 5-FU treatment) were utilised. Interestingly, the expression of Nrg1 in both models was 5-10 fold increased during the intestinal regenerative phase. This was reinforced by using an in vitro model of regeneration; we observed that Nrg1 significantly promoted organoid growth and the formation of colonies from single ISCs. Importantly, the intestinal regenerative response following damage was significantly improved when mice were treated with Nrg1. Taken together, our results suggest that Nrg1/ErbB signalling is a critical regulator of ISCs during intestinal homeostasis and tissue regeneration.

113 Establishing organoid reporter lines for optimising in vitro human lung alveologenesis

Jeng Q, Lim K, Sun D, Rawlins EL

Gurdon Institute, UK

We have previously established conditions for growing human embryonic lung organoids as self-renewing, undifferentiated progentiors from 6 – 20 weeks gestation human embryonic lung progenitor cells. These organoids can be differentiated into lung alveolar lineages in the presence of human embryonic lung mesenchyme. We aim to identify define media conditions for differentiating alveolar epithelium from human embryonic lung organoids. Media conditions will be optimised using a PerkinElmer Opera Phenix™ screening system. The onset of alveolar differentiation is to be indicated by the expression of a human-Surfactant Protein C–GFP (hSPC-GFP) transcriptional reporter inserted into the organoids on a PiggyBac construct.

114 ID1 in mouse epidermal progenitor cells

Kantzer CG, Genander M

Karolinska Institutet, Sweden

Keywords: Stem cell niche, Development, Epidermal progenitors

The choice between stem cell self-renewal and differentiation must be tightly controlled since alterations will lead to disease. However, the transcriptional networks orchestrating and maintaining this balance are poorly understood. The developing interfollicular epidermis (IFE) is an ideal model to study stem and progenitor cell fate as progenitors and differentiated cells can be identified by marker expression and distinct spatial distribution [2] . Our work aims at disclosing mechanisms that dictate progenitor cell fate by studying basic helix-loop-helix transcription factors and their modulators the ID proteins (inhibitor of DNA-binding-1) in the developing IFE. Immunohistochemical analyzes of the four family members of ID proteins revealed ID1 to be highly expressed in interfollicular epidermal progenitor cells, in the placodes of developing hair follicles at embryonic day (e) 14.5 and also the hair peg at e18.5. In contrast, ID1 expression was weaker in differentiated cells of the IFE. To study the role of ID1 in the IFE progenitor cells, we silenced ID1 expression in the embryonic skin using ultrasound-guided in utero microinjections of lentivirus co-expressing an shRNA [1] against ID1 and the reporter H2BGFP. Silencing of ID1 expression resulted in an 8- fold reduced proliferation at e14.5 when compared to control scrambled embryos, corresponding to an overall loss of infected cells over time. However, we failed to detect any differences in apoptosis. At e16.5, the ID1 shRNA targeted epidermis was significantly thinner than normal, lacked hair follicles and displayed an overall disorganized appearance. We found 7% ID1 shRNA targeted EdU+ proliferating progenitors in suprabasal layers. Furthermore, around 30% of infected and 32% of uninfected progenitor cells co-expressed basal progenitor marker K5 and the suprabasal differentiation marker K10, suggesting that ID1 acts non-autonomously. In vivo E-cadherin mRNA levels were downregulated in ID1 shRNA targeted epidermis, suggesting that ID1 keeps epidermal progenitor cells attached to their niche by enforcing cell adhesion.

115 Hair follicle stem cell fate and plasticity is controlled by oxygen tension in a TORC2-dependent manner

Kim CS, L'Hoest N, Ding X., Chacon-Martinez CA, Eming S, Wickström SA

Max Planck Institute for Biology of Ageing, Germany

Keywords: mTORC2, Hypoxia

Stem cells reside in a niche with distinct micro-environment that governs the fate of stem cells. Hair follicle stem cells (HFSCs) reside a specific niche termed the bulge where they go through the cycle of activation and quiescence to fuel hair follicle regeneration. The precise mechanisms that trigger hair follicle activation and in particular return to quiescence remain unclear. We performed transcriptional profiling of HFSCs and their progeny and observed differential expression of key genes involved in glycolysis and oxidative phosphorylation (OXPHOS), indicative of differences in the metabolic state of the two populations. This was confirmed by LC-MS analysis of key metabolites of OXPHOS and glycolysis. HFSCs cultured under 2% O2 in comparison to 20% O2 yielded an increased proportions of HFSCs compared to progenitors. Consistently, inhibition of OXPHOS led to the decrease in progenitors. Lineage tracing further indicated that 2% O2 increases the conversion of progenitors to HFSCs and decreases differentiation of HFSCs to progenitors, indicating that low oxygen tension, and thus the glycolytic state promotes the HFSC state. As the transcriptional profiling had further implicated the key component of tor complex 2 (TORC2), rictor as differentially regulated between HFSCs and progenitors, we hypothesized that rictor and TORC2 activity could be relaying this effect. Indeed HFSC cultures from Rictor-deficient mice failed to increase HFSCs in response to 2%. Further, unlike wild type controls, FACS purified rictor-deficient progenitors failed to convert to the HFSC state in the stem cell cultures. Collectively these data indicate that OXPHOS promotes HFSCs differentiation, whereas the glycolytic state, through mTORC2 signaling, promotes stemness, providing a potential mechanism for how the metabolic state of the niche controls HFSC plasticity during hair follicle cycling.

116 Profiling proliferative cells and their progeny in damaged murine hearts

Kretzschmar K, Post Y, Bannier-Hélaouët M, Mattiotti A,Drost J, Basak O, Li VSW, van den Born M, Gunst QD, Versteeg D, Lieneke Kooijman L, van der Elst S, van Es JH, van Rooij E, van den Hoff MJB, Clevers H

Hubrecht Institute, Netherlands

Keywords: cardiac regeneration, lineage tracing, single-cell transcriptomics

The capacity of the adult mammalian heart to functionally regenerate upon injury remains controversial. Different cardiac stem cell (CSC) populations have been reported to contribute to tissue renewal after insult, yet their functional significance for myocardial regeneration remains disputed. Furthermore, the proliferative capacity of resident non-cardiomyocyte cell lineages has been largely neglected. Here we perform single-cell messenger RNA sequencing and genetic lineage tracing using two Ki67 knock-in mouse models that allow sorting and unbiased mapping of proliferating cells and their progeny in homeostatic and regenerating murine hearts. We find that cardiomyocyte proliferation is largely restricted to the early postnatal growth phase, while non-cardiomyocyte cardiac cell lineages actively cycle also in the homeostatic and damaged adult myocardium. Proliferative post-damage fibroblasts display a gene-expression pattern similar to that of neonatal cardiac fibroblasts, while no significant numbers of cardiomyocytes re-enter the cell cycle upon damage. We find follistatin-like 1 (Fstl1), previously described as a cardiomyogenic factor of epicardial origin, to be specific to cardiac fibroblasts and to be strongly induced upon cardiac injury. Genetic lineage tracing from the Fstl1 locus reveals that these fibroblasts generate the fibrotic scar tissue, yet do not transdifferentiate into cardiomyocytes. Genetic deletion of Fstl1 in cardiac fibroblasts results in a severe phenotype with high mortality due to cardiac rupture upon cardiac injury. In sum, we find no evidence for the existence of a quiescent CSC population, for transdifferentiation towards cardiomyocytes, or for significant numbers of cardiomyocytes that can re-enter the cell cycle in response to cardiac injury and contribute to the myocardial lineage. However, cardiac fibroblasts resident in the myocardium of adult mice that proliferate upon myocardial infarction are critical to maintain post-damage tissue integrity, as they generate a fibrotic scar to minimise cardiac rupture.

117 The role of Nerve Growth Factor in limbal stem cell biology and corneal healing

Ghareeb A, Kolli S, Bojic S, Figueiredo F, Lako M

Newcastle University, UK

Keywords: Nerve growth factor, limbal stem cells, cornea

Nerve growth factor (NGF) has demonstrated great benefit in the treatment of neurotrophic corneal ulcers and potential benefit in the treatment of several other ocular conditions. There is evidence for several modes of action in promoting corneal healing, however only indirect evidence exists for NGF's effects on limbal stem cells (LSCs). Understanding the role of NGF in LSC biology will improve our understanding of the LSC niche and the development of stem cell-based therapeutics. We study the changes in cell signalling which take place upon LSC differentiation and the effects of NGF-blocking on primary human LSCs. Primary human limbal cultures derived from cadaveric corneoscleral rims were cultured on a layer of murine 3T3 fibroblasts for 40 days to allow differentiation. Differential expression of signalling proteins between days 10 and 40 was measured by protein microarray, with subsequent bioinformatic enrichment and network analysis. Expression of NGF and its receptors TrkA and p75NTR was also measured by Western Blot. The effect of addition of anti-NGF antibody on cell morphology, colony-forming efficiency and gene expression of putative differentiation markers was measured. Flow cytometry-based assays of proliferation and apoptosis were also used to study the effect of NGF blocking. Of 248 signalling proteins studied, NGF receptor p75NTR underwent the greatest fold-change in expression between the LSC and differentiated phenotypes, with expression decreasing 2.77-fold upon differentiation. NGF signalling exists at the top of a hierarchical network, controlling cell cycle, toll-like receptor, senescence regulatory pathways and VEGF signalling. Expression of NGF and TrkA also decreases upon differentiation. Primary human limbal cultures grown in the presence of anti-NGF antibodies show significant decreases in colony-forming efficiency ($p=0.039$), proliferation ($p=0.042$) and expression of putative stem cell markers ABCG2 and C/EBPδ ($p=0.03$, $p=0.04$, respectively). NGF maintains the LSC phenotype by promoting self-renewal. NGF signalling potentially coordinates the expression of several biological processes involved in corneal healing, making it a central paracrine signalling factor in the response to corneal epithelial injury. NGF, TrkA and p75NTR are markers of the stem cell phenotype.

118 Modelling Human Primary Liver Cancer in Organoid Cultures

Broutier L, Andrews T, Mastrogiovanni G, Francies HE, Gavarró LM, Bradshaw CR, Allen GE, Davies SE, van der Laan LJ, Wigmore SJ, Saeb-Parsy K, Garnett MJ, Hemberg M, Huch M

Gurdon Institute, UK

Keywords: Organoid, liver cancer, modelling

Adult somatic tissues have proven difficult to expand in vitro, largely because of the complexity of recreating appropriate environmental signals in culture. Recently this problem was overcome by the establishment of new primary cell culture systems, termed 'organoids', in which cells grow as 3D structures and spontaneously self-organize into stem cell/progenitors and properly differentiated functional cell types, which recapitulate at least some functions of the organ [1]. Dr. M. Huch recently established adult mouse and human healthy liver and pancreas organoid models [2-4]. Based on this near-physiological organoid culture, I then demonstrated the proof-of-concept that organoid technology can also recapitulate in vitro human primary liver cancer (PLC), the second most lethal cancer worldwide. Indeed, I have pioneered the technique of generating organoids from PLC biopsies (so-called tumoroids) and demonstrated that they faithfully recapitulate and maintain the histopathology and genetic profile of the original tumor, even after long term culture. Therefore, they provide an extremely powerful resource for analysing the biology of PLC. Moreover, I have evaluated tumoroids use as a drug screening platform and provided the proof-of-concept that PLC tumoroids are relevant for in vitro and in vivo drug testing and for identification of actionable therapeutic targets (Broutier et al., Nature medicine 2017). Recent advances in sequencing technologies have enabled high-throughput quantification of individual cell transcripts. These single-cell assays such as single cell RNA sequencing (scRNAseq) have enormous power to reveal differentiation hierarchies, lineage choice control and cell fate, and are therefore of great importance to study cancer stem cells (CSCs) biology. Then, moving forward I'm currently using my newly established PLC tumoroids and scRNAseq, to study the functional intra-tumoral heterogeneity and CSCs plasticity in human PLC.

119 Enhanced chondrogenesis in FOP-iPSC-derived paraxial mesoderm

Nakajima T, Shibata M, Nishio M, Nagata S, Alev C, Sakurai H, Toguchida J, Ikeya M

CiRA, Kyoto University, Japan

Keywords: Somitogenesis, iPSC, Disease modeling, Fibrodysplasia Ossificans Progressiva

Somite (SM) is a transient stem cell population that gives rise to multiple cell types such as dermatome (D), myotome (MYO), sclerotome (SCL), and syndetome (SYN). SM could also be an origin of mesenchymal stromal cells (MSCs), a postnatal cellular source of bone, cartilage and adipose. Although several groups have reported induction protocols of MYO and SCL from pluripotent stem cells, there is no report to induce SYN, D and MSCs through SM. Here we report the systematic induction of these cells from human induced pluripotent stem cells (iPSCs). We also successfully applied these protocols to disease modeling of fibrodysplasia ossificans progressiva (FOP), an inheritable disease characterized by heterotopic endochondral ossification in soft tissues after birth. Importantly, FOP-iPSC-derived MSCs showed enhanced chondrogenesis, while FOP-iPSC-derived SCL did not, possibly recapitulating normal embryonic skeletogenesis in FOP. These results consolidate the usefulness of multipotent SM for disease modeling and future cell-based therapies.

120 Detection of LGR5-positive cells revealed a new stem cell niche during odontogenesis

Olbertova K, Hrculak D, Hampl M, Dosedelova H, Vrlikova L, Korinek V, Buchtova M

Masaryk University, Czech Republic

Keywords: tooth, progenitor cells, odontogenesis

Tooth development is a complex process where the oral ectoderm interacts with surrounding mesenchyme. The prenatal and postnatal growth of tooth is driven by stem or progenitor cells. These cells are important for division, proliferation of cells or their differentiation to the odontoblasts, ameloblasts and other types of dental cells. Persistence of these cells in later stages enable odontoblasts self-renewal, which is essential for production of hard dental tissues. We propose the existence of stem cell niche in the dental stalk and in the dental lamina. To detect progenitor cells, we selected two progenitor markers. LGR5 (Leucine-rich repeat containing G-protein-couple receptor 5) represents a transmembrane receptor, which was identified as a marker of adult stem cells of numerous tissues. The second selected marker, SOX2 (SRY-Box 2) is a transcription factor, which controls division of stem and progenitor cells. We collected pre- and postnatal stages of transgenic mice LGR5-EGFP-IRES-CreERT2 and detected LGR5 signal by immunofluorescent staining. LGR5-positive cells were found in the area of epithelial thickening, dental stalk, and the base of the rudimentary successional dental lamina or surrounding mesenchyme. We observed asymmetrical expression of LGR5 in the lingual side of the dental stalk epithelium and also in the labial side of the surrounding mesenchyme. Double labelling with SOX2 was used to reveal their possible co-expression. SOX2-positive cells were detected similar to LGR5-positive cells in the lingual side of the dental stalk epithelium but mesenchymal expression was distinct. In conclusion, we observed stage-specific epithelial co-localization of LGR5- and SOX2-positive cells. Based on overlap of SOX2 and LGR5 in the lingual side of developing molars, we propose this area as a new stem cell niche for odontogenesis. This work was supported by the Czech Science Foundation (18-04859S) and Masaryk University (1403/2017).

121 Fixation and spread of somatic mutations in adult human colonic epithelium

Olpe C, Nicholson A, Winton DJ

Wellcome - MRC
Cambridge Stem Cell Institute , UK

Keywords: colon, mutations, dynamics

The mammalian intestine is a rapidly renewing tissue whose turnover is fuelled by the division of stem cells residing at the bottom of invaginations called crypts. Lineage-tracing experiments conducted in mice have shown that these stem cells constitute an equipotent population that replaces each other in a dynamic process called "neutral drift". Recently, we developed a method to investigate colonic stem cell dynamics in human tissue by detecting somatic nuclear DNA mutations in X-linked genes by immunohistochemistry. Applying this method of "clonal marks" to normal tissue samples from a large cohort of patients and using mathematical modelling to aid in the interpretation of an age-related increase in clone frequencies has established the baseline stem cell dynamics in normal human colon. We found that each human colonic crypt contains 5-10 (mean 7) functional stem cells that replace each other at a rate of 0.65 – 2.7/crypt/year (mean 1.3) – around 100x slower than in the mouse. For all our marks we detected patches of mutant crypts that, in addition, allow the rate of lateral expansion to be determined. Against these benchmarks biased stem cell behaviours advantaged within and beyond the crypt could be quantified. These include the cohesin complex member STAG2, the histone lysine demethylase KDM6A and the known cancer driver KRAS. As such we have shown by next-generation sequencing that ageing humans accumulate large patches of KRAS-mutant epithelium in their colons, which may predispose for neoplastic development. We are now using KDM6A-deficient clones to quantify the process of patch formation within the human colon, which we hypothesise occurs due to crypt fusion and fission events.

122 Characterisation of Equine Embryonic Stem Cell Derived Tenocytes Using Genome Wide Transcriptional Analysis

Paterson YZ, Henson F, Guest DJ

Animal Health Trust, UK

Keywords: Equine Tendon-Injury RNA-sequencing Scarring

Tendon injuries are a common occurrence in human and equine athletes. Adult tendon injuries heal via the formation of biomechanically inferior scar tissue resulting in high re-injury rates. Fetal tendon injuries however undergo total scar-less regeneration. Novel cell therapies should therefore try to recapitulate this scar-less fetal tendon regeneration. This study aims to build upon research into the use of equine embryonic stem cells (ESCs) to aid in tendon regeneration by determining if tenocytes derived from ESCs are more representative of the fetal or adult stage of tendon development. Using genome wide transcriptional analysis, we compared fetal, adult and ESC-derived tenocytes cultured and differentiated via our 3D collagen matrix system. 1,495 genes were differentially expressed between adult and ESC-derived tenocytes, whereas 1,342 genes were differentially expressed between fetal and ESC-derived tenocytes (log fold change ±2 ;q-value <0.01). Genes which were significantly upregulated in adult tenocytes tended to be expressed at similar levels in fetal and ESC-tenocytes and were highly associated with immune system processes and the recognition of foreign external stimulus. Interesting candidate genes included Interleukin 6 (IL-6) which has been implicated as a key inflammatory cytokine in wound repair. Genes which were significantly upregulated in fetal tenocytes tended to be even further upregulated in ESC-derived tenocytes and were highly associated with cellular adhesions. Interesting candidate genes included integrins ITGA6 and ITGB4 which are transmembrane glycoproteins which bind extracellular matrix proteins such as fibronectin and collagens and have been linked to fetal wound healing in skin. In summary, our initial work towards global gene expression profiling in fetal and adult tenocytes has yielded useful information into key over-represented pathways and novel genes that are likely to play a role in regulating tendon development and healing.

123 Adult cardiac progenitor cells and autophagy modulation for cardiovascular regeneration

Picchio V, Pagano F, Schirone L, Angelini F, Sciarretta S, Isotta C

La Sapienza University of Rome, Italy

Keywords: Cardiac stem cells, Autophagy, Heart regeneration

Ischaemic heart disease leads to remodelling and progressive heart failure (HF). Despite advances in medical treatments, new therapies against HF progression are needed. Cardiac cell therapy with resident cardiac progenitor cells (CPCs) is a promising regenerative option for HF under clinical evaluation. Autophagy is an intracellular degradation process of damaged components that can be cytoprotective or trigger cell death. ATG7 is one of the main autophagy regulators which can be also pharmacologically induced by the disaccaride trehalose. Autophagy promotes cardiomyocyte survival during ischemia, but its role in CPCs biology needs elucidation. In this project we investigate the effects of autophagy modulation (genetic/pharmacological) on resistance to stress, phenotype and regenerative potential of CPCs. CPCs are isolated from atrial explants of 4 weeks old C57BL/6J mice through selection by spontaneous spheroid growth as cardiospheres (CSs), and culture as CS-derived cells (CDCs) monolayers. CDC immuno-phenotype by flow cytometry is: CD45 neg, 4% CD31+, 65% CD90+, and 15% CD117+.This is consistent with a non-hematopoietic stromal profile including populations of endothelial and cardiovascular progenitor cells, further confirmed by clonal growth efficiency up to 60% at early passage. Preliminary experiments showed that 50mM trehalose treatment upregulated ATG7 gene expression after 5 days, whereas stress induced by hyperglycemia (50mM glucose) decreased ATG7 expression, as assessed by realtime PCR. For autophagy genetic induction, we have optimized transduction with 2 adenoviral vectors for either ATG7 overexpression (AdV-ATG7) or interference through short hairpin RNA (shATG7-AdV). Ongoing experiments aim at assessing how autophagy modulation through trehalose, ATG7-AdV or shATG7-AdV can affect: 1) CDC survival after stress, 2) CDC cardiovascular commitment, 3) CDC response to a cardiogenic microenvironment induced by rat neonatal cardiomyocyte-conditioned media.

124 Pharmacological inhibition of MAP4K4 rescues human stem cell-derived cardiomyocytes and suppresses ischemia-reperfusion injury in mice

Schneider M, Chapman K, Fielder L, Malfoshie E, Jenkins, M, Arabacilar P, Bellahcene M, Noseda M, Harding S, Jarvis A, Newton G, Perrior

Imperial College London, UK

Keywords: human iPSC-derived cardiomyocytes, myocardial infarction, apoptosis, drug discovery

Although directly suppressing cardiomyocyte death is a logical therapeutic strategy in acute myocardial infarction, no clinically approved counter-measures target the relevant signaling pathways. Historically, progress has been hampered by the lack of any preclinical target validation in a human context. Mitogen-Activated Protein Kinase Kinase Kinase Kinase-4 (MAP4K4) is activated in failing human hearts and mouse models of heart disease, including ischemia-reperfusion injury, engaging the TAK1-JNK pathway for cardiac muscle cell death. By gene silencing in human cardiomyocytes derived from pluripotent stem cells (hiPSC-CMs), we demonstrated that death induced by H2O2 or menadione requires MAP4K4. On this basis, we then developed highly selective small-molecule inhibitors of MAP4K4 through 3D field-point modeling, screening in silico, and iterative rounds of structure-based drug design. Our pharmacological inhibitors efficiently preserve survival, mitochondrial function, and calcium cycling in hiPSC-CMs. In proof-of-concept experiments, three MAP4K4 inhibitors of our lead series with suitable pharmacokinetic properties reduces infarct size by 50-60% in mice, even if given after the onset of injury. These experiments (1) implicate MAP4K4 as a well-posed target toward suppressing human cardiomyocyte death and (2) highlight the utility of hiPSC-CMs in target identification and cardiac drug development.

125 Insulin-producing beta-like cells derived from direct hepatocyte reprogramming ameliorate autoimmune diabetes

Shen CN, Chang FP, Shen CR, Chu EPF, Sytwu HK

Genomics Research Center, Academia Sinica, Taiwan

Keywords: lineage reprogramming, Autoimmune diabetes, Hepatocyte

Type 1 diabetes mellitus (T1DM) is characterized by complete loss of beta-cells due to T-cell mediated autoimmune attacking. Islet transplantation is a potential way to sustainably control blood sugar levels of T1DM patients'. However, the shortage of donor islets and poor islet graft survival limit the potential use of islet transplantation to treat T1DM patients. We recently discovered combination of Pdx1 and Ngn3 can convert liver cells to insulin-producing beta-like cells that exhibit the characteristics of pancreatic beta-cells. Treatment with PDGF-AA was found to facilitate Pdx1 and Ngn3-induced reprogramming of hepatocytes to beta-like cells with the ability to secrete insulin in response to glucose stimulus. Importantly, this reprogramming strategy could be applied to adult mouse primary hepatocytes, and the transplantation of beta-like cells derived from primary hepatocyte reprogramming could ameliorate hyperglycemia in diabetic mice. The efforts provided solutions to donor shortages and poor islet graft survival and functions. However, patients received the allogeneic islet transplantation were still suffering from side effects of the immunosuppressive medications. Hence, the possibility of producing immune-tolerable beta-cells would be a key challenge for developing cell-based therapeutics for T1DM patients. Since the liver has immune privileged properties, we therefore hypothesize beta-cells derived from hepatocyte reprograming mat possess immune-modulating capability. We then reprogrammed primary hepatocytes derived from Non-obese diabetic (NOD) mice with Pdx1/Ngn3/PDGFRa that resulted in efficient generation of glucose-responsive beta-like cells. Autologous transplantation of hepatocyte-derived beta-like cells to diabetic NOD mice significantly improved hyperglycemic status without needs of tolerogenic treatments. We further demonstrated induced expression of galectin-3 and galectin 9 by PDGF signaling in hepatocyte-derived beta-like cells could significantly suppress autoreactive T cells thus explain why hepatocyte-derived beta-like cells could ameliorate autoimmune diabetes. The findings raise the possibility of developing cell therapeutic strategies for patients with type 1 diabetes via autologous hepatocyte reprogramming.

126 Derivation of long-term expandable Small Intestinal Organoids from Human Pluripotent Stem Cells

Kramer PM, Soriano PC, **Simmini S**, Conder RK, Thomas TE, Eaves AC, Louis SA

STEMCELL Technologies

Keywords: hPSCs, intestine, organoids

Derivation and expansion of small intestinal organoids from human pluripotent stem cells (hPSCs) represents an attractive tool for drug screening, disease modelling and cell-replacement therapy. We have developed the STEMdiff™ Intestinal Organoid Kit, which is a specialized serum-free medium that promotes differentiation of human pluripotent embryonic stem (ES) and induced pluripotent stem (iPS) cells through the developmental stages of definitive endoderm, mid-/hindgut, and small intestine. Monolayers were generated with multiple hPSC lines maintained in mTeSR™1 on Corning®Matrigel®-coated dishes and then induced to differentiate into definitive endoderm. These cultures were then further differentiated into posterior endoderm, which gives rise to mid-/hindgut endoderm that releases 3-D spheroids into the culture medium. When these spheroids were embedded in Corning® Matrigel® and cultured in fully-defined STEMdiff™ Intestinal Organoid Growth Medium (OGM), they generated small intestinal organoids composed of a polarized epithelium patterned into villus-like structures, and a surrounding mesenchyme. These intestinal organoids can be maintained long-term through passaging or cryopreserved. Analysis of the monolayers at the definitive endoderm stage resulted in 81.6% ± 8.6% (n=21) cells expressing the endodermal markers FOXA2 and SOX17. Differentiation into posterior endoderm was confirmed with 71.4 ± 8.5% (n=13) of cells expressing the mid-/hindgut marker CDX2. The spheroids that detached into the medium were highly enriched for CDX2+/E-cadherin+/EpCAM+ epithelial cells and adjacent CDX2+/VIM+/EpCAM- mesenchyme. Organoids cultured for > 28 days in vitro consisted of enterocytes (Villin+), goblet cells (MUC2+), enteroendocrine cells (chromogranin A+), Paneth cells (LYSO+), and intestinal stem cells (LGR5+). In summary, STEMdiff™ Intestinal Organoid Kit supports the derivation of human small intestinal organoids from hPSCs in a highly efficient and reproducible manner.

127 Using human iPSC-derived osteoblasts for modelling osteoporosis

Stirmlinger N, Pfaender S, Tuckermann J, Boeckers TM

University of Ulm, Germany

Keywords: induced pluripotent stem cells, osteoporosis, osteoblasts

Osteoporosis is a disease which is caused by an imbalance between bone forming activity and bone resorption. Around 7.8 million patients, mainly of the elderly population, are affected which translates to roughly 15 % of the world's population (Wade et al., 2014). Current treatment strategies work against bone resorption, effectively blocking it and hence leading to fewer bone loss. However, these pharmaceuticals affect the bone's physiological performance causing it to become static which again promotes fractures. Therefore, we aimed to established a protocol for the cultivation of mature human iPSC-derived osteoblasts like it was first published by Kanke and colleagues (Kanke et al., 2014). These osteoblasts will then be used to test the effect of novel regulators of osteoblast differentiation that have previously been revealed a siRNA screen in MC3T3 cells. In this study we provide proof that our protocol enables us to induce the osteogenic pathway in human iPSC using qRT-PCR, western blotting, immunocytochemistry and osteoblast-specific stainings such as Alizarin Red and Safranin-O.

128 Notch/Wnt cross-signalling regulates stemness of dental pulp stem cells through a link between core pluripotency factors, metabolism and epigenetics

Uribe-Echevarria V, Agliano A, Luzuriaga J, García-Gallastegui P, Unda F, Ibarretxe G

The University of the Basque Country, Spain

Keywords: Dental Pulp Stem Cells, Stemness, Metabolism, Epigenetics

Dental pulp stem cells (DPSCs) from adult teeth express neural crest (NC) markers together with core transcriptional factors associated with stem cell pluripotency, such as Oct4a, Sox2, c-Myc, Rex1, Stella/ Dppa3, Ssea1/Fut4, Lin28 and Nanog. The possibility to boost the natural stemness features of DPSCs by mild methods, that do not involve gene and/or chromatin modification or gene transfection, is highly desirable for cell therapy 1,2. These stem cells posses promising properties such as high rate of proliferation, stemness potential, low immunoreaction and the possibility to develop personal therapies 3-5. Canonical Wnt and Notch are two highly conserved developmental signalling pathways that are involved in NC emergence and stem cell self-renewal. We determined that both pathways coordinate to regulate the expression of core pluripotency and NC factors in DPSCs6. Pharmacological inhibition of the Notch pathway for 48 h, by the γ-secretase inhibitor N-[N-(3,5-Difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT), abolished the expression of NC and core factors. This pluripotency network seems to be connected with metabolism which is mainly glycolytic and highly oxidative 7. Epigenetics plays also a relevant role preventing DNA from methylation and increasing acetylation marks in histones8. Genetic, metabolism and epigenetics would be connected by complex networks which allow cell reprogramming. In addition, Notch inactivation induced a silencing of the canonical Wnt signalling and a clear reduction in the stemness potential of DPSCs, as shown by a reduced ability to generate mature, fully differentiated osteoblasts and adipocytes. Conversely, pharmacological activation of the Wnt pathway for 48 h, by either the glycogen synthase kinase 3 beta (GSK3-β) inhibitor 6-bromoindirubin-3´-oxime (BIO) or the human recombinant protein Wnt-3a, not only largely increased the expression of NC and core factors, but also increased the efficiency of DPSCs to differentiate into mature osteoblasts and adipocytes6. These results showed that a short preconditioning activation of Wnt/Notch signalling by small molecules and/or recombinant proteins enhanced the stemness and potency of DPSCs in culture, which could be useful for optimising the therapeutic use of these and other tissue-specific stem cells.

129 The influence of Obesity and Diabetes on the function of adult stem cells of the Hypothalamic-Pituitary-Adrenal Axis

Werdermann M, Andoniadou CL, Berger I, Bornstein SR, Steenblock C

Technische Universität Dresden, Germany

Keywords: adult stem cells, metabolic disease, HPA-axis

The endocrine system involves communication among different tissues including the pancreas, adipocytes and components of the hypothalamic-pituitary-adrenal (HPA) axis. How these systems communicate can affect the progression of diabetes and obesity. For example, leptin produced in adipose tissue or insulin produced in the pancreas both modulate HPA activity. However, which signalling pathways are involved and also the role of adult stem cells (ASC)/progenitors in this dysregulation is still fairly unknown. The aim of the present study is to elucidate the influence of obesity and diabetes on the regulation of HPA homeostasis. In order to do this, we will characterise ASC populations of the pituitary as well as the adrenal gland and analyse their roles under metabolic disease. In vitro, we will study ASCs via the addition of leptin and insulin to primary cultures of Nestin+ cells isolated from the adrenal as well as the pituitary gland. Furthermore, tissues from normal and high-fat diet models will be examined to assess the role of ASCs in disease models. So far, we were able to demonstrate that insulin acts in a growth-promoting manner on both the cortical and medullary Nestin+ cells. Furthermore, cortical Nestin+ cells survived and proliferated in culture in the absence of insulin whereas a lack of insulin decreased the amount of medullary Nestin+ cells in vitro and attenuated the differentiation process. Moreover, we showed that the proliferation potential is elevated in cortical Nestin+ cells once insulin is added to the culture. In addition, mRNA expression levels revealed that insulin influences several differentiation aspects e.g. promoting steroidogenesis. Both diabetes and obesity influence the HPA-axis itself resulting in a hyperactivation of HPA-function. So far, it has not been addressed whether insulin and leptin affect the adult stem cell population in the pituitary and in the adrenal gland contributing to an exacerbation of an obese or diabetic phenotype.

130 Targeting BMPRII signaling in stem cell-derived vascular cells to identify a piperidine analogue for the treatment of pulmonary arterial hypertension

Yang J, Xing Y, Zhao S, Wei Q, Zhou F, Gong S, Zhao X, Al-Lamki R, Ortmann D, Du M, Pedersen R, Shang G, Si S, Morrell N

Institute of Basic Medical Sciences, CAMS, China

Keywords: BMPRII signaling, Pulmonary Arterial Hypertension, stem cell based drug screening

Rationale: Deficiency of bone morphogenetic protein type II receptor (BMPRII) signaling has been implicated in the pathobiology of pulmonary arterial hypertension (PAH). Targeting BMPRII signaling with small molecular compound to selectively improve the function of the vascular endothelium could be a desirable therapeutic intervention for PAH. Objective: To apply stem cell technology in drug discovery and identify compounds with novel chemical structure for the treatment of PAH. Methods and Results: Because BMP2 has been reported to enhance endothelial BMPRII signaling, we screened 8 160 small molecular compounds for their potential as BMP2 upregulators. And we performed an assay on the BMP downstream gene Inhibitor of DNA binding 1 (Id1) using a dual reporter driven specifically in human embryonic stem cell (hESCs) derived endothelial cells. A novel piperidine (BMP upregulator 1, BUR1) with half-maximal effective concentration (EC50) of 30.59 nmol/L was shown to increase endothelial Id1 expression. Microarray analyses showed that BUR1 induced BMP2 transcription and angiogenesis related factors. In combination with Immunoblotting results, we confirmed the enhancement of Id expression in human pulmonary artery endothelial cells (HPAECs). To strictly compare the signals between heterozygous autologous cells and wild type, we generated hESCs with the R899X heritable PAH mutation (hESCs-R899X). BUR1 effectively rescued deficient angiogenesis in BMPR2+/R899X ECs. In rats, BUR1 prevented and reversed PAH after the daily administration of 4.5 mg/kg in monocrotaline (MCT) rats. This treatment also effectively restored BMPRII signaling in the pulmonary arterial endothelium in Sugen/hypoxia PAH model. Conclusions: We provide a novel chemical structure type that increases BMP2 expression and targets the BMPRII signaling pathway. Using stem cell technology, we gained a clearer understanding of the genetic cause of PAH and identified a potential therapeutic treatment of PAH.

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131 Towards the generation of chimaera-competent marmoset embryonic stem cells

Slatery E, Ampartzidis M, Boroviak T

University of Cambridge, UK

Keywords: Naive pluripotency, nonhuman primate models

Conventional human and nonhuman primate pluripotent stem cells (PSCs) transcriptionally correspond to the postimplantation epiblast. Recently, several groups have reported culture conditions supporting a putative naïve pluripotent (preimplantation epiblast-like) state in human pluripotent cells (Gafni et al., 2013; Takashima et al., 2014; Theunissen et al., 2014; Ware et al., 2014; Duggal et al., 2015; Guo et al., 2017; Yang et al., 2017). However, naïve human PSCs cannot be tested for functional equivalence to the preimplantation epiblast in chimera contribution assays for ethical reasons. In my PhD project, I seek to generate naïve pluripotent marmoset PSCs and evaluate their ability to contribute to normal development in vivo. The specific aims of my proposal are to: (i) refine culture conditions promoting a naïve pluripotent state in marmoset PSCs; (ii) carry out joint profiling of chromatin accessibility, DNA-methylation and transcriptome in naïve marmoset PSCs for direct comparison to the in vivo preimplantation epiblast; (iii) functionally define the essential, primate-specific regulators of naïve pluripotency in gain- and loss-of function candidate screens, (iv) assess survival, incorporation and pluripotency maintenance of naïve marmoset PSCs in mouse blastocysts; and (v) examine the contribution of naïve marmoset PSCs to marmoset chimeras.

132 Probing transition points in cell fate decisions

Baye J, Mulas C, Hannezo E, Smith A, Chalut K

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: cell fate; transition; entropy

The dynamics of cell fate transitions in development and in the adult are still poorly understood. Conceptually, cell fate transtions arise when an external stimuli pushes a cell out of one stable state and into another. In this context, it has been suggested that a cell must cross a transition or tipping point in the process. The crossing of this transition point is pivotal to successfull cell fate dynamics and it is therefore of interest to locate it in a data-driven manner. I aim to identify such transition points with single cell RNA sequencing (scRNAseq) and in live-cell culture. I motivate entropy - the amount of uncertainty of a system - as a fundamental measure of the approach of a transition point. I show how entropy can be estimated with scRNAseq data in practice and discuss current experimental and data-related challenges. Finally I discuss the opportunities to leverage scRNAseq data to monitoring the approach of transition points in live-cell culture.

133 Temporal dynamics of Tet1 and Oct4 reactivation identify temporally distinct stages in late reprogramming

Bartoccetti M, Luo X, Khoueiry R, Xu J, Janiszewski A, Verfaillie C, Pasque V, Koh KP

KU Leuven, Belgium

Keywords: reprogramming, Tet1, epigenetics

Induced pluripotent stem cells (iPSCs), reprogrammed in vitro from somatic cells, have enormous potential in regenerative medicine. Current understanding of the temporal events in reprogramming has mainly focused on the early stages, while less is known of the later maturation phase, when the master pluripotency regulator OCT4/POU5F1 is reactivated and global DNA demethylation occurs. The Ten-Eleven-Translocation TET DNA oxygenases mediate DNA demethylation during reprogramming. Both Tet1 and Oct4 are regulated by distal and proximal cis regulatory domains active respectively in naive and primed pluripotent states [1, 2]. Here, we have generated murine transgenic lines harboring dual fluorescent reporters reflecting cell-state specific expression of Oct4 and Tet1. By crossing these reporter, we used time-course live cell imaging and flow cytometry to identify a deterministic trajectory of events marked by temporally distinct stages of Tet1 and Oct4 gene activation during reprogramming of mouse embryonic fibroblasts to iPSCs. First, Tet1 and Oct4 are activated by proximal regulatory elements at both genes, followed almost immediately by a second phase of activation of distal regulatory domains, in which naive-specific Tet1 gene activity clearly precedes that of Oct4. The sequential order is conserved in different reprogramming methods and media conditions, and occurs after entry into pluripotency marked by NANOG protein expression but prior to induction of DPPA4. By sorting reprogramming intermediates based on dual reporter patterns, we have identified and characterized temporally distinct late-stages of reprogramming associated with the reactivation of the pluripotency network. Moreover, clonal iPSC lines lacking Tet1 exhibit decreased genomic stability and fail to activate germ-line and meiotic genes normally upregulated with Tet1. Thus, Tet1 reactivation from its naive specific promoter may have an essential role in the establishment of high quality naive iPSCs.

134 Control of cellular identity during reprogramming by the Bcl11b/Bcl11a equilibrium

Huyghe A, Furlan G, Wang J, Yu Y, Braun SMG, Nefzger CM, Lainé A, Wajda P, Goddard I, Gadot N, Marie J, Liu P, Crabtree GR, Polo JM, Lavial F

Cancer Research Center of Lyon, France

Keywords: cellular identity, reprogramming, Bcl11a/b

A key challenge for developing embryos is to establish the identity of the cells by restricting progressively their plasticity. In contrast, such plasticity is regained during the conversion of somatic cells into induced pluripotent stem cells (iPS) (1) by the combined expression of Oct4, Sox2, Klf4 and c-Myc (OSKM). Cellular transformation also implies cellular identity loss and acquisition of embryonic features, leading to the hypothesis that oncogenes such as c-Myc and K-RasG12D might induce a reprogramming process in somatic cells to drive the acquisition of the malignant phenotype (2). For these reasons, a better understanding of cellular identity maintenance and cellular plasticity acquisition might have profound implications for regenerative medicine and cancer biology. The aim of this study is to characterize and compare the early stage of pluripotent reprogramming and malignant transformation in order to (i) define the reprogramming roadmaps leading to cellular identity loss and (ii) identify common molecular mechanisms controlling cellular identity. Combining FACS cell sorting with RNA-seq strategies led us to define a molecular signature shared by reprogramming cells in the early days of both iPS cells generation, induced by OSKM and malignant transformation triggered by c-Myc and Kras. In both scenarios, we showed that loss of cellular identity is correlated with a switch in the expression of the transcription factors Bcl11b and Bcl11a, components of the SWI/SNF complex known to instruct cell fate decision in the lymphoid system (3-5). Using gain- and loss-of-function approaches, we revealed that the modulation of this balance controls somatic cell identity loss and the emergence of reprogrammed derivatives. Moreover, the use of Bcl11-reporter mice allowed us to capture a novel and rare "intermediate state" emerging in the early days of both processes, and define a molecular roadmap of pluripotent and malignant reprogramming. The transcriptomic and epigenomic characterization of these novel cellular state might shed light on the molecular mechanisms constraining reprogramming and therefore iPS cells generation and tumorigenesis.

135 Setting the stage for gastrulation: Contact with the extraembryonic basement membrane promotes epithelial organisation in gastruloids; an in vitro model system for early post-implantation development

Baillie-Johnson P, Williams I, Nichols J

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: EMT, Gastrulation, Gastruloid, Polarity.

Gastruloids break symmetry to produce axially patterned, elongated structures containing all three germ layers, in the absence of the extraembryonic tissues [1]. The occurrence of germ-layer specification and cell emergence from gastruloids makes them a model system for studying gastrulation in vitro. Through immunostaining, groups of cells in gastruloids can be equated to corresponding regions of the gastrula, showing that the mesoderm and endoderm initially emerge in a spatially disorganised manner and some cells are shed into the medium. At this stage, gastruloids contain apico-basally polarised epithelia around small cavities and in regions at the surface of the tissue, but this organisation is discontinuous. The intervening tissue is a site of EMT, as indicated by the expression of E-Cadherin, N-Cadherin and Snail. Through a 24-hour period, the cells in the gastruloids transition from a state resembling the posterior epiblast to a mixture of all three germ layers that later becomes spatially organised. To improve the fidelity of this system and to corral the emergent mesendodermal cells into new tissues, an outer VE-like layer was generated by GATA4 induction and self-organised cell sorting. When enveloped by this layer, the internal cells develop into an epithelium that is apico-basally polarised in relation to the overlying basement membrane, as in the epiblast. Over time, a T/Brachyury-expressing primitive streak-like region forms in one part of the inner cell epithelium, demonstrating antero-posterior polarisation. Through comparison to uninduced gastruloids, I will use these complementary in vitro systems to dissect the regulation of cell fate specification at gastrulation and the crucial process of EMT.

136 The role of DNA hydroxylases Tet1 in embryonic cell fate decision

Luo X, Khoueiry R, Sohni A, Velde JV, Bartoccetti M, Veer BV, Koh KP

KU Leuven, Belgium

Keywords: Tet1, DNA methylation, lineage decision

TET (ten-eleven-translocation) genes are DNA hydroxylases that convert 5-methylcytosine to 5-hydroxymethyl-cytosine, an intermediate form involved in DNA demethylation. Previous studies have been showed that Tet1 and Tet2 can regulate 5hmC production and cell lineage specification in mouse embryonic stem cells (ESCs). Here, we showed that Tet1 null ESCs across different genetic backgrounds in two Tet1 knockout models consistently displayed increased upregulation of mesendoderm (ME) marker genes during embryoid body (EB) differentiation, accompanied by activation of Smad2 and impaired downregulation of Oct4. Knockdown of Oct4 can rescue the ME skewing phenotype in Tet1 knockouts. Moreover, Tet1–deficient ESCs showed abrogated expression of neuroectoderm related markers and defective formation of neurons during in vitro differentiation; these defects can be partially rescued by restoring the expression of Tet1 or its catalytic domain mutant, suggesting that TET1 uses both catalytic and non-catalytic mechanisms to regulate lineage genes. We further defined cell-state specific genomic regions bound by Tet1 in both primed epiblast-like cells (EpiLCs) and naive ESCs. Gene ontology (GO) analysis indicated that the primed state specific regions, but not the naive state specific regions are enriched in terms associated with lineage determination. In addition, further ChIP-seq analysis showed that Tet1 and Oct4 co-bind at inter-genetic regions in EpiLCs. In contrast, they strongly co-bind at promoter regions in naive ESCs. Collectively, these results suggest the importance of Tet1 in the regulation of pluripotency state transition and germ-layer specification. Further studies will provide insights into the physiological functions of Tet1 in cell fate choice and development.

137 Developmental roles of the deubiquitinase Usp9x

Macrae TA, Wong C, Nora EP, Larsen B, Bruneau B, Gingras AC, Ramalho-Santos M

Lunenfeld-Tanenbaum Research Institute, Canada

Keywords: embryonic stem cells, pluripotency, ubiquitination

Hypertranscription drives the rapid proliferation of the peri-implantation embryo. This state of permissive chromatin and elevated global transcriptional output is captured in vitro by pluripotent mouse embryonic stem (ES) cells. We recently showed that the permissive chromatin state and hypertranscription of ES cells are acutely sensitive to the translational output and proteome composition. Post-translational modifications, notably ubiquitination, further fine-tune translational output. A genome-wide screen indicated that the deubiquitinase Usp9x helps maintain the euchromatin/transcriptional state of ES cells. Usp9x has been implicated in the regulation of the switch from self-renewal to differentiation in multiple stem/progenitor cell types, but the underlying mechanisms remain unclear. We generated a system for tunable Usp9x expression by targeting an auxin-inducible degron (AID) motif to the Usp9x locus. Acute (< 8 hour) addition of auxin triggers swift yet incomplete proteasomal degradation of the AID-tagged protein, enabling separation of ES cells that lose (Usp9x-low) from those that retain (Usp9x-high) expression. RNA-sequencing revealed a remarkably rapid divergence in expression patterns between Usp9x-low and Usp9x-high cells. Whereas Usp9x-high cells resemble the pre-implantation epiblast, Usp9x-low cells correspond molecularly to a post-implantation, gastrulating epiblast. Consistent with this finding, Usp9x-low cells have a differentiated morphology and a severe self-renewal deficit. Intriguingly, the transcriptional rewiring of ES cells upon loss of Usp9x displays a high degree of similarity to perturbations of the function of Polycomb Repressive Complex 2 (PRC2), a key regulator of stem cell chromatin and biology. Studies are ongoing to identify the targets of Usp9x in ES cells, dissect the mechanism by which Usp9x interacts with PRC2 function, and investigate the role of Usp9x in the early mouse embryo. Our most recent findings will be presented.

138 Reprogramming human fibroblasts directly to naïve iPSC using mRNA

Guo G, Yang J, Bredenkamp N, Clarke J, Baker D, Smith A

Wellcome - MRC Cambridge Stem Cell Institute, UK

Keywords: human naïve iPSC , reprogramming, transgene-free

Pluripotent stem cells including iPSCs hold potential for both basic research and biomedical application due to their unlimited self-renewal and differentiation to somatic lineages. Human epiblast in the embryo, the founder of fetus and the origin of pluripotent stem cells, develops continuously from emergence in the inner cell mass to a single layered epithelial disc, which is primed for gastrulation. Human pluripotent stem cells representing the more primed post-implantation epiblast have been well studied. We have recently reported generation of human naïve stem cells by derivation from dissociated inner cell mass and by resetting primed human PSC. These cells have a molecular signature related to emerging naïve epiblast. Here we report establishment of transgene-free human naïve iPSCs by somatic cell reprogramming. We find that reprogramming is rapid and relatively efficient using unmodified RNA. We have implemented this approach on multiple human fibroblast cell lines and endothelial progenitor cells. Clonal naïve iPSC lines can be expanded in culture for more than ten passages and retain a normal karyotype. They express a panel of naïve specific transcription factors and surface markers. Direct production of transgene free naïve iPSCs offers a new platform to evaluate differentiation propensity of naïve versus primed pluripotency. We thank Sarah Eminli-Meisser (ReproCELL), Wing Chang (StemCell Technologies) and Amer Rana (University of Cambridge) for reagents and advice. This research is funded by the Medical Research Council.

139 Transcriptional Control of Lineage Decisions in Embryonic Stem Cells by NuRD

Montibus B, Ragheb R, Diamanti E, Reynolds N, Dunn S.J, Hendrich B

Wellcome - MRC Cambridge Stem Cell Institute, UK

Keywords: NuRD, Chromatin, Gene expression, Regulation

A cell's identity is determined by the genes it expresses, and those it keeps silent. Precise control of gene expression is essential for a cell to correctly execute cell fate decisions. One of the key players in regulation of gene expression is the Nucleosome Remodelling and Deacetylase (NuRD) complex. Embryonic stem cells (ES) lacking MBD3, a core subunit of the complex, are unable to differentiate properly. The aim of our project is to probe the direct role of NuRD in the transcriptional changes that occur as cells exit the ES cell state and commit to differentiate. To this purpose, we are studying in detail ES cells, WT and mutant for Mbd3, undergoing a switch between self-renewing and neural differentiation. As cell fate decisions occur at the level of individual cells, we used single-cell RNA-sequencing to carefully measure gene expression levels during differentiation. The analysis of these data indicates a defect in the ability of the NuRD-mutant cells to repress pluripotency genes but an overexpression of genes associated with trophectoderm. Moreover, the NuRD-mutant cells fail to properly induce the expression of differentiation genes. Thus, although NuRD was initially defined as a transcriptional repressor, this observation and previous work showing that NuRD is often found at site of active transcription, suggest that it could also act as a transcriptional activator. We next address this hypothesis by investigating genome-wide NuRD-dependent chromatin changes during differentiation using ChIP-seq. We also conducted a detailed molecular analysis at some key regulatory regions of differentiation-induced genes to determine, at the molecular level, how NuRD-dependent chromatin modification facilitates activation of these genes. Altogether, our results suggest that NuRD could play a new direct role in the induction of the expression of lineage genes during differentiation. Overall, this study supports the picture of NuRD being a global regulator involved both in activation and repression of gene expression.

140 Regulation of chromosome stability in mouse embryonic stem cells

Novo CL, Sheekey E, Rugg-Gunn PJ

Babraham Institute, UK

Keywords: embryonic stem cells, heterochromatin, genome organization

Constitutive heterochromatin domains are major contributors to genome stability and function. For example, disruption of the chromatin features that define pericentromeric (PCH) and centromeric (CH) heterochromatin lead to loss of centromere function and aneuploidy1-2. The establishment and organization of these heterochromatic loci relies largely on their chromatin identity rather than the underlying DNA sequence3. Thus, it is interesting that in pluripotent embryonic stem cells (ESCs) PCH is characterized by more dispersed chromatin fibers, less repressive epigenetic marks and higher transcription than somatic cells4 5. Furthermore, in ESCs the PCH of several chromosomes cluster together forming large nuclear foci that are often used to identify these cells6. Deletion of epigenetic regulators affects PCH identity in both somatic and ESCs but it only triggers severe chromosome mis-segregation and accumulation of genetic aberrations in somatic cells7-8, suggesting that ESCs tolerate, or even require, a less repressive and more transcriptionally active PCH identity. Indeed, we have shown that the pluripotent transcription factor NANOG can promote transcription and regulate PCH organization in ESCs9. Importantly, we found that Nanog deletion in ESCs leads to chromatin compaction and reorganization of the PCH domains without affecting pluripotency9, suggesting a potential role for PC transcription in genome organization of ESCs. Interestingly, during early mouse development PC transcripts are crucial for the establishment of heterochromatin and proper embryonic development10-12. Thus, we hypothesized that a NANOG-dependent role for PCH transcripts in maintaining the characteristically open ESCs genome. Here, we report the effects of directly targeting PC transcripts in ESCs using LNA-DNA gapmers to downregulate PC transcripts. We found that downregulation of PC transcripts seems to recapitulate the chromatin remodeling observed in Nanog-/- ESCs, supporting our hypothesis for a direct role of PC transcripts in genome organization of ESCs. Furthermore, similar to Nanog-/- ESCs, we found that downregulation of PC transcription also leads to genetic instability, as seen by a significant increase in chromosome fusions and chromosome gaps. These results suggest an important novel role for the NANOG-dependent PC transcription in the maintenance of the PCH organization typical of ESCs.

141 Epigenetic and genetic effects of sex on reprogramming and pluripotency

Pasque V

University of Leuven, Belgium

Keywords: iPS cells, naive pluripotency, X chromosome dosage, DNA methylation, chromatin

Pluripotency can be captured from early embryos and also re-established from somatic cells by reprogramming approaches. However, how sex affects early embryonic development, pluripotency and reprogramming processes remains poorly understood. We have recently isolated isogenic male and female mouse induced pluripotent stem cells (iPSCs). Here, I will present new studies combining DNA methylation profiling, transcriptional profiling, pluripotency exit kinetics, chromatin profiling, proliferation kinetics and genetic analyses, and functional experiments to investigate the transcriptional, epigenetic and genetic effects of sex on the induction, maintenance and exit from pluripotency. I will show that the transcriptional state, DNA methylation, exit from pluripotency and proliferation of female iPSCs differs from that of male iPSCs, partly mimicking early embryo development. I will present evidence that X chromosome loss in female iPSCs resolves sex-specific differences but does not restore imprint methylation. I will show that DNA hypomethylation and pluripotency exit can be molecularly uncoupled in female embryonic stem (ES) cells through manipulation of the X-linked MAPK inhibitor Dusp9. I will also present evidence that the open chromatin landscape of ESCs is modulated by sex at thousands of chromatin regions and reveal the transcriptional regulatory logic by which sex influences pluripotency. Defining the mechanisms regulating the establishment, maintenance and exit from pluripotency in vitro and in vivo and understanding how these mechanisms are influenced by sex will have important implications for development, reprogramming and pluripotency.

142 Modelling metabolic landscapes of naive and primed human ESCs

Pearce J, Le Novère N

Babraham Institute, UK

Keywords: metabolism, modelling, single cell, development

Human embryonic stem cell lines can exist in two forms: "naive" (representative of inner cell mass cells) or "primed" (similar to epiblast cells). They are defined by differences in their dependency on kinases and methylation machinery, amount of DNA methylation, X-activation status, and the metabolic profile of the cells. More specifically, naive cells exhibit a preference for oxidative metabolic pathways, compared to primed cells which prefer glycolytic metabolism- amongst other changes in major pathways. It has been suggested that these differences may be linked to the changes in DNA methylation observed during the naive-primed transition. I have used constraint-based modelling of genome scale metabolic reconstructions (in the form of flux balance analysis) to study the differences in metabolism between the naive and primed states.The models are constrained using transcriptomic data and optimised for a given objective. Many reaction fluxes are observed to differ between the naive and primed models, most of which are in line with biological observations. We observe a significant transcriptional heterogeneity between populations of naive and primed cells. Using constraints based on single cell RNA-seq data, we can produce populations of model metabolic landscapes. Implementation of single cell models would allow us to directly investigate the effect of transcriptional variability on the metabolism of the cells. Alongside the characterisation of the metabolic landscape in these cells comes the potential to identify factors which contribute to differing DNA methylation patterns during development.

143 Integration-free reprogramming of human umbilical arterial endothelial cells into induced pluripotent stem cells reserved Tie2 expression

Pei H, Li H, Xie X, Nie J, Qu M, Fan Z, Bai Y, He L, Nan X, Yue W, Pei X

Beijing Institute of Radiation Medicine, China

Keywords: Endothelial progenitor cell, umbilical arterial endothelial cells, induced pluripotent stem cell

Endothelial progenitor cell (EPC) is an attractive source of therapeutic transplantation for ischemic diseases such as ischemic myocardial infarction and hindlimb ischemia. However, scarcity of donor tissue and inability of proliferation remain major obstacles. Recent advances in induced pluripotent stem cells (iPSCs) technology have paved the way for generation of unlimited numbers of ECPs from easily accessible cell sources. Whereas, endothelial lineage restriction with high efficacy remains challenging during differentiation. Here we have derived a stable iPSC line reserved Tie2 expression from human umbilical arterial endothelial cells (HuAECs) by transduction of four human transcription factors (Oct4, Sox2, Klf4, and c-Myc) using sendai virus vector. The cell line showed morphology of typical human pluripotent stem cells, expressed endogenous Oct4, Sox2, Nanog and surface antigen SSEA4 and TRA-1-60. It maintained a normal 46,XX female karyotype. Furthermore, embryoid bodies formed from HuAECs-derived iPSCs expressed marker genes of all three germ layers. Upon injection into mice HuAECs-derived iPSCs formed a teratoma containing differentiated cell types of all three germ layer lineages. It thus possesses all the characteristics of an iPSCs line. Accidentally, angiopoietin receptor Tie2 remained high expression level in this cell line while all other specific endothelial markers were dramatically down-regulated, such as CD31, CD144, Tie1, VWF. Since Tie2 signals play a key role in blood vessel specification, the iPSC line reserved Tie2 expression is highly desirable, which will likely facilitate to lineage differentiate and generate sufficient EPCs for cellular therapies in ischemic cardiovascular disease.

144 A LINE1-Nucleolin partnership regulates early development and embryonic stem cell identity

Percharde M, Lin CJ, Yin Y, Guan J, Peixoto GA, Bulut-Karslioglu A, Biechele S, Huang B, Shen X, Ramalho-Santos M

Imperial College London, UK

Keywords: LINE1, retrotransposons, MERVL, totipotency

Transposable elements (TEs) comprise nearly half of mammalian genomes and are often described as genome parasites or 'junk DNA'. LINE1 retrotransposons are the most abundant TE class and their expression is thought to be largely deleterious for cells. However paradoxically, LINE1 is highly expressed during early development. Here we report that LINE1 plays essential roles in mouse embryonic stem (ES) cells and in pre-implantation embryos. This function is dependent upon LINE1 RNA and is independent of LINE1 retrotransposition activity. In ES cells, LINE1 acts as a nuclear RNA scaffold that recruits Nucleolin and Kap1/Trim28 to repress Dux, the master activator of a transcriptional program specific to the totipotent 2-cell embryo. LINE1 depletion causes inappropriate activation of Dux, along with genes and transcripts driven by the 2-cell specific LTR retrotransposon, MERVL. Furthermore, depletion of LINE1, Nucleolin or Kap1 induces the formation of 2-cell (2C)-like cells in culture. In parallel, LINE1 mediates association of Nucleolin and Kap1 with rDNA, promoting rRNA synthesis, hypertranscription and ES cell self-renewal. In embryos, LINE1 is required for correct Dux silencing, synthesis of rRNA, and exit from the 2-cell stage. Finally, we show that Dux loci are associated with Nucleolin at peri-nucleolar heterochromatin in ES cells, but released to the nucleoplasm in 2C-like cells. These results reveal an essential partnership between LINE1 RNA, chromatin factors and peri-nucleolar chromatin in the regulation of transcription, developmental potency and ES cell self-renewal. We propose that LINE1 forms an integral part of the transcriptional networks that regulate cellular potency during early mammalian development.

145 Towards dissecting the gene regulatory trajectories governing cellular reprogramming at single cell resolution

Radley A, Dunn S-J, Li A, Smith A

Wellcome - MRC Cambridge Stem Cell Institute, UK

Keywords: Pluripotency, single cell RNA sequencing, cellular reprogramming

Cellular reprogramming is a heterogeneous and asynchronous process. It is well established that the efficiency of productive reprogramming is low and alternative cellular idendities arise within the same culture condition. However, it is unclear how the starting cells adopt different routes in reprogramming process. Single cell RNA sequencing (scRNA-seq) offers the potential to reveal the transcriptomic differences of individual cells within a population, which could be exploited to dissect the cellular trajectories of both productive and unproductive reprogramming. We propose using scRNA-seq data alongside state of the art computational modelling to dissect the heterogeneity and asynchrony of reprogramming trajectories. Computational methods will allow the logical connection of transcriptome snapshots over time into differing trajectories. By identifying distinct cell populations along a reprogramming time course, we aim to elucidate transcriptomic events which correlate with the distinct cellular trajectories. A primary and critical source of technical noise that must be navigated is the high incidence of false negative dropouts in scRNA-seq data. Currently, I have focused on techniques to impute missing gene expression. My investigation reveals that a significant degree of biological structure can be recovered from noisy scRNA-seq data. Such imputed data will provide clearer insights into cellular trajectories in reprogramming.

146 Investigating NuRD in human pluripotent stem cells

Ragheb R, Gharbi S, Cramard J, Burgold T, Kloet S, Vermeulen M, Reynolds N, Hendrich B

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: NuRD, Regulation, Differentiation, Human induced pluripotent stem cells

Cell identity is determined by the genes they express, and those they keep silent. Precise control of gene expression is essential for a cell to correctly execute cell fate decisions. One of the key players in this regulation is the Nucleosome Remodelling and Deacetylase (NuRD) complex [1, 2]. Indeed, constitutive mouse mutants for chromatin remodelling components show very early defects in gene expression patterns and die at about the time of implantation [3, 4]. A comprehensive understanding of how cells control transcription during cell fate decisions would be advantageous in order to control or instruct cell fate decisions, such as in regenerative medicine applications and in cancer biology. NuRD has not been previously characterised in human pluripotent cells however this cell type has the potential to be of enormous therapeutic impact. We have created human induced pluripotent stem (iPS) cell lines in which we can test the biochemical make-up, genome-wide occupancy, and biological function of the NuRD complex. We have found that the core components of NuRD in human iPS cells are analogous to those in mouse cells. By comparing RNA-seq datasets between mouse embryonic stem (ES) cells, mouse epistem cells (EpiSC) and human iPS cells, and by using mutants in all three systems we will determine in which ways NuRD function might differ between these cell types. By integrating these different types of genomics and functional data, we will be able to define the function of this abundant chromatin remodeller in human cells.

147 OCIAD1 controls electron transport chain complex I activity to regulate energy metabolism in human pluripotent stem cells

Shetty DK, Kalamkar KP, Inamdar M

Jawaharlal Nehru Centre for Advanced Scientific Research, India

Keywords: Energy metabolism, Stem cells, Multipotent progenitors

Pluripotent stem cells (PSCs) derive energy predominantly from glycolysis and not the energy efficient oxidative phosphorylation (OXPHOS). Differentiation is initiated with energy metabolic shift from glycolysis to OXPHOS. We investigated the role of mitochondrial energy metabolism in human PSCs using molecular, biochemical, genetic and pharmacological approaches. We show that the carcinoma protein OCIAD1 interacts with and regulates mitochondrial complex I activity. Energy metabolic assays on live pluripotent cells showed that OCIAD1-depleted cells have increased OXPHOS and may be poised for differentiation. OCIAD1 maintains human embryonic stem cells and its depletion by CRISPR/Cas9-mediated knockout leads to rapid and increased differentiation upon induction, whereas OCIAD1 overexpression has the opposite effect. Pharmacological alteration of complex I activity could rescue the defects of OCIAD1 modulation. Thus, hPSCs can exist in energy metabolic sub-states. OCIAD1 provides a target to screen for additional modulators of mitochondrial activity to promote transient multipotent precursor expansion or enhance differentiation.

148 Understanding the Dynamics of Embryonic Stem Cell Differentiation

Strawbridge S, Kugler H, Martello M, Smith A

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: Single-Cell, Live Imaging

The two defining features of mouse embryonic stem (ES) cells are self-renewal and naïve pluripotency, the ability to give rise to all cell lineages in the adult body. In addition to being a unique and interesting cell type, pluripotent ES cells have demonstrated their potential for continued advancements in biomedical science. Currently, there is an improved understanding in the chemical signals and the gene regulatory network responsible for the maintenance of ES cells in the naïve pluripotent state. However, less is understood about how ES cells exit pluripotency. Here we study the dynamics and the factors affecting the irreversible exit from pluripotency. Expression of the reporter Rex1-GFPd2, which is inactivated upon exit from naïve pluripotency, was analyzed by quantitative long-term single-cell imaging over many generations. Exit is associated with increasing cell motility, decreased cell-cell contact, and an acceleration in cell proliferation. The onset of exit is associated with a sudden and irreversible inactivation of the Rex1- GFPd2 reporter. This inactivation is asynchronous, as it occurs at different times and in different generations during ES cell differentiation. However, examination of daughter cells generated from the same mother revealed a high level of synchronicity. Further investigation revealed that high levels of correlation in cell-cycle duration and Rex1-GFPd2 expression exist between differentiating sister and cousin cells, providing strong evidence that cell potency is inherited symmetrically in cell divisions during exit in vitro.

149 Dido is needed for reprogramming mouse embryonic fibroblasts to pluripotency

Talavera-Gutiérrez A, Fütterer A, Pons T, de Celis J, Gutiérrez J, Martínez-A C

Centro Nacional de Biotecnología - CSIC, Spain

Keywords: reprogramming, differentiation, self-renewal, reprogrammable mice, mouse embryonic fibroblasts, MEF, embryonic stem cells, ESC

Pluripotent cell differentiation and somatic cell reprogramming share similar, although inverse processes(1). So far we demonstrated that the death inducer obliterator (Dido) locus is needed for embryonic stem cell (ESC) differentiation. Mice with homozygous N-terminal deletion of the Dido gene (DidoΔNt) die shortly after birth or develop diseases linked to impairment of differentiation processes such as hematopoiesis(2), depending on their genetic background. In contrast, homozygous ablation of the Dido3-specific C terminus (Dido3ΔCt) causes lethal developmental defects at the onset of gastrulation (d8.5)(3). ESC from these mice maintain self-renewal capacity but their differentiation is impaired(3,4). To study the role of Dido in somatic cell reprogramming, we crossed DidoΔNt mice with reprogrammable mice (4F mice, bearing a doxycycline-inducible cassette with the four Yamanaka factors). We also crossed 4F mice with a Cre-loxP system-based conditional mouse that bears a floxed Dido exon 16. The Cre-induced E16 deletion in homozygosis produces the same phenotype seen in Dido3ΔCt mice. We show here that homozygous DidoΔNt mouse embryonic fibroblasts (MEF) have only a 2-fold decrease in reprogramming efficiency, whereas homozygous E16 deletion results in a dramatic impairment of their reprogramming capacity. DidoΔE16 MEF does not show differences in senescence compared to the wild type. Also, their slightly lower capacity to proliferate compared to wild type MEF cannot explain by itself their almost abrogated reprogramming ability. Small molecules reported to increase reprogramming efficiency by chromatin modification were also unable to restore DidoΔE16 MEF reprogramming. By combining ChIP-Seq, RNA-Seq and computational analysis of protein-protein interaction networks, we identified genes whose expression is altered in DidoΔE16 ESC differentiation and compared them to genes described in the process of reprogramming; by this we could identify common genetic programs and mechanisms involved in differentiation and cell reprogramming in which Dido is implicated.

150 The roles of mechanotransduction in pluripotency and cell fate decisions

Tan BX, Chalut K

Wellcome - MRC Cambridge Stem Cell Institute, UK

Keywords: mechanotransduction, substrate stiffness, cell fate decisions

The behaviour of pluripotent stem cells is regulated by multiple signals from the microenvironment. While the soluble biochemical cues involved in maintaining pluripotency have been studied extensively, the roles of mechanical cues, and their interaction with the former, are not well understood. Further elucidation of how mechanical properties of the extracellular matrix affect pluripotency will not only allow tighter control over pluripotent stem cell maintenance and differentiation in vitro, but also advance understanding of embryonic development. Here, we use a novel polyacrylamide hydrogel (StemBond) with tuneable stiffness and adhesive properties to study mechanotransduction in pluripotency and cell fate decisions.

151 The engineered activin A providing sustainable signalling in iPSC culturing

Wang X, Hyvonen M

University of Cambridge, UK

Keywords: TGF-beta superfamily, iPSC culturing, sustainable activity

Activin A, a member of TGF-beta superfamily, is widely used in the iPSC culturing to maintain the cell pluripotency and induce differentiation. Its signalling activity also induces the cells to produce follistatin, a secreted antagonist of activin A, which binds to activin A and inhibits its signalling in a negative-feedback loop. This leads to a gradual reduction of activin A bioactivity during the stem cell culturing. With the aim to develop an engineered form of activin A with more sustainable activities, we have designed a numbered activin A mutants that preserve the wild-type signalling activity, but resist inhibition by follistatin. We have used activin A-responsive luciferase assay to confirm that these mutants have more sustained signalling activities during the culturing of hiPSCs. These engineered forms of activin A could provide a more stable and sustainable signalling environment for hiPSC culturing. Moreover, there are potentials to reduce the amount of the engineered activin A added into the culture due to their longer half-life times.

152 Diploidization in human haploid pluripotent stem cells

Zuccaro MV, Egli D

Columbia University, USA

Keywords: Haploid, Diploid, Cytokinesis, Gamete

For mammals, allelic complementation and the presence of two sets of alleles contributes to the complexity of diploid genetics. The ability to study the genomes of diploid organisms in a haploid model would prove invaluable for research, especially for understanding the biology of disease and genetic variants. Human haploid pluripotent stem cells make this possible. In addition to existing in a haploid state, pluripotency provides the potential to differentiate into numerous disease-relevant tissue types, increasing the breadth of their relevance to studying disease. Haploid stem cells would directly contribute to the advancement of research, except for the caveat that these cells undergo spontaneous, irreversible diploidization. While this phenomenon has been observed in all mammalian haploid cell lines, the underlying mechanism remains largely unknown. This work aims to elucidate the mechanism of diploidization in haploid human pluripotent stem cells to maintain a stable haploid state.

153 Metabolic regulation of the stem cell epigenome and potential

Wong CW, von Meyenn F

King's College London, UK

Keywords: Metabolism, Epigenetics, Pluripotency

Pluripotency describes the potential of a cell to give rise to all somatic lineages. In vitro, pluripotent stem cells can be maintained in naïve and primed conditions, which reelct the progression of embryonic development towards lineage specification. Each state possesses unique properties and distinct epigenomic characteristics (1, 2). Acquisition of specific cell fates involves dynamic changes in the epigenetic landscape. The role of metabolic pathways is emerging to be a novel regulator of stem cell fate, likely affecting the regulation of the epigenetic machinery and the epigenome (3). Recent studies have linked a shift in cellular metabolism of oxidative phosphorylation towards glycolysis during the progression of the "naïve" to "primed" pluripotent state (4). These metabolic changes are potentially key developmental cues necessary in regulating cell fate decisions; however, a detailed understanding of how changes in cellular metabolism can regulate the shift in pluripotency and lineage specification ability remain elusive. Here, we propose to examine these changes in human embryonic stem cells using metabolomics to acquire quantitative information of intracellular metabolites and to combine this with a detailed characterisation of the impact of specific candidate metabolites and metabolic inhibitors on embryonic stem cell potential, epigenome and epigenetic modifiers. Understanding the metabolic requirement for specific pluripotent states may aid in the development of novel strategies to improve in vitro lineage-direct differentiation of stem cells in the future.

154 Physical mechanisms of epiblast and primitive endoderm segregation in the mouse embryo

Yanagida A, Achouri S, Revell C, Stirparo G, Cassani D, Paluch E, Chalut K, Nichols J

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: Early embryo development, Cell sorting

A fertilized egg undergoes a series of divisions and forms a blastocyst before implantation in the uterus. A blastocyst is composed of trophectoderm, an origin of a placenta and inner cell mass (ICM). ICM gives rise to epiblast (EPI), an origin of a foetus and primitive endoderm (PrE), an origin of a yolk sac. EPI- and PrE-specific transcription factors are expressed in the inner cell mass (ICM) in a random "salt and pepper" pattern at E3.75. These two lineage progenitors segregate into the appropriate cell layers and differentiate into mature EPI and PrE by E4.5. However, how each ICM cell segregates into two lineages is still unclear. Physical forces are thought to regulate embryo development. However, its involvement in the early embryo, specifically in EPI and PrE segregation is not yet understood. To assess the individual cell physical properties in the embryos and whole actin cytoskeleton network regulating them, we performed single cell RNAseq, measured the single cell surface tension, extracted interfacial tension and quantified cell membrane dynamics. Through the characterisation of physical properties, we found that differential interfacial tension and membrane dynamics between EPI and PrE might drive their sorting. Understanding the mechanical properties of EPI and PrE will provide an important avenue for approaching the cell specification and morphogenesis in the embryo.

155 Dynamics of cell phase transitions

Mulas C, Nett I, Hodgson A, Baye J, Corominas-Murtra B, Hannezo E, Chalut K, Smith A

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Stem cell states or phases have been well characterised over the years. Our efforts have focused on studying mouse embryonic stem (ES) cell differentiation as a model of early development. The gene regulatory network, the metabolic profiles and epigenetic landscapes that characterise the different states have been mapped. We have recently shown that ES cells have to acquire the capacity to respond to lineage inducing signals over time, in a 'formative' phase that precedes lineage choice. However, we know very little about how cells transition from a naïve to a formative phase.

The aim of this project is to try to understand how cells irreversibly reconfigure their identity to enter a new cell phase. First, we show that the signalling pathways that modulate the transition are highly dynamic due to feedback systems. Interfering with such feedbacks alter the kinetics of the transitions without compromising developmental potential. Second, by combining live imaging with functional and molecular characterisation, we present a novel approach that can be used to study cell transitions in unsynchronised systems. We hope that by combining information about the signalling dynamics, molecular signatures and functional properties of cells at different stages of transition we can place cells within a phase diagram and identify potential parallels between phase transitions in Biology and Physics.

156 Identifying factors that influence stem cell reprogramming

Hladkou S, Silva JCR

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Pluripotency is the ability of a cell to give rise to the derivatives of all three germ layers during differentiation. Naturally, pluripotency is restricted within the early embryo only, represented by two sequential morphologically, physiologically and epigenetic pluripotent stages – naïve (embryonic stem cells, or ESCs) and primed (postimplantation epiblast stem cells, or EpiSCc). By nuclear reprogramming - a technology allowing a differentiated cell to artificially regain pluripotency - EpiSCs can also be de-differentiated into ESCs. The reprogrammed cells represent a flexible biological tool for regenerative therapies. Currently reprogramming is moderately efficient, however reliable and safe medical strategies require decent understanding of the physiological and genetic networks underpinning cell identity change. In my project, I've performed a whole genome CRISPR knockout screen to identify genes working as barriers for reprogramming from EpiSC to ESC, discovered a JAK/STAT3 pathway regulator SOCS3 as a key hit, and investigated the interplay between JAK/STAT3 signalling and core pluripotency genes in ESC and EpiSCs.

Theme 3: Haematopoiesis

157 Dissection of Endothelial-to-Haematopoietic Transition at the single cell level identifies cell-cycle regulation as a determinant of haematopoietic commitment

Canu G, Ortmann D, Athanasiadis E, Garcia-Bernardo J, Cvejic A, Vallier L

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: Stem cells, haematopoiesis, cell-cycle

Haematopoietic stem cells (HSC) are responsible for the maintenance of blood homeostasis and are involved in a diversity of diseases including leukemia. Understanding their embryonic development is essential to produce these cells in vitro from human pluripotent stem cells (hPSC), but also to develop new therapies. During development, they first arise in the aorta-gonad-mesonephros (AGM) region of the embryo from a population of haemogenic endothelial cells lining the ventral portion of the dorsal aorta which undergo endothelial-to-haematopoietic transition (EHT). During this process, both erythro-myeloid and lymphoid progenitors are produced, ultimately culminating with the generation of the first HSCs capable of multilineage differentiation and long-term engraftment. Little is known about the molecular mechanisms driving this process, especially in human where the AGM region is not easily accessible in vivo. Here we used differentiation of hPSCs as a model system to uncover the mechanisms by which haemogenic endothelium generates early HSCs. By combining this approach with single cell RNA-sequencing (scRNAseq), we were able to dissect this fundamental stage of development and gain information about cell state and dynamics during EHT. Of particular interest, we identified that cell-cycle progression is essential for endothelial cells to undergo the transition to the haematopoietic fate. Specifically, we demonstrated that most of the endothelial cells at this stage reside in the G1 phase, with a direct correlation between an active cell-cycle and their ability to engage cell fate decision towards the haematopoietic fate. We also confirmed the importance of the cell-cycle by functional validations and identified CDK4/6 and CDK1 as key molecules affecting this process. Finally, we used scRNAseq to identify potential pathways disrupted upon inhibition of these regulators. Ultimately, we propose here a direct connection between cell-cycle machinery, cell fate decision and capability of haemogenic endothelial cells to undertake the haematopoietic fate during EHT. These results will have major impact in the improvement of protocols for the production of functional HSCs in vitro, but also for understanding key mechanisms regulating HSCs, therefore helping with the development of new therapies.

158 A single cell hematopoietic landscape resolves eight lineage trajectories and defects in Kit mutant mice

Hamey FK, Dahlin JS, Pijuan-Sala B, Shepherd M, Lau WWY, Nestorowa S, Weinreb C, Wolock S, Hannah R, Diamanti E, Kent DG, Göttgens B and Wilson NK

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: Single-cell; Hematopoiesis; Transcriptional landscape

Hematopoietic stem and progenitor cells (HSPCs) are responsible for maintaining the adult blood system. Fate decisions driving the differentiation of these cells must be properly balanced, as their dysregulation causes a multitude of diseases. However, the differentiation journeys towards specific hematopoietic lineages remain ill defined, and system-wide disease interpretation remains challenging. Here, we have profiled 44,802 mouse bone marrow HSPCs using single cell RNA-Sequencing to provide a comprehensive transcriptional landscape with entry points to eight different blood lineages (lymphoid, megakaryocyte, erythroid, neutrophil, monocyte, eosinophil, mast cell and basophil progenitors). To assess how this landscape is altered when perturbed, we performed transcriptional profiling of 13,815 HSPCs from the c-Kit mutant (W41/W41) mouse model. This revealed the absence of a distinct mast cell lineage entry point, together with global shifts in cell type abundance. Proliferative defects were accompanied by reduced Myc expression. Possible compensatory processes included upregulation of the integrated stress response pathway and downregulation of pro-apoptotic gene expression in erythroid progenitors, thus providing a template of how large-scale single cell transcriptomic studies can bridge between molecular phenotypes and quantitative population changes.

159 Aging of bone marrow microenvironment promotes myeloid bias of hematopoietic progenitors and is a target in age-related myeloproliferative neoplasms

Ho YH, Del Toro R, Rivera-Torres J, Rak J, Korn C, García-García A, Kubovcakova L, Macias D, Arranz L, González-Gómez C, Wittner M, Nienhold R, Waller A, Foster HR, López-Otín C, Johnson RS, Nerlov C, Vainchenker W, Ghevaert C, Louache F, Skoda RC, Andrés V, Méndez-Ferrer S

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: Aging, bone marrow microenvironment, myeloid bias

Hematopoietic stem cells (HSCs) residing in the bone marrow (BM) accumulate during aging but are functionally impaired. However, the role of HSC-intrinsic and -extrinsic aging mechanisms remains debated. Premature aging in Hutchinson-Gilford progeria syndrome (HGPS) recapitulates physiological aging features, but whether these arise from altered stem and/or niche cells is unknown. Here we show that the murine BM microenvironment promotes stem/progenitor cell myeloid bias in normal aging and HGPS. During physiological aging, HSC supporting niches decrease near bone (endosteal BM) but expand in the BM further from bone. Increased sympathetic noradrenergic innervation in BM microenvironment promotes Beta2-adrenergic-receptor(AR)-interleukin-6-dependent myeloid bias. Reduction of endosteal niches decreases Beta3-AR-nitric-oxide-NO-dependent inhibition of cytokines driving myeloid expansion. However, chronic treatment with Beta3-AR-agonist does not rejuvenate overall hematopoiesis, but decreases exacerbated megakaryopoiesis in mice and humans with myeloproliferative neoplasms (MPNs). Therefore, niche aging promotes stem/progenitor myeloid bias and might represent a therapeutic target in age-related myeloproliferative disorders.

160 Kat2a loss promotes transition from self-renewal into differentiation with metabolic reprogramming in Acute Myeloid Leukaemia

Kulkarni R, Domingues AF, Giotopoulos G, Zeka K, Parcival B, Castro C, Gupta S, Foerner E, Adao, RR, Griffin J, Grootveld M, Huntly B, Prabakaran S, Pina C

University of Cambridge, UK

Keywords: AML, transcriptional heterogeneity

We have recently identified histone acetyl-transferase Kat2a, the orthologue of classical yeast noise regulator Gcn5, as a vulnerability in cultured Acute Myeloid Leukaemia (AML) cells. Herein, we investigate Kat2a requirements in vivo by initiating MLL-AF9-driven AML in a Kat2a conditional knockout (KO) background. We find that loss of Kat2a prolongs AML survival through erosion of functional AML stem-like cells. We aim to decipher changes in AML gene regulatory networks (GRN) and how they are impacted by transcriptional noise, through the integrated analysis of single-cell RNA-seq (scRNA) and ChIP-seq data obtained from Kat2a KO and wild-type (WT) mouse models of AML. Cluster analysis of scRNA data with the RaceID-StemID protocol decipher global and genotype-specific population sub-structures, and identify a distinct cellular organisation of KO AML, with profound depletion of cells with transcriptional stem characteristics, namely high information entropy. We computed pairwise distance as a measure of transcriptional heterogeneity. We found that Kat2a loss associates with a global enhancement of transcriptional heterogeneity in KO cells. The analysis of ChIP-seq data from KO and WT leukaemias show unique signatures of differential chromatin regulation patterns at promoter elements. Integration of ChIP-seq and scRNA targets shows that WT-enriched, stem-like clusters, have unique associations involving metabolic genes, as well as cell cycle and chromatin organisation, which are reflected in a defect in mitochondrial metabolism. Overall, our data position Kat2a as a novel regulator of AML-LSC, and indicate that Kat2a may act through enhanced transcriptional heterogeneity associated with metabolic reprogramming in leukaemic cells.

161 Characterization of splicing in the haematopoietic landscape using combined short and long read single-cell sequencing

Mincarelli L, Wrzesinski T, Lipscombe J, Haerty W, Macaulay I C

Earlham Institute, UK

Keywords: splicing, single cell sequencing, haematopoiesis

Haematopoiesis has been widely used as model of stem cell biology, and single-cell RNA sequencing analysis has recently provided low-resolution snapshots of gene expression in thousands of cells across the haematopoietic hierarchy. Current high-throughput single-cell RNA sequencing methods are based on short-read (Illumina) counting of unique 3' tag sequences. This enables identification of cells types within a complex population of cells but has limitations in terms of resolving transcriptional heterogeneity in closely related cell types and lacks any information on cell-specific isoform expression – therefore these methods are missing key aspects of transcriptional regulation and cell fate choice, and perhaps the resolution to distinguish functionally distinct sub-populations of stem and progenitor cells. In the present work we introduce a novel approach using the 10x Chromium to combine short-read (Illumina) and long-read (Pacific Biosciences) of the same single-cell RNA-sequencing libraries. We demonstrate the feasibility of this hybrid approach using haematopoietic cell lines and applying it in mouse bone marrow stem and progenitor cells (Lin- cKit+) to define sub-populations of cells and in parallel annotate them with information on long-read based isoform expression.

162 Single-cell transcriptome analysis identifies CD44 as a novel marker and regulator of the endothelial-haematopoietic transition

Oatley M, Vargel Bölükbaşı Ö, Svensson V, Shvartsman M, Ganter K, Pavlovich P, Natarajan K, Teichmann S, Lancrin C

European Molecular Biology Laboratory (EMBL), Italy

Keywords: Embryonic haematopoiesis, Endothelial-haematopoietic transition, CD44, Hyaluronan

Haematopoietic stem and progenitor cells (HSPCs) arise from the vasculature of the aorta gonad mesoneprhos (AGM) through an endothelial to haematopoietic transition (EHT). Understanding this transition is critical to the in vitro culturing and eventual clinical use of HSPCs. Recent reprogramming efforts have demonstrated the importance of an endothelial intermediate. To investigate the formation of HSPCs in the embryo we employed single-cell RNA sequencing analysis and identified CD44 as a marker of cells transitioning between endothelial and haematopoietic identities. CD44 expression was found to increase at embryonic day 10.5 as haematopoietic stem cells begin to emerge and was shown to mark both haematopoietic clusters and endothelial cells of the AGM. Single-cell qPCR further revealed that CD44 could be used to distinguish the key cellular stages of EHT. Specifically, by using CD44 as a marker in the embryonic vasculature we could identify two distinct endothelial cell populations, pre-HSPC type I and type II cells. With single-cell OP9 cultures, we could use CD44 to robustly differentiate between cells in the vasculature with haematopoietic potential and without. Colony forming unit (CFU) and lymphocyte assays showed that CD44+ cells had high multi-potent haematopoietic potential. Interestingly, by inhibiting CD44+ cells ex vivo with a CD44 blocking antibody we could extinguish this haematopoietic potential. Furthermore, we found that the addition of the CD44 blocking antibody or an enzyme that degrades the ligand of CD44, hyaluronan, we could impair EHT in our in vitro embryonic stem cell differentiation culture system. This demonstrates a new role for CD44 in embryonic haematopoiesis. This knowledge will be valuable to develop new methods of in vitro blood cell production for regenerative medicine.

163 Minimal culture medium reveals components essential for haematopoietic stem cell function

Oedekoven CA, Belmonte M, Diamanti E, Bastos HP, Bode D, Shepherd M, Pijuan Sala B, Laurenti E, Kent DG

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: Haematopoietic Stem Cells, Heterogeneity, Quiescence

Recent descriptions of single cell gene expression datasets from highly purified long-term haematopoietic stem cells (LT-HSCs) have significantly advanced our understanding of the molecular state of HSCs. Emerging concomitantly, however, is the knowledge that LT-HSCs share a significant amount of their transcriptome with cells that do not possess the functional properties of durable self-renewal and multi-lineage cell output. To discern which genes and pathways drive individual HSC properties and which are bystanders is a major outstanding challenge for the field. We have recently demonstrated that single HSCs (EPCR++CD150+CD48-CD45+Sca-1++, >50% LT-HSCs by single cell transplantation) can be cultured without undergoing division for a period of 7–10 days with minimal cytokine stimulation (21% single cell survival, 99.2% undivided). Limiting dilution assays of HSCs cultured for 7 days (7dHSCs) estimated the durable multi-lineage HSC frequency at 22%. Single cell transplantation experiments had a similar frequency with both primary (15/31) and secondary (3/3) transplantations demonstrating that single 7dHSCs retained HSC function. Since 7dHSCs share the functional properties of freshly isolated HSCs, they represent a robust comparator population to identify which genes are indispensable for the maintenance of HSC self-renewal and quiescence. We generated RNA-sequencing datasets from freshly isolated HSCs and 7dHSCs and identified 960 genes and a number of biological processes that were significantly down-regulated in 7dHSCs, suggesting that these are dispensable for HSC function. Major down-regulated processes included the response to oxidative stress, ligand-independent apoptosis, and cellular senescence. Interestingly, in all the majority of mice successfully transplanted with single 7dHSCs, a lymphoid-deficient (or α-HSC) program was observed, suggesting that α-HSC are more resilient to the stress induced by cytokine depletion and represent a potential mechanism of obtaining the molecular program of α-HSC. Together, these data highlight the importance of studying HSCs in different contexts to identify common molecular features of their functional properties.

164 Purifying the elusive CD34-negative haematopoietic stem cells

Popova S, Ariza-McNaughton L, Montaner B, Anjos-Afonso F, Bonnet D

The Francis Crick Institute, UK

Keywords: HSC, human, haematopoiesis

Twenty years after the self-renewal and differentiation potential of CD34-negative haematopoietic cells was first reported, their role in adult haematopoiesis still remains elusive. Considering their highly quiescent nature, yet robust repopulation capacity, it is easy to speculate that the CD34-/CD38-/CD93+ population serves as an HSC (haematopoietic stem cell) reservoir contributing to blood regeneration only under conditions of stress and injury rather than steady state haematopoiesis. One of the main goals of this project is to elucidate the role of the CD34-/CD38-/CD93+ HSCs in human haematopoiesis. Currently the repopulating cell frequency of the CD34-/CD38-/CD93+ population established at 12 weeks in NSG mice is quite low in comparison to other defined CD34+HSCs. Our goals here were: first, to test whether using the immunodeficient mouse strains NSG-S (which produced human IL-3, GM-CSF and SCF) or NSBGW (NSG-KitW41/w41) could modify the kinetic of engraftment of these cells; second, to further purify the stem cell in the CD34-/CD38-/CD93+ fraction. Bringing the purity up is essential as it would be challenging to draw meaningful conclusions from data representative of a population where only one out of a several thousand cells is estimated to be a true stem cell. Here we report on the comparison of the engraftment kinetics between different immunodeficient mice and a new marker that increases the CD34-negative HSC frequency by 40-fold. These data represent a step forward in a better understanding of the molecular mechanisms regulating CD34-/CD38-/CD93+ compared to CD34+ HSCs, as single cell RNAseq could now be performed. We also aim in the future to explore the role of the CD34-/CD38-/CD93+ population during ontogeny in an attempt to both study their role in the establishment of the haematopoietic system and uncover molecular mechanisms that can be exploited to manipulate this population in vitro.

165 The role of MEK1 in hematopoietic stem cell division

Schirripa A, Baumgartner C, Baccarini M

Max F. Perutz Laboratories, Austria

Keywords: Hematopoietic Stem Cells, Asymmetric Division, MEK1

Hematopoiesis crucially depends on the function of hematopoietic stem cells (HSC) which are able to produce new blood cells and to maintain the HSC compartment. Asymmetric division is a process that produces two daughter cells with distinct fates: one which enters the path of differentiation and one which retains the self-renewal ability and returns to quiescence to maintain the HSC compartment. During ageing, HSCs progressively lose their regenerative capacity and the ability to maintain this balance, becoming more prone to self-renew rather than differentiation. The cell-intrinsic mechanisms and the signaling involved in HSC fate decision among quiescence, self-renewal and differentiation are largely unknown. We have recently discovered a central player regulating self-renewal and differentiation. This protein, MEK1, is part of the evolutionary conserved RAS-RAF-MEK-ERK signal transduction cascade involved in the regulation of a variety of cellular programs such as cell cycle progression, apoptosis, proliferation and differentiation. Within the cascade, MEK1 phosphorylates and activates ERK. ERK, in turn, phosphorylates MEK1 on a key regulatory residue that mediates negative feedback mechanisms dimming the activity of both the ERK and the AKT pathway. It is the phosphorylation of this MEK1 residue which mediates the return to quiescence of activated HSCs and therefore prevents HSC exhaustion. MEK1-deficient cells show a greater propensity towards differentiation at the expense of self-renewal. We will use single cell analysis of signalling pathways to address the question of how asymmetry is established during HSC division, and how aging and MEK1 ablation impact this process.

166 Studying the role of Lyl1, Tal1 and Lmo2 in the Endothelial to Hematopoietic Transition

Serina Secanechia YN, Bergiers I, Le S, Lopez Anguita N, Arnold C, Ganter K, Zaugg J, Lancrin C

European Molecular Biology Laboratory (EMBL), Italy

Keywords: hematopoiesis, hematopoietic stem-cells, embryogenesis

Hematopoietic Stem-Progenitor Cells (HSPCs) first appear in the vertebrate embryo at mid-gestation, in a region named aorta-gonad-mesonephros (AGM). They were shown to originate from an endothelial precursor, the Hemogenic Endothelium (HE), through the process of Endothelial to Hematopoietic Transition (EHT), whereby HE cells progressively turn off the expression of endothelial genes and lose their endothelial phenotype, while turning on hematopoietic gene expression and acquiring a hematopoietic phenotype. This process has been described in different animals, however, the exact mechanisms and molecular players that regulate it are still poorly understood. A complex of seven transcription factors (i.e. the "heptad complex") was proposed to act as a master switch of the transcriptional program that drives HSPC generation. However, a recent work performed in our lab revealed that only two out of these seven factors, namely Runx1 and Gata2, possess a clear hematopoietic-promoting function in vitro, while the heptad factors Erg and Fli1 were shown to promote an endothelial cell fate. A clear role could not be identified during this study for the remaining heptad factors Lyl1, Tal1 and Lmo2. My preliminary results using the same in vitro system strongly suggest a pro-hematopoietic function of these factors. To understand mechanistically how Lyl1, Tal1 and Lmo2 exert their role, I am investigating the changes that occur at the chromatin and transcriptional level in cells undergoing EHT in vitro when these factors are overexpressed, and when the heptad factors are overexpressed without Lyl1, Tal1 and Lmo2.

167 Single cell approaches identify the molecular network driving malignant hematopoietic stem cell self-renewal

Shepherd MS, Li J, Wilson NK, Li J, Oedekoven CA, Fink J, Prick JCM, Pask DC, Hamilton TL, Löffler D, Rao A, Schroeder T, Göttgens B, Green AR, Kent DG

Wellcome - MRC Cambridge Stem Cell Institute, UK

Keywords: Single cell, haematology

Recent advances in single cell technologies have permitted the investigation of heterogeneous cell populations at previously unattainable resolution. Here we apply such approaches to resolve the molecular mechanisms driving disease in mouse hematopoietic stem cells (HSCs), using JAK2V617F mutant myeloproliferative neoplasms (MPNs) as a model. Single cell gene expression and functional assays identified a subset of JAK2V617F mutant HSCs that display defective self-renewal. This defect is rescued at the single HSC level by crossing JAK2V617F mice with mice lacking TET2, the most commonly co-mutated gene in MPN patients. Single cell gene expression profiling of JAK2V617F-mutant HSCs revealed a loss of specific regulator genes, some of which were restored to normal levels in single TET2/JAK2 mutant HSCs. Of these, Bmi1 and, to a lesser extent, Pbx1 and Meis1, overexpression in JAK2-mutant HSCs could drive a disease phenotype and retain durable stem cell self-renewal in functional assays. Together, these single cell approaches refine the molecules involved in clonal expansion of MPNs and have broad implications for deconstructing the molecular network of normal and malignant stem cells.

168 Single-cell transcriptional analysis reveals ILC-like cells in zebrafish

Strzelecka P*, Athanasiadis E*, Hernández P*, Robalo A, Collins C, Boudinot P, Levraud J-P, Cvejic A

Wellcome - MRC Cambridge Stem Cell Institute, UK

Keywords: zebrafish, ILC-like cells

Innate lymphoid cells (ILCs) have been studied extensively in mouse and human but so far have not been described in any other species [1]. Using single-cell RNA sequencing [2, 3], we generated a comprehensive atlas of cellular states of innate and adaptive lymphocytes collected from various organs in homeostasis and following immune challenge. Our profiling of 14,080 individual cells from the gut of wild-type and rag1-deficient [4] zebrafish revealed previously unappreciated diversity of innate lymphocytes. We examined the biological relevance of this heterogeneity and characterised how distinct cell populations respond to immune challenges. Our analysis revealed the presence of helper ILC-like cells in zebrafish, including a rorc-positive subset which could be induced to express either il22 or il13 following immune challenge with inactivated bacteria or helminth extract, respectively. A survey of cell surface receptors suggested that cytokine-producing ILC-like cells express a specific repertoire of novel immune-type receptors, involved in recognition of environmental cues. We further highlighted novel markers of ILCs in zebrafish and generated a cloud repository for their future in-depth exploration.

169 Platelet-derived microparticles enhance megakaryocytes differentiation and platelet generation via miR-1915-3p

Xie X, Qu M, Zou X, Fang F, Zeng Q, Fan Z, Chen L, Yue W, Pei X

Institute of Health Service and Transfusion Medicine, China

Keywords: Platelet-derived microparticles, megakaryocyte differentiation, miR-1915-3p

Thrombosis leads to platelet activation and consumption, which requires replenishment from hematopoietic stem/progenitor cells (HSPCs). Platelet-derived microparticles (PMPs) are released from activated platelets. The amount of PMPs elevates during thrombosis. Analysis of the relevance between megakaryocytic progenitors, platelets and PMPs gives hints that messages carried by PMPs might feed back for the production of platelets from HSPCs. Here we first revealed that megakaryocytic differentiation and platelet production were promoted by the increment of PMPs in vivo. During mouse acute liver injury, we found elevated bone marrow megakaryocyte density following increased level of circulating PMPs, but a positive response from a typical megakaryocyte regulator, TPO, was not detected in these cases. Similarly, exogenous PMP injected into irradiated mice could elevate the number of megakaryocytes and platelets, without affecting TPO levels in peripheral blood and liver. In vitro studies also demonstrated that PMPs could be internalized by HSPCs and drive the cells toward megakaryocytic fate. miRNA is one of the abundant contents of PMPs. Through elaborated miRNA analysis, a PMP highly enriched miRNA, miR-1915-3p was found could be transported to target cells and suppressed Rho GTPase family members B (RHOB) expression, thereby induced megakaryocytic differentiation. As a consequence, more megakaryocytes and platelets could be generated to replace the platelets lost by activation. In conclusion, PMP might be a complement to classic TPO mediated homeostasis of platelets during pathophysiologic states.

170 Use of pluripotent stem cells and stem cells-derived neurons in support of drug discovery and target validation

Iovino M, Nancolas B, Cook K, Anton J, Clark N, Lazari O, Mitchell P, Waddell I, Fischer DF

Charles River Laboratories, UK

Keywords: neurons, drug discovery, neurodegenerative diseases

Recent advances in the field of stem cell biology have opened up previously unprecedented opportunities to apply fundamental scientific discoveries of monogenetic and complex diseases in drug discovery. Importantly, the possibility of generating induced pluripotent stem cells from patient derived samples combined with the ability to differentiate them into any desirable cell/tissue type highlight some of the major strengths these models represent. Although technological challenges still exist, the use and incorporation of stem cell based assays in drug discovery and early safety screenings offers the potential for a more innovative and potentially safer medicine as well as opening up strategies to treat rare & orphan disorders. Charles River have developed a suite of assays using both pluripotent stem cells and stem cell-derived neurons from patients and healthy controls in support of drug discovery programs. Herein we report examples of high and medium throughput compatible assays for small molecule HTS, target validation and hit compound de-convolution as well as high content and molecular biology approaches used for a thorough quality control during routine cell culture and differentiation. Moreover using high content algorithms in fixed-cells and real time format we have developed low-throughput, high value assays to further support hit-to-lead and lead optimization programs.

171 The Role of Neural Activity in Myelination

Jia J, Karadottir RT, Martin K

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: Myelin, Activity, DREADD

In the central nervous system (CNS), oligodendrocytes (OLs) wrap membranes around axons to produce myelin. Their functional roles not only include rapid neurotransmission, synchronization, and functional maintenance, but also motor learning and plasticity.1 In the adult brain, ~5% of the total cellular population consists of oligodendrocyte precursor cells (OPCs), stem cells that proliferate and differentiate to produce new OLs. While myelination is largely a post-natal process that occurs rapidly during development, it has been recently shown that new OLs form throughout adulthood in both grey and white matter.2 Research has shown that neural activity plays a central role in early myelin development and promotes oligodendrogenesis and adaptive myelination.3,4 Thus, it becomes important to understand how neural activity affects myelin. In this project, we used chemogenetic tools to alter the activity of neurons in order to observe the activity-dependency of myelin. Using the optic nerve—a CNS tract that becomes fully myelinated during adulthood (>p70)—as a model, we studied the importance of neural-glial communication in the maintenance and turnover of myelin.5 To address how activity-dependent changes in myelin occurs, we first established and characterized tools to change retinal ganglion cell (RGC) activity. First, we tested the RGC targeting efficiency of AAV2 DREADD viruses through intravitreal injections and showed significant and reliable infection rates across the retina. Second, we provided preliminary data on the activation of DREADDs through a clozapine dose response curve.6 As the alteration of neural activity lies at the foundation of this project, the robust characterization of these chemogenetic tools will allow us to observe myelin's dependency on neural activity in future experiments. Elucidating the role of neural activity on myelin may lead to the development of better therapeutics and a greater understanding of plasticity and during adulthood.

172 Investigation of the effects of ependymal cell ablation in the function of postnatal brain neural stem and progenitor cells within the Subependymal Zone stem cell niche of the rat

Kakouri P, Koutsakis C, Meri D, Andreopoulou E, Dimitriou C, Patsoni M, Franklin RJ, Kazanis I

University of Patras, Greece

Keywords: ependymal cells, neural stem cells, brain

Ependymal cells are multiciliated cells that line the ventricular system of the CNS. They form an important structural and functional element of the Subependymal Zone (SEZ/ also known as ventricular-subventricular zone) stem cell niche that is located at the lateral walls of the lateral ventricles of the mammalian brain and we have previously shown that the number of epnedymal cells regulates the number of neural stem cells (NSCs) that survive within the postnatal niche (Kazanis and ffrench-Constant, 2012). Recently we developed a new method for isolating NSCs from the SEZ of live rats via liquid biopsies of cerebrospinal fluid ("milking of the SEZ", see Dimitriou et al. poster). This process involves the intracerebroventricular injection of a 'release cocktail' containing neuraminidase, a toxin that kills selectively ependymal cells, and a β1-integrin blocking antibody. Here, we investigate if the ablation of ependymal cells leads to aberrant function of NSCs, as judged by immunohistochemical analysis of the SEZ at different time-points, ranging from 7 days to 3 months. We assess the presence of mitotic (PH3+) cells and of neuroblasts (Dcx+ cells) throughout the affected SEZ, and in more detail within and outside areas of direct ependymal loss. Our results show both transient and chronic effects of ependymal damage with induction of astrogliosis within the SEZ as well as a surprising endurance of ependymal cells outside the SEZ.

173 G protein-coupled neurotransmitter signalling in the oligodendrocyte lineage

Kamen Y, Káradóttir RT

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: Oligodendrocyte precursor cells; G protein-coupled receptors

In the Central Nervous System, oligodendrocytes produce myelin, which is essential for fast neurotransmission and normal brain function. The importance of myelin is made clear by white matter disorders such as multiple sclerosis, where most motor and cognitive symptoms arise from the degeneration of the myelin sheath. Neuronal activity can regulate oligodendrocyte precursor cell (OPC) differentiation and myelination in health and disease, and recent evidence indicates that new myelin occurs during learning and memory. Similar to neurotransmission, G protein-coupled receptors (GPCRs) may mediate activity-dependent neuron-OPC signalling. In particular, blocking certain neurotransmitter-activated GPCRs in OPCs increases remyelination in white matter disease models. Blocking these receptors also modulates experience-dependent myelination. However, the underlying GPCR signalling mechanisms and their effect on OPC cell fate remain unclear. Using whole-cell patch-clamp and calcium imaging in acute brain slices, I examine downstream signalling in the oligodendrocyte lineage following activation of individual neurotransmitter-gated GPCRs. Studying GPCR signalling in OPCs will significantly contribute to our understanding of OPC and myelin biology both in health and disease.

174 Neural stem and progenitor cells isolated from live rats by "milking" the Subependymal Zone neural stem cell niche exhibit restricted self-renewing capacity

Dimitriou C, Andreopoulou E, Patsoni M, Koutsakis C, Meri DK, Kakouri E, McClenahan FK, Anestakis D, Franklin RJM, **Kazanis I**

University of Patras, Greece
University of Cambridge, UK

Keywords: Neural stem cells, brain, stem cell therapy

Cell replacement therapy is an attractive treatment option for CNS disorders that involve cell type-specific defects, such as hypomyelinating disorders, Parkinson's disease and amyotrophic lateral sclerosis. Foetal neural stem cells or patient-specific iPSCs are promising sources but necessitate aggressive immunosuppression and raise concerns regarding tumorigenesis, respectively. We have developed a method that enables the release of postnatal brain neural stem and progenitor cells (pbNSCs) from the subependymal zone (SEZ) neurogenic niche into the cerebrospinal fluid, facilitating their isolation via liquid biopsies of live rats; a process we have named "milking of the SEZ". Here, we present an overall assessment of the validity and efficiency of this method (cell yields and profiles at various time-points ranging from 3 days to 3 months post-release) after single or multiple biopsies. The cell-type profile of isolated cells matches that of the SEZ niche and even accurately reflects changes in the source area, for example after the co-injection of FGF-2 that results in increased numbers of Sox2+ cells. Surprisingly, the collected cells showed limited capacity for self-renewal, even though they formed neurospheres and/ or adherent cultures similarly to pbNSCs isolated via the typical experimental dissociation of dissected SEZs. Our results indicate that pbNSCs isolated via "milking" the SEZ demonstrate behaviors more acurately reflecting those recently described using a range of in vivo labeling strategies (Calzolari et al., 2015; Obernier et al., 2018). We also present data suggesting that pbNSCs might be circulating in the rodent and human CSF under normal or pathological conditions.

175 Looking for adult neural stem cells (aNSC) in the pacific oyster Crassostrea gigas: identification and expression of stem cell markers in the central nervous system

Kellner K, Le Saux A, Adeline B, Cherif--Feildel M, Heude Berthelin C, Lelong C

University of Caen-Normandie, France

Keywords: Crassostrea gigas, adult Neural Stem Cells, Immunocytochemistry, Sox2, Oct, KLF, Myc, ALDH

Adult neurogenesis has been largely documented in invertebrate species presenting regeneration processes, for example in planaria (1) and molluscan species, mainly gastropods (2 for review) and cephalopods (3) in relation to their capacity to regenerate body parts, including central and peripheral nervous system. Regeneration of the peripheral nervous system was also observed in bivalve species (3). These observations led to postulate that neural stem cells are present along the whole life in lophotrochozoan species, and that molecular mechanisms involved in neural regeneration may be ancestral. Here, we aimed to identify neural stem cells in the bivalve Crassostrea gigas (e.g. Magallana gigas). Indeed for this species, the availability of whole genomic and numerous transcriptomic data should be an asset to identify conserved molecular markers of aNSC and to investigate their expression. The nervous system of the oyster Crassostrea gigas include numerous peripheral nerves and ganglia pairs, the main one being the visceral ganglia located at the proximity of the posterior adductor muscle. Among the most widely used markers for studies of stem cells and adult neural stem/progenitor cells in vertebrate, some appeared to be conserved in the oyster, as for example Sox2, Ascl1, KLF4, Pou3F4, Aldh. Immunolabelling using heterologous antibodies selected based on epitope conservation led to identify some labelled cells in visceral ganglia of oysters. Moreover, the expression of these markers were investigated. This work may help to characterize conserved mechanisms involved in aNSC and more broadly to identify and characterize the various adult stem cells types in this species. The isolation of such stem cells should be of great interest for further establishment of bivalve cell lines, a key obstacle for bivalve in vitro studies.

176 GDNF redirects fate determination of hiPSC-NPCs following cervical spinal cord injury

Khazaei M, Ahuja CS, Li L, Wang J, Nakashima H, Nagoshi N, Fehlings MG

Krembil Research Institute
University Health Network, Canada

Keywords: Neural progenitor cells, GDNF, spinal cord injury

Transplantation of neural progenitor cells (NPCs) is a promising therapeutic strategy for traumatic spinal cord injury (SCI); however, challenges remain including poor graft-host integration, low transplanted cell survival, and disproportionate differentiation towards glial lineages. Here we show that glial cell-derived neurotrophic factor (GDNF) promotes cell survival and integration while counteracting the pro-astrocytogenic effect of injury- induced factors produced as a result of SCI. Taking advantage of these benefits, we have engineered human induced pluripotent stem cell derived-NPCs (hiPSC-NPCs) using the PiggyBac transposon system to robustly express GDNF. When transplanted into a C6/7 clip-contusion model of rodent cervical SCI, GDNF-expressing hiPC-NPCs survived in significantly greater numbers than control hiPSC-NPCs and demonstrated a differentiation bias towards a neuronal fate. At 8 weeks post-injury, GDNF secretion was found to significantly reduce cystic cavitation within the parenchyma while promoting preservation of endogenous cells. Transplanting GDNF expressing NPCs also resulted in improved functional recovery. These results represent an important step in optimizing hiPSC-NPC transplantation therapy by redirecting the fate of transplanted cells towards neurons and enhancing graft survival.

177 Unveiling lineage decisions in zebrafish neurogenesis

Than-Trong E, **Kiani B**, Alunni A, Simons BD, Bally-Cuif L, Rulands S

Max Planck Institute for Physics of Complex Systems,
Germany

Zebrafish have the remarkable capacity to regenerate neural tissue rendering these animals excellent model systems for understanding neurogenesis. These tissues have been demonstrated to host specialised precursor cells, called neural stem cells (NSCs), which fuel the ongoing production of neurons into discrete brain regions. In order to understand how neural maintenance is achieved in this system, we performed a quantitative clonal analysis of the fate of precursor cells. Lineage tracing in growing tissues is complicated by the fact that labelled clones fragment into disconnected clusters, rendering the retrospective analysis of cell fate highly ambiguous. Combining statistical inference with biophysical modelling we reconstructed the clonal origin of labelled cells, revealing that progenitor containing clones persist over the lifetime of the animal. Using stochastic modelling allowed us to unveil lineage relationships and proliferation kinetics in the adult zebrafish pallium.
The developed mathematical scheme can also be used to decipher the fate behaviour and potency of progenitors using inducible genetic labelling methods in other growing tissues.

178 The effects of Apolipoprotein E (APOE) polymorphism on human hippocampal neurogenesis

Lee H, Thuret S

King's College London, UK

Keywords: Hippocampal neurogenesis, Alzheimer's disease, APOE

Apolipoprotein E (APOE) polymorphism is the most common genetic risk factor for late-onset Alzheimer's disease (AD). Growing body of evidence suggests that APOE polymorphism may differentially affect adult hippocampal neurogenesis in the dentate gyrus (DG), which has been implicated in AD progression in its own right. However, the impact of APOE isoforms on hippocampal neurogenesis at a cellular/molecular level is yet to be fully understood. In the current study, a time-course characterization was performed to examine the changes in neurogenic properties of isogenic human iPSC lines that differ only in APOE genotype differentiating into hippocampal neural progenitor cells (NPC) and then dentate gyrus granule cells (DGC). We found that compared to APOE3/3 cells, APOE4/4 cells showed significantly different patterns of expression for hippocampal NPC markers such as PAX6, NEUROD1, and EMX2 as well as for PROX1 which is the marker for mature DGCs. Interestingly, the gene expression pattern of APOE and neurogenic markers such as MAP2 and DCX did not significantly differ between APOE3/3 and APOE4/4 cells. The percentage of cells expressing Ki67, a marker for cell proliferation, was found to be the highest in APOE4/4 cells during DGC differentiation, suggesting an abnormal phenotype of increased proliferation during the neuronal differentiation stage. Based on these findings, we are now aiming to study whether gene-environment interaction can affect these phenotypes differentially depending on APOE genotype.

179 Dynamic interplay between the ageing CNS progenitor cells and surrounding ECM

Molotova A, Yin C, Axpe E, Caliscan B, Lee J, Williamson C, Segel M, Guy J, Holmqvist S, Hill M, Wright A, An H, Chalut K, Franklin R

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: ageing, rejuvenation, ECM, OPC, glia

Ageing is the leading cause and the major risk factor of neurodegenerative disorders. Atrophy of the ageing brain incorporates degeneration of the CNS white matter composed of axons ensheathed with myelin. Loss of myelin, also known as demyelination, is caused by a direct injury to an oligodendrocyte cell, which synthesises and maintains the myelin sheath. Lost oligodendrocytes can be replaced from a population of glial progenitor cells called oligodendrocyte progenitor cells (OPCs), which migrate to the site of injury and differentiate into myelinating oligodendrocytes. However, in the ageing brain OPCs exhibit a reduced response to injury thereby leading to atrophy of chronically demyelinated axons. Increasing evidence supports the notion that the brain extracellular matrix (ECM) properties regulate OPC function and dynamic ECM changes with age direct OPC fate. This project is investigating the physiological and biophysical changes in the ageing brain ECM and the molecular mechanism behind OPC response to changes in their biophysical and biochemical environment. Moreover, this project is exploring how modulation of the ECM properties alone can rejuvenate OPCs and hence, become an attractive new therapeutic target.

180 Enriched cultures of pluripotent stem cells derived substantia nigra dopaminergic neurons provides platform for Parkinson's disease modelling and drug discovery

Garcao P, Oosterveen T, Moles E, Patrick K. Soleilhavoup C, **Panman L**

MRC Toxicology Unit, UK

Keywords: Parkinson's disease, substantia nigra dopaminergic neurons, neuronal lineage specification

Pluripotent stem (PS) cells offer important opportunities for disease modelling, drug discovery and cell replacement therapy. However, it is important that cell types of the desired identity are generated. Dopaminergic neurons can be broadly subdivided into two major groups, which form the substantia nigra (SN) and ventral tegmental area (VTA). Despite their communalities in developmental origin and gene expression profile, only SN dopaminergic neurons degenerate in Parkinson's disease. Therefore, access to enriched cultures of SN dopaminergic neurons will offer important opportunities for modelling Parkinson's disease. Although, dopaminergic neurons have been efficiently generated from embryonic stem (ES) cells, it is unclear how enriched cultures of either SN or VTA subpopulations can be obtained. We have established novel mouse and human embryonic stem (ES) cell differentiation protocols that results in the generation of dopaminergic cultures highly enriched for SN neurons. The SN enriched cultures display the predicted sensitivity to mitochondrial toxicity, which is not observed in cultures mainly consisting of VTA neurons. In addition, applying our differentiation protocol to a PD patient specific iPS cell line resulted in increased levels of alpha-synuclein selectively in SN enriched cultures. Altogether our platform provides us with a model system that can be used to model PD disease and to get novel insight into the selective vulnerability of SN neurons.

181 Motor neuron differentiation from ALS patient's pluripotent stem cells

Pollini D, Loffredo R, Cardano M, Quattrone A, Provenzani A

University of Trento, Italy

Keywords: Amyotrophic Lateral Sclerosis (ALS), Matrin 3, Stem cell derived from patients

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease of the motor neuron system, leading to a progressive muscle paralysis and respiratory failure within 2-5 years from diagnosis (1). ALS is categorized in sporadic form (sALS) (90–95% of cases) which has no obvious genetically inherited component and familial ALS (fALS) (5–10%) associated to genetic dominant inheritance factor (2). Mutations in Matrin3 gene were been found to be associated with fALS cases. Our previous work shows as the silencing of Matrin3 affects neuronal differentiation. In order to study the role of Matrin3 mutations in motor neuron differentiation process, we generated iPS cells from sALS and fALS patients' fibroblasts. fALS cells carry Q66K-Matrin3 mutation. Reprogramming was achieved with a non-integrative reprogramming system in 25 days. After checking stemness, we differentiated ALS patients-derived stem cells into motor neurons (3) and evaluated some motor neuron differentiation markers and neurites morphology in ALS patient-derived cells in comparison with healthy donor- derived cells.

182 MKRN3's influence on GnRH-neuron differentiation and its protein-protein interaction partners

Yellapragada V, Xiaonan L, Lund C, Kansakoski J, Pulli K, Vuoristo S, Lundin K, Tuuri T, Varjosalo M, Raivio T

University of Helsinki , Finland

Keywords: MKRN3, GnRH, Puberty

MKRN3 is clinically one of the most relevant gene underlying Central precocious puberty (CPP). CPP is a condition of puberty occurring at unusually early age. CPP, if left untreated would cause psychological and behavioral disorders and recently timing of puberty has also been identified as a risk factor for breast cancer in women. In our study, we generated a pluripotent stem cell based MKRN3 knock out cell lines which were then differentiated to GNRH1 expressing neurons based on a protocol previously published by our lab. Simultaneously, we have identified novel high confident protein-protein interaction (PPI) partners for MKRN3 and our PPI analysis revealed potentially important links between MKRN3 and puberty timing. The results of our study unveil the role of MKRN3 in GnRH neuron development and expression and it also presents the protein interaction map of MKRN3 for the first time in mammalian cells. This results would allow us to further investigate the mechanistic role of MKRN3 in the complex process of puberty initiation. .

183 Investigating the role of SQSTM1 and OPTN in mitochondrial function and clearance in cortical neurons

Poon A, Soutar M, O'Sullivan G, Plun-Favreau H, Wray S, Dawson L.A, McCarthy JM

Astex Therapeutics Ltd, UK

Keywords: SQSTM1, frontotemporal dementia, mitochondria

Mitochondrial dysfunction is a common feature of numerous neurodegenerative diseases. Furthermore, several disease-associated mutations have been identified in genes regulating the selective degradation of damaged mitochondria by autophagy (aka mitophagy), suggesting the involvement of mitophagy in driving disease pathogenesis. Selective mitophagy is critically important for neuronal survival as it maintains an optimal cellular energy production whilst avoiding the toxic accumulation of damaged/ dysfunctional mitochondria, which can lead to cell death. The discovery of mutations in the PINK1 and Parkin genes in Parkinson's disease has facilitated mechanistic understanding of the mitophagy process. For inducing selective mitophagy, PINK1 and Parkin interact with various autophagic adaptor proteins including SQSTM1 and OPTN both of which have been shown to be mutated in patients with frontotemporal dementia. The precise role of SQSTM1 and OPTN in mitochondrial function and clearance remains unclear, particularly whether SQSTM1 in neuronal mitophagy is compulsory. To address this knowledge gap, we are assessing mitophagy in neurons with or without genetic alterations in SQSTM1 and OPTN. We have optimised the culture medium composition, treatment strategies and biochemical methods to detect endogenous levels of mitophagy. In parallel, we are assessing mitochondrial function in neurons with or without SQSTM1 and OPTN using high-content/ throughput screening systems (e.g., Opera Phenix and Seahorse XF Analyzer). Here we outline our phenotypic screening workflow and present preliminary data on the effects of SQSTM1 deletion on mitochondrial function and clearance in neurons.

184 Personalized single motor neuron transcriptomics in axon degeneration diseases

Sainio MT, Mäenpää L, Tenhunen E, Woldegebriel R, Jenni Lahtela, Pirkko Mattila, Auranen M, Ylikallio E, Palmio J, Tyynismaa H

University of Helsinki, Finland

Keywords: axon, neuropathy, differentiation, single cell

Inherited Charcot-Marie-Tooth neuropathies (CMT) are clinically and genetically heterogeneous axon degeneration diseases. The patients suffer from length-dependent degradation of peripheral neurons leading to loss of neuronal connections to their target muscles. The identified disease genes function in multiple cellular pathways but the studies of disease mechanisms have often been complicated by the lack of human neuronal models with endogenous expression levels of the mutant proteins. We use patient-specific iPSC, and differentiate those into cultures containing spinal motor neurons in order to study the pathomechanisms caused by different patient mutations. We currently use the droplet based 10x Genomics Chromium system to capture single motor neurons to profile the patient-specific transcriptomes of these cells. As a proof of principle, we profiled single motor neurons from a patient with an early-onset CMT and a novel homozygous nonsense mutation in NEFL, which encodes for a strictly neuron-specific intermediate filament subunit. We captured high quality transcriptomic data from about 100 and 300 control and patient neurons, respectively. The transcriptome fingerprints revealed that NEFL was the most downregulated transcript in patient neurons in comparison to control neurons. We confirmed the neuronal NEFL loss by western blotting and immunocytochemistry, and thus show for the first time that the consequence of homozygous NEFL nonsense mutations is the absence of NEFL, instead of its aggregation, which is commonly associated with neurodegeneration. This particular setting allowed a rare opportunity to investigate the transcriptomic alterations of human neurons lacking NEFL, which is largely considered to be essential for axonal architecture. In the following years we aim to utilize more advanced differentiation protocols, coupled with emerging axon isolation and electrophysiological techniques to reveal axon specific mechanisms of CMT.

185 Characterisation of a SOX2-positive population in the postnatal adrenal medulla.

Santambrogio A, Russell JP, Brennand APB, Lodge EJ, Steenblock C, Bornstein SR, Andoniadou CL

King's College London, UK

Keywords: Sox2, adrenal

The adrenal gland is an endocrine organ responsible for the stress response and is involved in the regulation of the immune system and metabolism. The adrenal gland is composed of an outer cortex and an inner medulla, which have distinct functions. It is a highly dynamic organ, capable of responding to the variable physiological demand requiring production of steroids and catecholamines. While both stem and progenitor cell populations in the adrenal cortex have been identified and characterised (1), investigation of the adrenal medulla cell hierarchy is still ongoing (2). The adrenal medulla derives from the neural crest, from which a common progenitor generates both sympathetic neurons and neuroendocrine chromaffin cells (3). SOX2 is a marker of multiple stem cell/progenitor lineages throughout the body, including the CNS (4) and another major endocrine organ, the pituitary gland (5). It is found to be upregulated in many tumours including pheochromocytomas and paragangliomas, tumours of the adrenal medulla (6). Here we identify and characterise SOX2-positive cells in the murine adrenal medulla during homeostasis. SOX2-positive cells do not colocalise with other known progenitor markers in the medulla. Using genetic lineage tracing in the juvenile postnatal animal, we identify SOX2-positive cells to be an expanding population which gives rise to chromaffin cells. Taken together our data point towards a new candidate progenitor cell population of the adrenal medulla.

186 Characterisation of oligodendrocyte iron pools and their contribution to development and disease

Stockley JH, Nobuta H, Wernig M, Rowitch DH

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: Myelin, Iron, iPS

In the central nervous system of brain and spinal cord, Oligodendrocytes are essential for the rapid communication between neurons by providing metabolic support and wrapping neuronal axons in a fatty substance called myelin. Leukodystrophries are rare genetic diseases that prevent the formation of oligodendrocytes. Pelizaeus-Merzbacher's disease (PMD) is rare type of luekodystrophry as a result of point mutations, duplications or deletions of the essential myelin gene PLP1. Using iPS cells from patients of PMD we show oligodendrocytes fail to develop when PLP is mutated. Furthermore, we found that iron chelation can rescue PMD phenotype as efficient as gene correction. Oligodendrocytes contain the highest levels of iron of all CNS cells, however very little evidence has been presented on how iron is stored, mobilised and what it is essential for. We present here a new understanding of import into oligodendrocytes and are validating how iron is used in oligodendrocytes from human iPS cells and primary rat cultures.

187 Assembly of brain region domains in vitro to study human development and disease

Andersen J, Birey F, Makinson CD, Islam S, Wei W, Fan CH, O'Rourke NA, Steinmetz LM, Huguenard JR, Pașca SP

Stanford University , USA

Keywords: Cortical spheroid, organoid, interneuron migration

The development of the central nervous system involves an elaborate succession of events including the migration of GABAergic neurons from the ventral to the dorsal forebrain and their ultimate integration into cortical circuits. Genetic or environmental perturbations to these processes have been proposed to contribute to neuropsychiatric disorders, including epilepsy and autism spectrum disorders. However, progress in dissecting the molecular programs underlying these key developmental processes has been remarkably slow. Neural cells derived from pluripotent stem cells (hPSC) hold promise as a platform to model some of these events that occur mostly in mid-to-late gestation and are therefore largely inaccessible for functional studies in humans. To address this, we have developed three-dimensional spheroids from hPSCs that resemble either the dorsal or ventral forebrain and contain cortical glutamatergic or GABAergic neurons. These subdomain-specific forebrain spheroids can be assembled in vitro to generate two-region human forebrain structures that recapitulate specific cell-to-cell interactions in forebrain development. In particular, using this system we are able to model the saltatory migration of interneurons towards the cortex. Moreover, we show that this system can be used to identify key transcriptional changes associated with the integration of interneurons, as well as to model and explore key disease processes using hiPSCs derived from patients. Altogether, this novel in vitro platform has great potential for understanding some of the unique features of human development and modeling human disease.

188 Activation of an antiviral signalling pathway in human NPCs mimics phenotypes of neurodevelopmental disorders

Warre Cornish K, Perfect L, Srivastava D, Price J

King's College London, UK

Keywords: Neurodevelopment, Inflammation, IFNγ, Neurodevelopmental Disorders

The proinflammatory cytokine IFNγ is produced by mammalian cells in response to viral infection, stimulating establishment of an antiviral cellular state (1). IFNγ is also involved in diverse neural processes including neurogenesis, synaptic excitability and control of social behaviour (2, 3, 4). Maternal immune activation during pregnancy leads to upregulation of cytokines, including IFNγ, in offspring's brains and is associated with an increased risk of autism, schizophrenia and other neurodevelopmental disorders in offspring (5, 6, 7). Here we demonstrate a previously unreported mechanism through which IFNγ perturbs neurodevelopment and hypothesize a role for this mechanism in the pathology of neurodevelopmental disorders. We investigated the effects of IFNγ on neurodevelopment using neural progenitors (NPCs), derived from human induced pluripotent stem cells. NPCs were treated with IFNγ for a defined period, before induction of cell cycle exit and maturation into post-mitotic neurons. IFNγ treated neurons displayed increased neurite outgrowth, mimicking a phenotype observed in autism neurons (8). RNA sequencing revealed highly significant overlap between genes upregulated with IFNγ treatment and genes upregulated in brains of individuals with autism and schizophrenia. Among the most significant were multiple genes of the HLA class I complex. HLA proteins were enriched in neuronal growth cones following IFNγ treatment. HLA induction by IFNγ has been shown to be regulated by a nuclear protein complex known as the PML body. Disruption of PML with arsenic trioxide (As2O3) effectively removed the IFNγ-induced increase in neurite length, reduced MHC gene expression changes and removed HLA enrichment in growth cones. Furthermore, PML showed a significant spatial association with nascent HLA transcript. We thus propose a mechanism whereby IFNγ activates PML-dependent HLA transcription leading to altered growth cone dynamics and increased neurite outgrowth.

189 Precision gene editing in iPSC-derived macrophages for early drug discovery

Escudero-Ibarz L, Bagri J, Armesilla-Diaz A, Ashby C, Santiváñez-Pérez J, Mohamet L

GlaxoSmithKline (GSK), UK

Dysfunction of macrophage homeostatic functions can result in inflammatory disease and immune disorders, making them a key therapeutic target. The traditional approach to compound screening is the use of immortalised cells, which lack biological relevance, or primary cells that are expensive at scale and exhibit poor donor reproducibility. Moreover, advances in gene editing of human macrophages have been hampered due to viral delivery systems and their limited proliferative capacity. We have developed a robust cellular platform to generate human macrophages at scale using induced pluripotent stem cell (iPSC) technology. Moreover, we have successfully implemented highly efficient precision gene editing in both iPSCs and iPSC-macrophages by using CRISPR RNP. The use of engineered iPSC-macrophages provides a great opportunity to accelerate early-stage drug development pipelines by generating physiologically and clinically relevant models to help reduce attrition, time and ultimately costs.

190 A resource of healthy iPSC lines derived from the personal Genome Project Canada

Hildebrandt MR, Piekna A, Wei W, Dedeagac A, Pasceri P, Meng G, Reuter MS, Tayebi N, Kinnear C, Rozycki M, Liu J, Kamath B, Mital S, Scherer SW, Ellis J

The Hospital for Sick Children,
University of Toronto, Canada

Keywords: whole genome sequencing, healthy control resource, disease modeling

Reliable healthy control cells are essential to identify disease phenotypes using patient derived iPSCs. Many control lines are potentially compromised by unknown background genetic variants that may predispose to a phenotype or mask it (1). It is crucial to have access to the highest caliber lines that are subjected to whole genome sequencing (WGS) to allow full annotation of disease variants (2). We accessed blood samples from healthy volunteers enrolled in the Personal Genome Project Canada who had undergone whole genome sequencing to identify genomic variants of clinical or unknown significance to determine usability as control lines (3). Their prospective status as healthy controls will be tracked through current and future medical records. We generated multiple iPSC lines by Sendai virus-mediated reprogramming from three individuals lacking pathogenic variants in neuronal and cardiac disease causing genes. Final iPSCs were then subjected to WGS to ensure few or no novel pathogenic variants arose during reprogramming. IPSC characterization was performed by immunostaining for pluripotency markers, in vitro differentiation into embryoid bodies, and demonstration of normal karyotypes. To demonstrate their utility as controls, we directed differentiation into cell types of all three germ layers (cortical neurons, ventricular cardiomyocytes and hepatocytes). These cells were characterized by qRT-PCR, flow cytometry and imaging. Furthermore, neurons and cardiomyocytes were subjected to functional characterization using multi-electrode array studies, while hepatocytes were tested with enzymatic assays. To demonstrate the use of these cells as a platform for gene editing, indel mutations were introduced by CRISPR/Cas9 into MECP2 (neurons) and MYBP3C (cardiomyocytes) and will be compared to control isogenic cells. In conclusion, we generated control iPSC lines as a highly characterized resource for disease modeling and gene editing studies.

191 Routine monitoring of common genetic abnormalities in human pluripotent stem cells using the hPSC genetic analysis kit

Hirst AJT, Zhang A, Hills M, Hunter AL, Thomas TE, Eaves AC, Louis SA, Lee VM

STEMCELL Technologies, UK

Keywords: Pluripotent Genetic Karyotype

Chromosomal aberrations in cultured human pluripotent stem cells (hPSCs) such as numerical aneuploidy, chromosomal rearrangements and sub-microscopic changes have been widely reported. Genetic variants can affect hPSC growth rates, cell survival and differentiation potential. Recurrent genetic abnormalities observed in hPSCs are also observed in human cancers, an observation that raises concerns for downstream clinical applications. The hPSC Genetic Analysis Kit is a qPCR-based method designed to rapidly detect the most common genetic abnormalities observed in hPSC cultures. Specifically, primer-probe assays were optimized to detect the minimal critical regions on chromosomes 1q, 8q, 10p, 12p, 17q 18q, 20q and Xp, as well as a control region on chromosome 4p. These regions represent approximately 70% of all reported abnormalities in hPSC cultures. Amplification efficiencies for all primer-probe sets were measured at ≥ 90% (n = 2). Abnormalities were detected in 4 different hPSC lines each containing a 1q duplication, 10p deletion, 12 trisomy or 20q duplication ($p < 0.001$), with no other genetic abnormalities detected in other regions ($p > 0.1$). Duplication of 20q11.21 is a submicroscopic abnormality often missed when using G-banding karyotyping. As a case study, we analyzed the WLS-4D1 human induced pluripotent stem cell (hiPSC) line using G-banding, fluorescent in situ hybridization (FISH), and the hPSC Genetic Analysis Kit. Although this hiPSC line was found to be karyotypically normal by G-banding, duplication of 20q11.21 was detected using the hPSC Genetic Analysis Kit and confirmed by FISH. To determine assay sensitivity, fluorescently-labelled hPSC lines known to be abnormal for 10p, 12p and 20q were mixed with unlabelled normal diploid hPSC at varying ratios. Results indicate that our qPCR-based approach was able to detect genetically abnormal hPSCs when present at a minimal frequency of 30% ($n = 3$; $p < 0.05$). In summary, the hPSC Genetic Analysis Kit offers researchers a reliable, fast and cost-effective tool to routinely monitor and pre-screen the hPSC lines in their laboratory for recurrent genetic abnormalities.

192 Elastic beads as in vivo tension sensors

Hofemeier A, Wallmeyer B, Betz T

University of Münster, Germany

Keywords: tension sensor, cell fate, cell migration

Mechanical tension has recently been recognized as a key element to understand many biological processes such as cell fate determination or collective cell migration during embryogenesis. However, direct experimental access to determine tension in vivo in a non-destructive way remains a major challenge. Here, we present a novel experimental approach that allows direct measurement of stress inside in vitro and in vivo tissue. By injecting fluorescent polyacrylamide (PAA) beads of known size and elasticity in the tissue, we are able to measure the deformation of their surface and obtain the resulting displacement vector. Solving the inverse elastic problem yields an approximation of the stress field inside the tissue. Furthermore, we show two applications of this novel technique. Firstly, PAA beads are injected into mouse muscles to examine forces exerted during muscle contraction on muscle stem cells, a cell type known to respond to changes of mechanical properties. Secondly, PAA beads are injected into zebrafish embryos to investigate the role of tissue stress in collective cell migration during embryogenesis.

193 Lessons elucidated from the Paolo Macchiarini regenerative medicine research scandal

Joyce M, Turner L

University of Minnesota Bioprinting, USA

Keywords: Paolo Macchiarini Missconduct

Dr. Paolo Macchiarini was able to proceed to human clinical trials of a bioprosthetic trachea that many experts argue, should not have been used in humans without robust evidence of safety, and efficacy obtained from pre-clinical research. How was Dr. Macchiarini able to proceed with his research after a majority of his initial patients suffered serious adverse events and then died? Who, and within which institutions, permitted Dr. Macchiarini to increase the number of human patients in his trial while also including healthier patients, that were not in immediate danger of death? There is nothing that prevents similar circumstances from testing IRB's and research institutions around the world. Lessons in how to prevent bioethical violations in scientific research can be elucidated by studying the events that allowed Macchiarini to proceed with experiments that were not supported by pre-clinical research. The fields of tissue engineering, and regenerative medicine show great potential. However, if scientific research is not conducted in safe, ethical approved ways, public support, will turn from being in favor of progression, to favoring restrictive and repressive measures that can stifle and limit innovations. To maintain a fair and innovative environment for these sciences to progress in, it is imperative that the scientific community speak out when it discovers unethical practices. It is also imperative that there are institutional mechanisms in place to handle allegations of scientific misconduct in a fair and responsible manner. This approach requires participation from researchers, institutions, academic journals, regulatory bodies, sources. Such stakeholders in biomedical research must work with one another to preserve the reputation of revolutionary fields.

194 High efficiency cloning of human pluripotent stem cells using CloneR™ to facilitate gene editing and disease modelling

Kardel MD, Hirst AJ, Macri VS, Chang Y, Zhang A, Norberg JC, Hunter A, Thomas TE, Eaves AC, Louis SA, Lee VM

STEMCELL Technologies, UK

Keywords: hPSC, gene editing, disease modelling

Recent advances in gene-editing techniques such as CRISPR/Cas9, have led to more accessible and cost effective methods to generate variant human pluripotent stem cell (hPSC) lines for a wide range of research areas. However a major hurdle for gene editing in hPSCs is the extremely low cloning efficiency of these cells (< 5%), making the generation of clonal hPSC lines an inefficient process. To address this hurdle, we have developed a novel hPSC cloning supplement, CloneR™. CloneR™ significantly increases the cloning efficiency of both human embryonic stem (ES) and induced pluripotent stem (iPS) cells to 15-40% without the need for single-cell adaptation. The supplement is compatible with both mTeSR™1 and TeSR™-E8™media on a range of different cell culture matrices. This breakthrough enables researchers to plate hPSCs using single-cell deposition (1 cell/well), minimizing the occurrence of mosaic colonies whilst generating a large number of clones for downstream applications. Clonal hPSC lines derived using CloneR™ display a normal diploid karyotype and retain all characteristics of their respective parental hPSC lines including morphology, growth rate, undifferentiated marker expression, and ability to differentiate to all three germ layers. To demonstrate the utility of CloneR™ we generated a clonal hPSC line following CRISPR/Cas9 gene-editing of the KCNH2 (hERG2) to successfully model Long QT Syndrome in vitro. In summary, supplementing TeSR™ media with CloneR™ will facilitate gene editing in hPSCs through the rapid and highly efficient generation of clonal cell lines.

195 Pipeline for producing genetically modified hIPSC lines for disease modeling

Lenaerts A, Kadiwala J, Tamang P, Shao Y, Vallier L

University of Cambridge, UK

Keywords: hIPSC derivation, genome editing

The development of methods allowing the generation of induced pluripotent stem cells (IPSCs) from somatic cells has brought enormous progress in the fields of stem cell biology and regenerative medicine. Human iPSCs are currently widely used for disease modelling, drug discovery and development of cell therapy. The combined use of gene editing technologies with IPSCs allows for genetic background-controlled disease models and drug screens. Furthermore, mutation corrected IPSCs hold the promise to overcome immune rejection problems in therapeutic interventions. Here, we will describe the pipeline developed by the NIHR Cambridge BRC hIPSCs core facility to either induce or correct mutations in hIPSCs. Indeed, we have established a gene editing protocol for IPSCs using the CRISPR-Cas9 technology. This protocol is optimised for efficient selection of guide RNA, robust selection of genetically modified hPSCs, rapid genotyping and functional characterization. Moreover, for essential genes, we have successfully implemented an inducible knockdown system in IPSCs. This system allows to conditionally knockout genes not only in undifferentiated cells but also in their differentiated derivatives1. Finally, we will discuss the potential deleterious effect of CRISPR/Cas9 on genomic instability. To conclude, our pipeline has been successful for a diversity of applications including large genome deletions of a super enhancer2 and correction of disease-causing mutations in patients with a diversity of mono-genetic disorders. The Cambridge BRC hIPSCs core facility is funded by the NIHR.

196 iPSC and CRISPR/CAS9 technologies enable precise and controlled physiologically relevant disease modeling for basic and applied research

Lo J, Sommer A, Dantara H, Wang Z, Pijanowski L, Yu Z, Tummala P, Rajeswaran P, Nguyen D, Cao C, Chen-Tsai RY

Applied StemCell, USA

Keywords: Gene Editing, Model Engineering, CRISPR KO, TARGATT

Human-induced pluripotent stem cell (hiPSC) technology has provided unique ways to understand and potentially treat human diseases using cells from individual patients. When combined with genome editing techniques such as the clustered regularly interspaced short palindromic repeat (CRISPR)/Cas system, it is now possible to establish highly-controlled experimental models via correction of disease-causing mutations in patient lines or introduction of the same genetic defects into healthy iPSCs. Typically, isogenic cell pairs that differ in a single genetic change are further differentiated into target cell types that are relevant to the disease and then used to interrogate disease phenotypes or to screen novel therapeutic agents in high-throughput format. Here, we illustrate this process by showing the generation of hiPSCs from healthy donors and patient populations using fibroblasts and PBMCs as the somatic cell source. Using CRISPR/Cas9 or our proprietary TARGATT™ technology, customized gene-edited hiPSC lines can be efficiently generated, including lines with monoallelic or biallelic gene mutations, large-fragment knock-in and conditional/inducible expression models. Finally, differentiation of hiPSCs towards neural lineages is presented as an example of an experimental platform with potential for drug screening and neurotoxicity assays.

197 SUPRAPRIMATE: An online resource for cross-species single cell transcriptome data integration

Madrigal P, Boroviak T

University of Cambridge, UK

Keywords: single cell transcriptome, primate embryogenesis, web resource, bioinformatics

Early human and non-human primate development substantially differs from the rodent paradigm. This divergence has important implications for our understanding of germ cell specification, placental development, embryonic stem cells and pluripotency in the embryo. Although multiple studies have focused on single cell RNA-seq profiling of preimplantation stages, little is known about the earliest postimplantation stages in primates. Moreover, integration of cross-species data is still in its infancy. Our goal is to build an integrative platform for rodent an primate development from zygote to gastrulation. To this end, we have developed SUPRAPRIMATE, an online portal for the integration and visualization of single-cell RNA-seq data of rodent and primate pre- and postimplantation embryonic development. The portal is implemented as a Shiny web application in R, and currently includes data from mouse and cynomolgus monkey. Common marmoset data generated in our lab will be added soon. Furthermore, SUPRAPRIMATE allows the user's own data to be integrated into the developmental framework to examine the developmental stage of in vitro cultured cells. We aim to use this resource to delineate primate- and rodent-specific genes in early embryogenesis and to determine culture conditions supporting authentic lineage identity.

198 Cell therapy for bone defects using umbilical cord MSC-derived osteoblasts

Lee S, Choo S, Ahn SY, Lee J, Lim GI, Ulijn RV, Kwak CK, **Park H-S**

Kyunghee University/CEFO Co., Ltd

Keywords: Stem cell, 3D culture, Osteoblasts, Differentiation

Currently extensive research into physical properties in stem cell differentiation has been made and we ventured to investigate a strategy ensuring rapid and efficient protocol for osteogenic differentiation of mesenchymal stem cell (MSC) using synthetic gels with optimal stiffness. We have shown that optimizing stiffness of the synthetic gel allows osteogenic differentiation of MSC more efficiently. Osteogenic differentiation occurred in as early as 5 days after differentiation induction, without individual variation and no variation in between different tissue sources of MSC. Neither bone morphogenic protein (BMP) nor Wnt3a induced an additive effect on osteogenesis, which implied that the stiffness of synthetic gel plays a preferential role in cell fate determination towards osteogenesis. Expression of MHC did not change after differentiation. However, loss of surface proteins of MSC and gain of surface proteins of osteoblasts were observed sequentially over time, during osteogenic induction period. In rat tibial partial defect model, grounded bone was completely regenerated within 5 weeks by transplanted human osteogenic cells. In rat calvarial defect model, transplanted human osteogenic cells healed rat calvarium to form human bone, confirmed by immunohistochemistry using human nuclei specific antibody. These results suggested that mature osteoblasts derived from MSC might effectively facilitate recovery from bone injury by cell replacement.

199 Developing light sheet imaging pipelines for cancer research

Reichelt S, Shah G, Sims R, O'Holleran K

Cancer Research UK
Cambridge Institute, UK

Keywords: Light sheet microscopy, high-throughput imaging, cancer research, intestinal adenocarcinomas, spheroid, mouse embryo

In-vitro cell culture techniques are rapidly moving towards three-dimensional (3D) models to better mimic the tissue architecture and environment in vivo. These physiologically relevant models such as mouse and patient cells derived organoids provide a platform for understanding behaviour of cells in various physiological and perturbed conditions. However, appropriate imaging technology, to visualise and understand the dynamic interactions of cells within organoids in 3D, is still lacking. Therefore, alongside rapid advancements in the field of organoid technology, there is a need for parallel development of imaging techniques that enable long-term imaging of living samples. In recent years, light sheet microscopy has proved to be an excellent technique for fast volume imaging of growing tissues, organs and embryos of model organisms. Owing to its capability to image large samples with minimal phototoxicity, visualisation of dynamic biological processes across spatio-temporal scales has become possible, revolutionising the fields of cell and developmental biology. Despite the wide applicability of light sheet microscopy, cancer research still relies on conventional imaging techniques using fixed and sectioned tissues, primarily due to the challenges posed by samples used to study cancer biology. In contrast to robustly developing embryos, 3D cell culture and organoids are very fragile and susceptible to contamination, challenging their long-term survival during the course of imaging. At the CRUK CI, we are working towards developing dedicated experimental setups and image processing workflows for live organoid imaging. In collaboration with several groups, we have established novel ways of growing the organoids and cells directly inside polymer tubes used for light sheet imaging. We have successfully imaged co-cultures of tumour cells, growing organoids and whole mouse embryos for over 24-48 hours of development at high resolution, tracking the dynamics and division of individual cells therein. Further, to understand the structure and organisation of cells within large tumours, we use clearing techniques to make the samples transparent and image whole tissues using light sheet microscopy. Our dedicated image-processing and analysis pipelines are used to reconstruct 3D models. I will present our on-going efforts and discuss the prospects light sheet microscopy brings to the cancer biology and translational research communities at large.

200 Site-specific seamless vector transgenesis in human LINE-1 elements

Suki R, Harshyaa M, Farid JG , Desmond W, Dario C, Peter D

Nanyang Technological University, Singapore

Keywords: Genome manipulation, Phage λ-integrase, Seamless vector, LINE-1 elements

Genome manipulation is an important component of gene/cell therapy and molecular medicine, particularly where insertion of a large transgene cassette at a safe harbor site in the human genome is required. In this respect, several tools like viral vectors, ZFNs, TALEN and CRISPR-Cas system are currently being used, however, issues like insertional mutagenesis, off-target events, and small cargo size have limited their usage. We have developed a novel transgenesis tool based on phage λ-integrase that catalyzes conservative site-specific integration of large plasmid DNA into a safe harbor site, termed attH4X, found in 1000 human Long INterspersed Elements-1 (LINE-1) (1). To further improve this tool with respect to safety concerns and targeting efficiency, we used our λ-Int platform to produce seamless vector via in vitro intramolecular recombination and subsequently achieved ex vivo targeting to the LINE-1 element for site-specific genome integration. Furthermore, we utilized this platform for successful transgenesis and expression of therapeutic protein like anti-CD19 chimeric antigen receptor in human embryonic stem cells, demonstrating its potential utility in therapeutic applications.

201 Reconstruction of cellular reprogramming landscapes and trajectories by analysis of large-scale single-cell gene expression

Jian S, Geoff S, Marcin T, Brian C, Vidya S, Peter B, Lia L, Stella M, Konrad H, Aviv R, Rudolf J, Eric L

Broad Institute/Whitehead Institute, USA

Keywords: reprogramming, single cell

Understanding the molecular programs that guide cellular differentiation during development is a major goal of modern biology. Here, we developed an approach, WADDINGTON-OT, for inferring developmental landscapes, probabilistic cellular fates and dynamic trajectories from large-scale single-cell RNA-seq (scRNA-seq) data collected along a time course. We demonstrated the power of WADDINGTON-OT by applying it to study around 300,000 scRNA-seq profiles collected during reprogramming of fibroblasts to iPSCs by the Yamanaka factors. We applied this strategy to an additional 200,000 scRNA-seq profiles of other reprogramming cocktails, with the goal of discovering the inherent mechanisms of iPSC reprogramming. We construct a high-resolution map of reprogramming that rediscovers known features; uncovers new alternative cell fates; predicts the origin and fate of any cell class; and implicates regulatory models in particular trajectories. Our approach provides the first high resolution roadmap of different reprogramming cocktails and a general framework for cell fate conversions in natural and induced settings.

202 Development of hiPSC reporters for chemical safety assessment in vitro

Snijders KE, ter Braak R, Niemeijer M, van den Berk L, van de Water B

Leiden University, Netherlands

Keywords: hiPSC reporters, CRISPR/Cas9, Toxicology

Despite rigorous testing procedures, unpredicted adverse effects remain a major concern for drug development and in clinical practice. There is an impending need for an vitro model that carries an increased human relevance and predictive accuracy during chemical safety assessments. We established a Bacterial Artificial Chromosome (BAC)-GFP reporter model based in the liver cancer HepG2 cell line. High Content Imaging (HCI) allows for the visualization of different stress pathway activation upon compound exposure on a single cell level. While this model has been proven to be sensitive and accurately predicts toxic side effects we are currently developing a second generation reporter model using fluorescently tagged human induced pluripotent stem cells (hiPSCs). hiPSCs are excellent candidates for in vitro safety screening since they rapidly divide, have undisrupted metabolic activity and can be differentiated into a whole range of cell types. This overcomes many of the limitations found in current in vitro models such as availability, inter-donor variability and stability. Coupling hiPSCs with a clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system allows for the endogenous tagging of multiple stress-related genes. Scar free integration of the fluorophore leads to minimal disruption of the gene and maintenance of a representative stress response in vitro. We anticipate that our hiPSC cellular stress response reporters in combination with HCI may play a key role in future safety assessment of chemicals.

203 Improving endoplasmic reticulum stress resistance for enhancing therapeutic potential of cell therapy

Toietta G, Baldari S, Gentile A, Capogrossi M, Di Rocco G

IRCCS Regina Elena National Cancer Institute, Italy

Keywords: cell and tissue-based therapy; Duchenne muscular dystrophy; endoplasmic reticulum stress; protein disulphide isomerase

Cell therapy for degenerative muscle diseases aims at rescuing muscle damage by delivery of myoblast precursors. Thus far, this strategy has been mostly unsuccessful due to massive loss of donor cells shortly after transplantation. Several strategies have been used to increase transplanted cell survival but only with limited success (Baldari et al., 2017). The endoplasmic reticulum (ER) is an organelle involved in protein folding, calcium homeostasis, and lipid biosynthesis. Protein Disulfide Isomerase (PDI) is a molecular chaperone induced and activated by ER-stress. PDI is upregulated by hypoxia in neuronal, cardiac and endothelial cells, supporting increased cell survival to hypoxic stress and protection from apoptosis in response to ischemia (Severino et al., 2007). We investigated the involvement of ER-stress associated proteins and in particular of PDI in the muscle system and in its degenerative pathologies. We determined that overexpression of PDI confers a survival advantage to human myoblasts injected into murine dystrophic muscle and to endothelial cells administered upon hindlimb ischemia damage, improving the therapeutic outcome of the treatments. Collectively, these results suggest that overexpression of PDI may protect transplanted cells from hypoxia and other possibly occurring ER-stresses and consequently enhance their regenerative properties. Financial Support: Ministero della Salute – Ricerca Finalizzata RF2011-02347907

204 A novel differentiation system to generate hepatocytes from hPSCs for drug screening and disease modelling

Tomaz RA, Wesley B, Zacharis K, Ortmann D, Vallier L

Wellcome - MRC
Cambridge Stem Cell Institute, UK

Keywords: hPSCs, hepatocytes, gene targeting

Human pluripotent stem cells (hPSCs) have emerged has a promising tool for modelling diseases "in a dish", given their ability to expand indefinitely and differentiate into any tissue. Differentiation conditions for hepatocyte-like cells (HLCs) have been successfully established1,2,3. However, current methods mimic different steps of embryonic development and are limited by the lack of knowledge of signaling pathways operating in critical steps of differentiation/maturation, ultimately producing fetal-like cells. Forced expression of transcription factors (Forward Programming) has recently emerged as a robust and alternative route for conversion of hPSCs into somatic cell types, circumventing the need to mimic development4. The aim of this study was to apply the forward programming system to the generation of hepatocytes. hPSCs were genetically engineered with an optimized inducible overexpression (OPTi-OX) platform relying on the Tet-ON system4. The transgenes tested were a combination of four known hepatocyte-specific transcription factors: HNF4a, HNF1a, HNF6 and FOXA3. The four factors were robustly overexpressed in hPSCs upon treatment with 1ug/ml of doxycycline. After 15 days, dox-treated hPSCs expressing the four factors acquired a cobble-stone like morphology, binucleation, displayed CYP3A4 metabolic activity, and expressed hepatocyte markers such as Albumin, AFP and a1AT. This phenotype persisted up to 25 days in culture. These results represent a first step towards the development of novel and faster methods for production of hepatocytes in vitro. In addition, this system can be further adapted in order to include additional transcription factors, with the potential to induce the acquisition of mature adult-like hepatocyte phenotype. Finally, these findings could be transferred to other cell types generated from hPSCs and thus provide a universal approach for generating mature cell types.

205 Mass spectrometry quality control of stem cell cultures

Vanhara P, Kucera L, Prokes L, Jureckova L, Pena-Mendez EM, Havel J, Hampl A

St. Anne's University Hospital Brno ,
Czech Republic

Keywords: Mass spectrometry, quality control

Pharmaceutical, bioindustrial or biomedical applications of pluripotent stem cells require stringent quality control of cell cultures. In routine, long-term cultures, the major risks involve cross-contamination by other cell type(s) and gradual phenotype shifts. Precise biotyping of cultured cells is thus required for unambiguous characterization of cell states or their products. Analysis of repetitive DNA sequences, such as Short Tandem Repeats (STR), is a widely accepted, simple, and commercially available technique to authenticate cell lines, however, providing only qualitative information that is dependent on the reference databases for interpretation. Similarly, karyotyping or analysis of gene or protein markers of e.g. pluripotency or differentiation focus on a particular type of instability, such as genome abnormalities or adverse phenotypic traits, and do not provide sufficient sensitivity. Therefore, there is a need for robust, feasible, and sensitive tools for determining or validating cell phenotype and revealing potential divergences from the optimal cell state. First we modeled two common scenarios that typically occur in long term cultures of human embryonic stem cell (hESC) - induction of hESC differentiation and cross-contamination by other cell types (mouse embryonic fibroblasts or mouse ESCs). Then we analyzed the cultures by intact cell mass spectrometry and analyzed the mass spectra for informative regions. Input matrices of spectral data allowed correct clustering of cell samples and even classification of unknown spectra by artificial neural networks. Taken together, we show that intact cell mass spectrometry, when linked to proper mathematical methods, is a tangible and routinely applicable tool for unraveling and even quantifying hidden heterogeneity in stem cell cultures. This study was supported by the National Program of Sustainability II (project no. LQ1605, MEYS CR) and the Czech Health Research Council (project no. 18-08-00299, MH CR).

206 The "Helene Medium": specialized stem cell culture medium

Yang CF, Saito M, Shirakawa K, Matsuoka T

STEMCELL Co. Ltd, Japan

Keywords: cell culture, transplantation

The stem cell culture requires many different conditions from normal or cancer cell culture, such as serum concentration, culture methods, and most of all, the culture medium. In our clinic in Japan, we provide stem cell treatments to our patients. We need more efficient and safer stem cell culture; thus, we have developed a specialized stem cell culture medium for primary stem cell culture. The "Helene Medium" is designed specially for stem cell growth, with better growth rate, stable cell growth, less chance of differentiation. We grow the stem cells in our medium and two other commercial mediums, examine the growth rate, cell morphology and passage number to evaluate the cell quality. Besides, serum usage in stem cell culture might lead to cell differentiation. We also test the different concentrations and types of serum. Stem cells could grow easily in Helene Medium but other cell types such as fibroblast cells, are difficult to grow. In our research, we have found that compared with other commercial mediums, stem cells cultured by our medium grow faster and with more stable cell morphology. Also, the expression of clusters of differentiation (CD) shows that stem cells are able to keep their potency during cell culture.

207 Development of 3D bioprinting technology for in vitro culture of corneal cells

Chen JA, Wu CH, Liang CM

National Defense Medical Center, Taiwan

Keywords: Limbal Mesenchymal Stem Cell, 3D bioprinting, Corneal Transplantation

An estimated 10 million people worldwide suffer from corneal opacity loss of vision, so there is a high demand for corneal substitutes. To provide an alternative to artificial corneal transplantation, various methods have been employed in the field of tissue engineering, but current research results are far from delivering functionally similar corneal tissue substitutes. Most methods of designing corneal stroma in the past have been limited to 2D cultures in which the composition is optimized to maintain cell growth and tissue on the surface of the material. In contrast, the 3D bio-printing tissue system provides a more similar structure to the natural tissue and further guides cell tissue and tissue development. Cell adhesion molecules involved in intracellular signaling and differentiation interact with surrounding matrices in three dimensions, and thus the aspects of the culture environment strongly influence the organization and response of the cells. The project use 3D bioprinting technology combined with collagen-based bio-ink and corneal limb mesenchymal stem cells to make artificial corneal tissue constructs. Limbal Mesenchymal Stem Cell can form human corneal stromal tissue equivalents in vitro and has the immune privilege and mesenchymal stem cell characteristics suitable for corneal replacement and in vitro models.

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