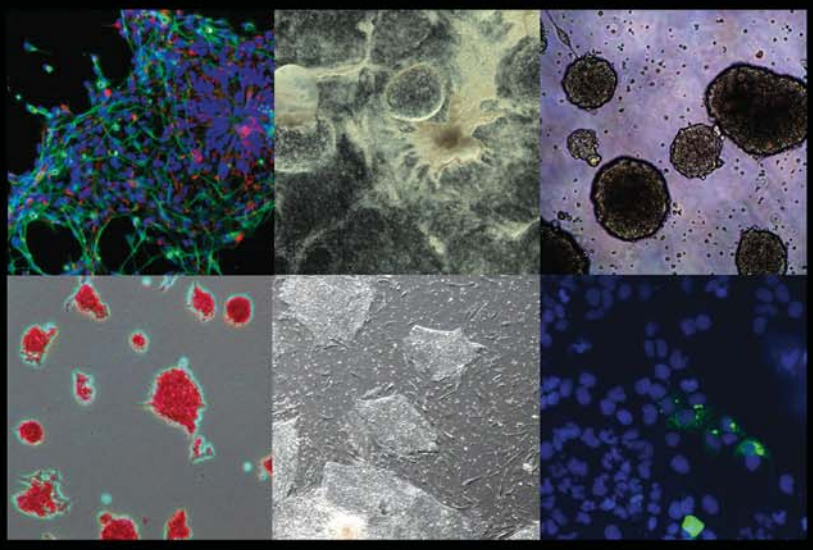
International symposium
26 - 28 May 2010
Gulbenkian Foundation
Lisbon

Stem Cells in Biology and Disease



Speakers

Peter Andrews
Yves Barde
Nissim Benvenisty
Oliver Brüstle
Elena Cattaneo
Tariq Enver
Magdalena Götz
Domingos Henrique
Ron McKay
Christine Mummery
Andras Nagy
Martin Pera
Marc Peschanski
Angel Raya
Tom Reh
José Silva
Andrew Smith
Austin Smith
John Stingl
Lorenz Studer
Maarten van Lohuizen
Marius Wernig
Shinya Yamanaka

Ethics Workshop:

Do we still need human embryonic stem cells?

Friday 28 May, 2pm

estools
Advances with Human Embryonic Stem Cells

FUNDAÇÃO CALOUSTE GULBENKIAN

SIXTH FRAMEWORK PROGRAMME

It is a great pleasure to welcome you to this International Symposium organised by ESTOOLS. The ESTOOLS consortium, launched in 2006 with 4 years funding from the European Union, has comprised 21 leading groups from the European Research Area, specialising in stem cell biology.

To mark the final months of ESTOOLS we have brought together at the Gulbenkian Foundation in Lisbon a wide range of researchers who work on human ES and iPS cells, both from ESTOOLS and from around the world, to provide an update on the state of this rapidly evolving field. We have also included a Workshop in which we will address the ethical issues raised by the development of iPS cells.

A corpus of over 80 publications that you can see on our website links you to the work of ESTOOLS. Our work has spread right across the themes that need to be well understood so that human-origin stem cells can be of eventual use in regenerative medicine, disease modelling and drug discovery: we have studied how to multiply, differentiate, modify and reprogram these cells, all the while conscious of the ethical frameworks within which we conduct our research. In your delegate bag is a folder that holds a summary of our work in these areas. The folder also signposts the ESTOOLS training programme that has embraced interns from within the network and for one-year fellowships, as well as attendees at our schools and workshops.

Beyond fundamental scientific research and training, ESTOOLS has developed new methodologies to connect this science with the public of school age and beyond. The folder has more information about these, and at Lisbon we have taken this further by supporting the creation and first staging of an innovative theatrical production "*Staminalia: a dream and a trial"* by Valeria Patera. Our will activities in Lisbon will close with a workshop for local school students.

I hope you will enjoy the next few days, and find the events informative and stimulating.

Peter W Andrews
University of Sheffield
Co-ordinator of ESTOOLS

Contents

Here to help

Registration:
Peri Sykes,
Gillian Hind

Speakers:
Angela Ford

Powerpoints:
Ras Trokovic, Veronika Ganeva

Posters:
Sebastien Duprat

Operations:
Andrew Smith,
Katia Hervy

Press:
Sergio Pistoi

Symposium Programme

Any last-minute changes to this programme will be announced and posted on the notice-board outside the conference room.

Wednesday 26 May

11:00 Registration - POSTER PUT UP

12:30 Lunch

13:25 Opening words: **Antonio Coutinho** (Instituto Gulbenkian de Ciência) and **Peter Andrews**

13:30 Keynote speech - *Human pluripotent stem cells in disease and drug discovery*
Christine Mummery - Leiden University

14:30 SESSION 1: Cancer stem cells - Chair: Peter Andrews

14:30 – 15:00 Maarten van Lohuizen - Netherlands Cancer Institute
Role of Polycomb repressors in stem cells, cancer and development

15:00 – 15:30 John Stingl - Cancer Research UK Cambridge Research Institute
Mammary stem and progenitor cells: Understanding the cellular context of breast tumours

15:30 – 15:45 Selected talk: Christina Scheel - Whitehead Institute for Biomedical Research

15:45 – 16:15 Tariq Enver - University of Oxford
The Stem Cell Biology of Childhood Leukaemia

16:15 Coffee

16:45 SESSION 2: Fate switching and induced pluripotency - Chair: Austin Smith

16:45 – 17:15 Marius Wernig - Stanford University
Inducing cell fate changes with transcription factors

17:15 – 17:45 José Silva - University of Cambridge
Unblocking the path to induced pluripotency

17:45 – 18:00 Selected talk: Samer Hussein - Mount Sinai Hospital, Toronto

18:00 – 18:30 Angel Raya - Centre for Regenerative Medicine, Barcelona
Using iPS cells to treat and model human disease

18:30 – 20:30 POSTER SESSION

Thursday 27 May

09:00 SESSION 3: Neural stem cells - Chair: Yves Barde

09:00 – 09:30 Magdalena Götz - Helmholtz Zentrum München
Adult neural stem cells - heterogeneity and molecular fate determinants for neurogenesis

09:30 – 10:00 Domingos Henrique - Instituto de Medicina Molecular, Lisbon
From ES cells to Neurons and Notch

10:00 – 10:15 Selected talk: Silvia Nicolis - University of Milano-Bicocca

10:15 – 10:45 Oliver Brüstle - University of Bonn
Conversion of ES and iPS cells into stable neural stem cell ground states

10:45 Coffee

11:30 SESSION 4: Neurodegenerative disease modelling, prospects for neural repair - Chair: Elena Cattaneo

11:30 – 12:00 Yves Barde - University of Basel
Embryonic Stem Cells: A powerful Discovery Tool to Study the Nervous System

12:00 – 12:30 Tom Reh - University of Washington
Stem cell strategies for retinal repair: regeneration or replacement?

12:30 – 12:45 Selected talk: Philipp Koch - University of Bonn & Hertie Foundation

12:45 – 13:15 Marc Peschanski - I-STEM, INSERM, Paris
Prospects for epidermic cell therapy using human pluripotent stem cells

13:15 Lunch

14:45 Keynote speech - *Controlling stem cells in health and disease*
Ron McKay - National Institute of Neurological Disorder and Stroke

15:45 SESSION 5: Technological advances in manipulating pluripotent stem cells – Chair: Nissim Benvenisty

15:45 – 16:15 Andras Nagy - Mount Sinai Hospital, Toronto
Transposon- reprogrammed induced Pluripotent Stem cells are powerful exploratory tools

16:15 – 16:45 Lorenz Studer - Memorial Sloan-Kettering Cancer Center
Tools for Modeling Neural Development and Disease in Human Pluripotent Stem Cells

16:45 – 17:00 Selected talk: Tobias Cantz - Hannover Medical School

17:00 – 17:30 Andrew Smith - University of Edinburgh
Versatile gene targeting strategies for genetic modification of human ES cells

17:30 Coffee

19:30 THEATRE EVENT *Staminalia – a Dream and a Trial*
(in the Gulbenkian Foundation Park *anfiteatro* or in the auditorium)

20:15 departure immediately after theatre by buses to the CONFERENCE DINNER
see details p.144

Friday 28 May

09:00 Keynote speech - *Induction of Pluripotency by Defined Factors*
Shinya Yamanaka - Kyoto University

10:00 SESSION 6: Control of stem cell state and pluripotency - Chair: Tariq Enver

10:00 – 10:30 Peter Andrews - University of Sheffield
The evolution of stem cell fate

10:30 – 11:00 Martin Pera - University of Southern California
Extrinsic Regulation of Human Pluripotent Stem Cells

11:00 – 11:15 Selected talk: Veronique Azuara - Imperial College London

11:15 – 11:45 Austin Smith - University of Cambridge
Design principles of pluripotency

11:45 Poster prizes presentation / Symposium closing words: Peter Andrews

11:50 Coffee available

12:35 – 14:00 Lunch

Scientific Committee:

Peter Andrews
Yves Barde
Tariq Enver
Magdalena Götz
Domingos Henrique
Austin Smith

For the opportunity to hold these meetings in Lisbon and for their assistance ESTOOLS are very grateful to the Fundação Calouste Gulbenkian, to Ana Godinho (Instituto Gulbenkian de Ciencia) and to Domingos Henrique (Instituto de Medicina Molecular).

Workshop Programme

ESTOOLS 3rd Ethics Workshop:
do we still need human embryonic stem cells?

Each talk includes 5 minutes Q&A.

14:00 **Opening words**: **Göran Hermerén** - Lund University / European Group on Ethics in Science & New Technologies

14:10 SESSION A: Scientific aspects: comparison of ES to iPS cells
Chair: Göran Hermerén

14:10 – 14:35 *Differential Modeling of Fragile X Syndrome by Human ES and iPS Cells*
Nissim Benvenisty - Hebrew University of Jerusalem

14:35 SESSION B: Ethical, societal and regulatory aspects, including public perception.
Chair: Demetrio Neri **-** University of Messina

14:35 – 15:00 *Regulating stem cells*
Margarida Menezes-Ferreira - INFARMED National Authority of Medicines & Health Products, Portugal

15:00 – 15:25 *From ES to iPS cells: the Reprogramming of Scientific and Societal Choices*
Giuseppe Testa - European Institute of Oncology, Italy

15:25 – 15:45 To be confirmed

15:45 Coffee

16:10 SESSION C: IPR, patentability and commercialisation
Chair: Kate Millar - University of Nottingham, Centre for Applied Bioethics

16:10 – 16:35 *The ECJ-case and strategies for stem cell patenting*
Clara Sattler de Sousa e Brito - Yale Law School

16:35 – 17:00 *Consequences of the G2/06 decision of the Enlarged Board of Appeal on Patent Applications concerning human ES cells*
Christof Friedrich - EPO Munich

*Patentability of embryo-derived versus induced pluripotent stem cells*Aliki Nichogiannopoulou - EPO Munich

17:00 ROUND TABLE - Chair: Göran Hermerén

Participants: Eurico Reis (Conselho Nacional de Procriação Medicamente Assistida) and other participants from the Workshop

18:00 Closing words: Peter Andrews

Symposium Faculty

Peter Andrews
Centre for Stem Cell Biology, University of Sheffield

My research interests have focused on the biology of human ES cells and also their malignant counterparts, embryonal carcinoma cells, the stem cells of teratocarcinomas. One aspect of this work has been a focus on the genetic stability of ES cells and their tendency to evolve in culture to a state resembling that of EC cells. Over the past few years I have co-ordinated ESTOOLS and also the International Stem Cell Initiative.

Talk: The evolution of stem cell fate

Yves-Alain Barde
Biozentrum, University of Basel, Switzerland

Yves-Alain Barde studies the developing nervous system of vertebrates with a focus on mechanisms regulating the number of neurons and their shape. Neurotrophins and their receptors are used to get insights into such mechanisms as these molecules emerged as major regulators of brain development and function. They are also involved in an increasing number of conditions associated with brain dysfunction in humans. During the last decade his group developed a method to generate defined classes of neurons from embryonic stem cells that turned out to be uniquely useful to study function and dysfunction of neurons under well-defined conditions.

Talk: Embryonic Stem Cells: A powerful Discovery Tool to Study the Nervous System

Oliver Brüstle
Director, Institute of Reconstructive Neurobiology, LIFE & BRAIN Center, University of Bonn

Oliver Brüstle is head of the Institute of Reconstructive Neurobiology at the University of Bonn, Germany and scientific director of the LIFE&BRAIN GmbH. His research focuses on pluripotent and neural stem cells and their use for disease modeling and nervous system repair.

Talk: Conversion of ES and iPS cells into stable neural stem cell ground states

Elena Cattaneo
Professor of Pharmacology, Università degli Studi di Milano

Elena Cattaneo is Director of the Center for Stem Cell Research of the University of Milan (UniStem). Since 1997 she has been a Coalition Investigator of the Huntington's Disease Society of America (H.D.S.A.) and since 2008 Coordinator of the European Union funded project NeuroStemcell. Her research is focussed on Huntington's disease and the ultimate goal is to identify stem cells, molecules and pathways that are suitable for therapeutic intervention.

CHAIR

Tariq Enver
Weatherall Institute of Molecular Medicine, University of Oxford

Tariq Enver is Professor of Stem Cell biology at the Weatherall Institute, The University of Oxford in the Molecular Haematology Unit in Oxford. His research explores the cellular and molecular aspects of cell fate decision making in both normal and leukaemic blood, stem and progenative cells.

Talk: The Stem Cell Biology of Childhood Leukaemia

Madgalena Götz
Institute of Stem Cell Research, Helmholtz Center Munich and Institute of Physiology, University of Munich, Germany

Our main interests are the cellular and molecular mechanisms of neurogenesis. Since our discovery of radial glial cells as the source of neurons in the developing brain, we examine the fate and potential of glial cells in the adult brain with the final aim to re-install neurogenesis after brain injury. We use functional genomics as well as in vitro and in vivo assays to tackle these questions at the Institute for Stem Cell Research at the Helmholtz Center and the University of Munich.

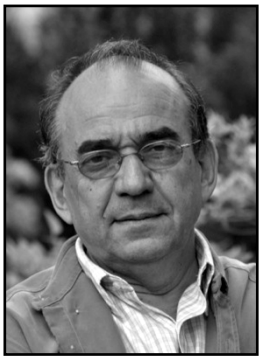
Talk: Adult neural stem cells - heterogeneity and molecular fate determinants for neurogenesis

Henrique Domingos

Instituto Medicina Molecular, Lisbon

Our lab is particularly interested in understanding the regulatory principles governing the generation of neurons in vertebrate embryos and how Notch signalling functions to maintain neural progenitors in the embryonic and adult brain. We are located at Instituto Medicina Molecular but affiliated also with the Gulbenkian Institute and Champallimaud Foundation.

Talk: From ES cells to Neurons and Notch



Ronald D. G. McKay

Senior Investigator, National Institute of Neurological Disorder and Stroke, Bethesda

Ron McKay received a B.Sc. in 1971 and a Ph.D. in 1974 from University of Edinburgh, where he studied under Edwin Southern examining DNA organization and chromosome structure. He received postdoctoral training at University of Oxford working with Walter Bodmer examining the first restriction-fragment-length polymorphism (RFLPs) in the human genome. In 1978, he became a senior staff investigator at Cold Spring Harbor Laboratory concentrating on two areas: developing the first immunoassay for DNA-protein complexes and establishing the field of molecular neuroscience. Joining the MIT faculty in 1984, Dr. McKay identified neural stem cells as a tool to study brain development and function. In 1993 he joined the NIH as chief of the Laboratory of Molecular Biology at NINDS. His laboratory studies pluripotent and somatic stem cells with a particular focus on regeneration of the nervous system.

Talk: Controlling stem cells in health and disease

Christine Mummery

Professor of Developmental Biology, Dept. of Anatomy & Embryology, Leiden University Medical Center

Christine Mummery studied Physics and has a PhD in Biophysics from the University of London. She received a postdoctoral fellowship from the Royal Society (UK) for research at the Hubrecht Institute where she became group leader and in 2002, Professor of Developmental Biology. Her research concerned mouse development and differentiation of mouse and human embryonic stem cells. She pioneered studies differentiating and characterizing cardiomyocytes from human embryonic stem cells and was among the first to inject them in mouse hearts and assess their effect on myocardial infarction. In 2008 she was appointed chair of the Department of Anatomy and Embryology, Leiden University Medical Centre. Here she continues research on heart development and the differentiation of pluripotent human cells into the cardiac and vascular lineages. Immediate interest of her lab is in using stem cell derived cardiomyocytes and vascular cells as disease models, for drug discovery and future cardiac repair. In 2007, she spent sabbatical leave as a joint Harvard Stem Cell Institute/Radcliffe fellow. She presently serves on Ethical Councils of the Netherlands Academy of Science and Ministry of Health, providing specialized advice on human embryos and stem cell research. She is a member of several Scientific Advisory Boards and has written a popular book on stem cells. She is also editor/ editorial board member of Stem Cell Research, Cell Stem Cells, Stem Cells and Differentiation, elected member of the board of ISSCR and president elect of the International Society of Differentiation.

Talk: Human pluripotent stem cells in disease and drug discovery

Andras Nagy

Mount Sinai Hospital

Dr. Nagy is currently a Senior Scientist at the Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Professor in the Department of Molecular Genetics at the University of Toronto, and Investigator at the McEwen Centre for Regenerative Medicine. He also holds a Tier I Canada Research Chair in Stem Cells and Regeneration. His research focuses on several areas of interest, which include 1) Functional studies of genes belonging to families with known roles in vessel formation; 2) Development of sophisticated genetic manipulation tools in the mouse model; 3) Applying genetics to cancer research; 4) Derivation, differentiation and genetic modification of both mouse and human Embryonic Stem cells; 5) Reprogramming of somatic cells to pluripotent stem cells. In 2005, Dr. Nagy's lab created the first two Canadian hES cell lines which have opened a new area of research leading toward ES cell based therapies for incurable diseases. Using a novel method, they generated induced pluripotent stem cells (iPS) from both mouse and human somatic cells using the piggyBac transposon system. The transposon system also allows for the removal of the reprogramming factors from the iPS cell in a seamless manner, which is a leap forward for the safe use of these stem cells in future stem cell-based therapies. These are the first non-viral transduction iPS cell lines which keeps them at a leadership position in this highly competitive and promising field of research.

Dr. Nagy¹s research is currently funded by the National Cancer Institute of Canada, Genome Canada, Stem Cell Network, NSERC, and the National Institutes of Health USA.

Talk: Transposon- reprogrammed induced Pluripotent Stem cells are powerful exploratory tools

Martin Pera

University of Southern California

I am Professor of Cell and Neurobiology and Director of the Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research at the University of Southern California. I am interested in the control of growth and differentiation of pluripotent human stem cells.

Talk: Extrinsic Regulation of Human Pluripotent Stem Cells

Marc Peschanski

I-STEM (INSERM/AFM), Genopole Evry France

I am currently head of the Institute for Stem cell Therapy and Exploration of Monogenic diseases (I-STEM, INSERM/UEVE unit 861, associated to the Association Française contre les Myopathies) in Evry (Paris district), dedicated to the exploration of therapeutic potentials of pluripotent stem cells in rare diseases of genetic origin, using embryonic stem cells as well as iPS. I-STEM has grown rapidly since its opening in 2005 and comprises nowadays close to 90 people in 10 research teams interested, in particular, in neurological diseases, myopathies, retinopathies and genodermatoses. I coordinate in parallel the "STEM-Pole", a network that federates 80 stem cell research teams in the Paris district.

Talk: Prospects for epidermic cell therapy using human pluripotent stem cells

Angel Raya
ICREA Research Professor at the Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain

Angel Raya directs the Control of Stem Cell Potency group at the IBEC, located within the Barcelona Science Park in Barcelona, Spain. Research in his laboratory uses a variety of experimental models (heart regeneration in zebrafish, reprogramming of somatic cells, differentiation of pluripotent stem cells), to address one basic question: How can a discrete and defined degree of developmental potency be imposed onto a somatic cell so that it regains the ability to regenerate a functional tissue or structure?

Talk: Using iPS cells to treat and model human disease

Thomas A. Reh
Biological Structure, University of Washington, Seattle, WA, USA

I have been studying the development and regeneration of the retina for over 25 years, and have focused on the mechanisms which control cell fate during development and the process of de-differentiation that underlies retinal regeneration. More recently, we have applied our understanding of retinal developmental biology to direct the differentiation of human embryonic stem cells to retinal progenitors and photoreceptors. The stem cell derived photoreceptors may be useful for cell replacement therapy for retinal degeneration.

Talk: Stem cell strategies for retinal repair: regeneration or replacement?

José Silva
Wellcome Trust Centre for Stem Cell Research at the University of Cambridge

José Silva has been researching the basic biology of nuclear reprogramming, originally by using cell fusion of pluripotent cells with somatic cells and more recently by using induced pluripotency. His current work is focused on the investigation of the molecular events and mechanisms underlying induced pluripotency.

Talk: Unblocking the path to induced pluripotency

Andrew JH Smith
Institute for Stem Cell Research (ISCR), School of Biological Sciences, and MRC Centre for Regenerative Medicine, University of Edinburgh, Scotland, UK

Andrew Smith is a Research Investigator located in the ISCR and is also a member of the MRC Centre for Regenerative Medicine. His major research interest is the application of innovative genetic engineering strategies in embryonic stem cells for functional genetics and disease modelling. Recently he and his colleagues developed a recombinase strategy, RMGR, for large scale syntenic replacement of the mouse ES cell genome with human sequence to create an accurate recapitulation of a human disease.

Talk: Versatile gene targeting strategies for genetic modification of human ES cells

Austin Smith
Director, Wellcome Trust Centre for Stem Cell Research, University of Cambridge

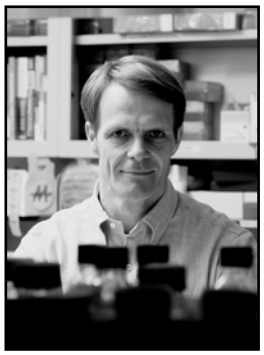
Professor Smith is Director of the Wellcome Trust Centre for Stem Cell Research. He coordinated the European Commission integrated project EuroStemCell (2004-2008) and currently coordinates the EuroSyStem (2008-2012). Professor Smith is a Fellow of the Royal Society of Edinburgh, an elected member of EMBO, and a Fellow of the Royal Society of London. He was awarded the Louis-Jeantet Prize for Medicine in 2010. His research interests are centred on the biology of embryonic stem (ES) cells and in particular the molecular basis of pluripotency. His approach is to identify and manipulate pivotal extrinsic and intrinsic regulators of the pluripotent state, comparing cultured ES cells with resident pluripotent cells in the mammalian embryo.

Talk: Design principles of pluripotency

John Stingl
Cancer Research UK Cambridge Research Institute

The main research focus of my laboratory is the identification and hierarchical characterization of the cells that make up the normal mammary epithelium. I am interested in characterizing the molecular mechanisms that regulate mammary cell differentiation, understanding the cellular context in which human breast tumours occur and determining the source of heterogeneity within and between human breast tumours. The laboratory is based at the Cancer Research UK Cambridge Research Institute on the Addenbrookes Hospital site, Cambridge, United Kingdom.

Talk: Mammary stem and progenitor cells: Understanding the cellular context of breast tumours



Lorenz Studer
Developmental Biology & Department of Neurosurgery, Director, SKI Center for Stem Cell Biology

My lab is interested in using human pluripotent stem cells for applications in regenerative medicine and for human disease modeling in the nervous system and beyond. We have pioneered many of the currently available protocols to direct pluripotent stem cells into specialized CNS and PNS cell fates. We have also developed powerful tool for lineage marking and high throughput chemical screening in human ESCs and iPSCs. My lab is located at the Sloan-Kettering Institute in New York City, and I am the director of the recently established SKI Center for Stem Cell Biology.

Talk: Tools for Modeling Neural Development and Disease in Human Pluripotent Stem Cells

Maarten van Lohuizen
Division of Molecular Genetics, The Netherlands Cancer Institute, Amsterdam

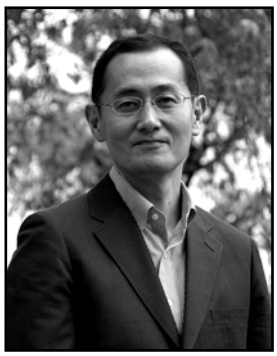
Our lab has a long-standing interest in epigenetic gene regulation dictated by chromatin modifications. We study the mechanism of stable inherited transcriptional repression by Polycomb-group (Pc-G) protein complexes, and the effects of deregulation of Pc-G genes on development, cell cycle control, cancer formation and stem cell maintenance. In addition, we are performing large-scale genetic screens in primary cells and in cancer-predisposed mice to identify cancer-relevant combinations of collaborating oncogenes and tumor suppressor genes.

Talk: Role of Polycomb repressors in stem cells, cancer and development

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

My laboratory is interested in the epigenetic regulation of embryonic and neural stem cell function as well as the transcription factor-mediated induction of specific cell types such as induced pluripotent stem (iPS) cells or induced neuronal (iN) cells. In particular, we are focussing on the potential medical applications of such cell fate conversions as well as the mechanistic regulation of these processes on the molecular level.

Talk: Inducing cell fate changes with transcription factors



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

Shinya Yamanaka has been serving as a Director of CiRA, Center for iPS Cell Research and Application at Kyoto University, Japan. The ultimate goal of his laboratory is to generate clinical grade pluripotent stem cells from somatic cells by direct reprogramming. They have shown that pluripotent stem cells can be induced from mouse fibroblast cultures by retrovirus-mediated introduction of four factors in 2006 and they designated these cells as iPS cells for induced pluripotent stem cells, and in 2007, they also succeeded in generating human iPS. They are trying to understand molecular mechanisms during induction of pluripotency, and to improve the method for overcoming safety issues toward future clinical application.

Talk: Induction of Pluripotency by Defined Factors

Ethics Workshop Faculty

Nissim Benvenisty
Stem Cell Unit, Department of Genetics, Institute of Life Sciences, The Hebrew University of Jerusalem

Prof. Nissim Benvenisty is the Herbert Cohn Chair in Cancer Research and the director of the Stem Cell Unit at the Hebrew University. Prof. Benvenisty earned his M.D. and Ph.D. degrees from the Hebrew University. Following a postdoctoral fellowship at Harvard University, he joined the Department of Genetics at the Hebrew University. Prof. Benvenisty was awarded several prizes among them the Foulkes Prize (London), the Hestrin Prize, and the Teva Prize.

Talk: Differential Modeling of Fragile X Syndrome by Human ES and iPS Cells

Christof Friedrich
European Patent Office, Munich

Christof Friedrich is Patent Examiner in the field of Biotechnology at the EPO in Munich. He serves as an expert for applications concerning animal cells in the department of biotechnology.

Talk: Consequences of the G2/06 decision of the Enlarged Board of Appeal on Patent Applications concerning human ES cells

Göran Hermerén

Professor Emeritus, Department of Medical Ethics, BMC Biomedical Centre, Lund University, Sweden

Göran Hermerén has been (and is) involved in ethics workpackages of a number of EU-funded projects on various aspects of stem cell research over the years, such as EuroStem, EuroStemCell, ESTOOLS and NeuroStemCell. He has published widely on ethical aspects of research and health care, also on priority-setting, scientific fraud and ranking orders of values. Finally, he is involved in national and international ethics committees, being the president of the European Group of Ethics, the chair of the advisory board of the German reference center for ethics in the life sciences, the chair of the ethics committee of the Swedish research council and member of the national council on medical ethics in Sweden since its start.

CHAIR

Margarida Menezes-Ferreira

National Authority of Medicines and Health Products, Portugal

Margarida Menezes Ferreira graduated in Biology at the Faculty of Sciences of the University of Lisbon. She had her PhD degree in Medical Biochemistry at the Aix-Marseille II University in France 1981 and did post-graduate research in molecular endocrinology at the National Institutes of Health USA until 1984. She published several research papers in peer-reviewed journals amongst which two of them were selected for the Year Book of Endocrinology.

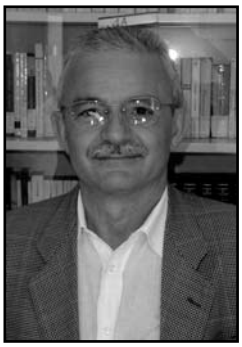
Since 1996 she is part of INFARMED, the Portuguese regulatory authority for medicines and health products having developed the strategic plan for the installation of the Biologics, Biotechnology and Microbiology Departments of the National Laboratory for the Control of Medicines and Health Products and coordinated its implementation.

Since 1999 to the present she has been the INFARMED appointed member of the Biologics Working Party and, since 2007, of the Cell based Products Working Party at the European Medicines Agency (EMA), participating in the drafting of European guidance and scientific advice on biological medicinal products including cell based therapies. Presently and since its start in 2009 she is the alternate member representing Portugal in the Committee for Advanced Therapies.

She also collaborates with the Faculty of Pharmacy from the University of Lisbon in several post-graduate courses and with the “Instituto Superior Técnico” at the Technical University of Lisbon in the PhD program MIT-Portugal lecturing on Regulatory aspects related to Biotechnology and Cell based Therapies.

Since 1998 she has been a member of the National Vaccination Commission; since 2004 she has been Member of the Board of the Biotechnology College at the Biologists Association (Colégio de Biotecnologia da Ordem dos Biólogos).

Talk: Regulating stem cells



Demetrio Neri
University of Messina, Italy

Demetrio Neri is full professor of Bioethics at the University of Messina, member of the Italian National Bioethics Committee. Main work area: bioethical issues concerning the beginning and the end of human life.

CHAIR

Aliki Nichogiannopoulou
European Patent Office, Munich

Aliki Nichogiannopoulou is Director of Biotechnology at the EPO in Munich. Her directorate is in charge of patent applications concerning immunology and antibodies. She is involved in the process of harmonizing the examination of patent applications concerning human embryo-derived cells at the EPO.

Talk: Patentability of embryo-derived versus induced pluripotent stem cells

Clara Sattler de Sousa e Brito
Visiting Fellow, Information Society Project, Yale Law School

Clara Sattler de Sousa e Brito is a visiting fellow at Yale Law School and a patent attorney with the law firm Liermann-Castell. At the center of her research are problems in the intersecting areas of law, ethics and modern technologies. In particular the interaction between innovation, social norms and IP in the life sciences (e.g. synthetic biology or stem cells) is of specific importance to her.

Talk: The ECJ-case and strategies for stem cell patenting

Giuseppe Testa
European Institute of Oncology, Milan, Italy

Giuseppe Testa heads the laboratory of Stem Cell Epigenetics at the European Institute of Oncology in Milan. His lab studies the epigenetic regulation of cell fate determination; key contributions include the development of novel genome engineering tools and the characterization of the role of histone demethylation in neurogenesis. In parallel, he trained in Bioethics and Biolaw and co-founded in Milan the first interdisciplinary PhD program on 'Foundations of the Life Sciences and Their Ethical Consequences' (Folsatec).

Talk: From ES to iPS cells: the Reprogramming of Scientific and Societal Choices

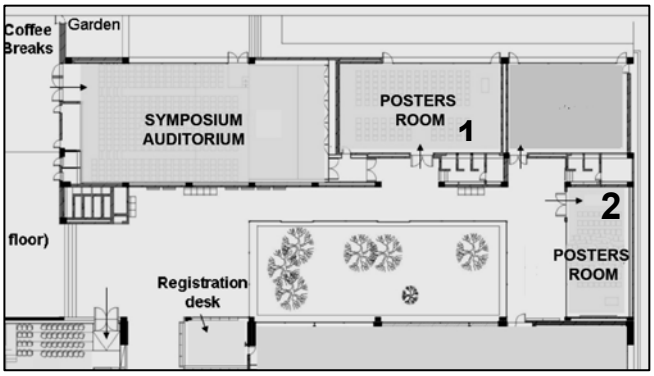
Poster Session

Wednesday 26 May, 18:30 – 20:30

Poster put-up

Please ensure you put up your poster before 13:15 on Wednesday 26 May.

Your will find your poster number (Poster ID) in the abstracts index page 17.



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Poster ID - 1

Formation and Maintenance of Stem Cells in the Breast by Paracrine and Autocrine Signals

Christina Scheel[1*], Christine L. Chaffer[1], Elinor NgEaton[1], Sophia Hsin-Jung Li[1], Ferenc Reinhardt[1], Kong Jie Kah[1], Wenjun Guo[1], Andrea Richardson[2], Robert A. Weinberg[1]

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Passage through an Epithelial-Mesenchymal Transition (EMT) has been associated with the acquisition of stem-cell properties in both immortalized and neoplastic human mammary epithelial cells (HMECs). However, the signaling mechanisms that induce and maintain the resultant mesenchymal/stem-cell (SC)-state and its position within the normal hierarchy of the mammary gland have been unclear.
We describe a constellation of 3 autocrine signaling loops that function in concert to maintain HMECs in the mesenchymal/SC state. They involve the actions of Transforming Growth Factor (TGF)-beta, as well as canonical and non-canonical Wnt ligands, which act in concert and impinge on the expression of EMT-transcription factors, notably ZEB1. We find that activation of these signaling pathways in epithelial HMECs is blocked by a series of secreted inhibitors, notably Wnt antagonists and Bone Morphogenetic Protein (BMP) ligands. Inactivation of these inhibitors causes immortalized and transformed HMEC to become responsive to the EMT-inducing signals of paracrine TGF-beta together with canonical Wnt ligands and Wnt5a.
Establishment of autocrine signaling involving the same factors that previously triggered entrance into the EMT stabilizes the resultant mesenchymal/SC-state. Interruption of these autocrine loops by exposure of mesenchymal/SC to secreted Wnt antagonists, BMP ligands or TGF-beta inhibitors acutely inhibits migration and self-renewal abilities. Continued inhibition promotes differentiation via a Mesenchymal-Epithelial Transition (MET)-like process.
When applied to short-term cultures of primary HMECs, induction of EMT correlates with an expansion of basal cell populations enriched in self-renewal abilities. In contrast, inhibition of autocrine TGF-beta signaling promotes differentiation along the luminal lineage, as assessed by expression of cell-surface markers and self-renewal assays.

Poster ID – 2

Cancer Stem Cells and Drug Resistance: The BCRP Expression in Human Hepatocellular Carcinoma

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Background
The cancer stem cells (CSCs) are related with drug resistance to chemotherapies against cancer, including liver cancer. Both normal stem cells and CSCs have the capacity to export the toxic compounds out of the cells, accounting for drug resistance. The Breast Cancer Resistance Protein (BCRP), one of the ABC transporters members, is proposed to be a determinant molecule for this activity. Recent evidences indicate the relationship between the BCRP expression, stem cells and cells differentiation, including in the hepatic oval and liver cancer cells.

Objective
To investigate the BCRP gene and functional expression in correlation with drug resistance and cells differentiation in human liver cancer.

Methods
For the *in vivo* study, a total of 75 tissues samples of hepatocellular carcinoma, hepatoblastoma, biliary atresia, and normal liver were collected, covering tumoral lesion and distal region in individual patients. For the *in vitro* study and drug toxicity assay, cell lines HepG2 and Huh7 as differentiated HCC and JHH6 as undifferentiated HCC were employed as models. The BCRP mRNA expression was studied by quantitative real time PCR normalized to references genes 18S and B-actin. Doxorubicin (DOX) was used as a toxic compound for drug toxicity assay. The BCRP functional assay was measured by Hoechst 33342 efflux using spectrofluorimeter.

Results
In vivo data showed that BCRP mRNA was present in all tissues samples with different degree of positivity. A higher BCRP expression was observed in the tumoral compared to normal in individual patients, indicating a BCRP up-regulation in less differentiated tissues. Of notice was the correlation between BCRP expression and the type of liver malignancies. From *in vitro* models, the JHH6 expression was two folds increased than in HepG2 or Huh7, and was correlated with cell differentiation. After exposure to DOX for 24 hours, all cell lines showed BCRP up-regulations. The Hoechst 33342 export was highest in the JHH6 and decreased in HepG2 and Huh7 indicating that the cells capacity in exporting DOX was inversely related to the degree of differentiation.

Conclusion
In human liver BCRP gene was overexpressed in tumoral tissues compared to normal tissues and the extent of expression was related to the degree of differentiation both *in vivo* and *in vitro*. Functional tests indicated that higher BCRP-related drug resistance in less differentiated cells which was further increased after exposure to DOX.

Poster ID – 3

Sox2 in Breast Cancer Stem Cells.

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The recent observation that the tumor suppressor p53 pathway activation reduces reprogramming efficiency has lead to the speculation that cancer stem cells may arise through an altered reprogramming-like mechanism.
Many solid tumor types, including breast cancer, exhibit a functional hierarchy of cells of which only a small subpopulation called stem-like cells can give rise to the differentiated cells that comprise the bulk tumor. These cells phenotypically resemble the adult stem cells of the organs they are derived from. This has led to the hypothesis that cancer is a disease of aberrantly transformed stem cells.

The presence of cancer stem-like cells has been suggested previously in the carcinoma cell line MCF7 and others as cells capable of making serial clonogenic tumor spheres in non-adherent culture conditions.
We first analyzed regulation of different key pluripotency regulatory genes upon sphere formation and observed that Sox2 became upregulated. Next we tested whether Sox2 overexpression stimulated sphere formation, effect that was contingent with continuous expression of the transgene. This data suggests that Sox2 plays a significant role in breast cancer stem-like cell generation.
We then studied how Sox2 expression is controlled at promoter activation level. We observed that non-adherent sphere formation increases reporter activity by 16 fold in the upstream R1 enhancer and not in the core promoter. This enhancer has been demonstrated to be active in hESC and in oligodendrocyte precursors.

Since Sox2 is one of the necessary factors for maintaining the undifferentiated state of stem cells, particularly embryonic and neural stem cells, its uncontrolled expression in adult cells may induce an altered reprogramming-like mechanism that when encountering a permissive environment (as inactivation of the p53 pathway) may give rise to cancer initiating cells.

Poster ID – 4

Sphere formation ability in breast tumors shows heterogeneity in histologic tumor subtypes. Novel functional model in zebrafish.

A. Eguiara[1], C. Quevedo[2], L. Martinez Indart[3], M. Diaz-Nunez[1], K. Elorriaga[4], C. Callol[2], R. Rezola[4], A. G. Martin[1*]

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Breast cancer stem cells have been defined according to several parameters such as surface marker expression, ability to efflux dyes, growth in non-adherent conditions. Sporadic breast cancer has been clasified by gene expression analysis in several molecular subtypes with distinct clinical outcomes (Basal, Her2, Luminal A and B). There is no clear evidence, however, on the cell of origin of the different phenotypes. By using the unique ability of stem cells to grow as floating spheres in culture, we have assessed the presence of stem cells in primary tumor specimens and correlate with clinical, phenotypic and histological features of the tumor.

We analyzed 142 fresh breast carcinoma fragments collected at Instituto Oncologico between 2007-2010, for which clinical-pathological data was obtained through routine diagnostic procedures. Although aprox. 40% of the tumors were capable of forming spheres in vitro, irrespective of the total number of cells isolated from the original tumor, we didn't observe association between the ability to form spheres and clinical-pathological features of the tumor. We did not find association with the surface marker profile at the level of the CD44+CD24- population or linage markers (Muc1, CD10 or ESA) either.

Functional analysis of cancer stem cells to date involves difficult, expensive and time consuming technology. We have established a simple, fast, sensitive and cost effective functional model based on xenografts in the theleost zebrafish. We have analyzed tumor mass formation and cell migration of xenografted cells and have observed that cells (continuos breast carcinoma cell lines) grown in non-adherent conditions (spheres) showed significantly increased mass formation and migration to the tail of the fish compared to the unselected adherent growing counterparts. Therefore our results show that zebrafish can be a useful functional animal model for rapid analysis of cancer cell populations.

Poster ID – 5

DNMT3B plays a role in RA-induced neuronal differentiation of human embryonal carcinoma cells

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Dynamic change of global DNA methylation has been identified to be crucial for early embryogenesis especially before preimplantation stage, during which embryonic stem cells are derived. However, the role of de novo DNA methylation during ES cell self-renewal and differentiation mostly remains unknown. In this study, we used a doxycycline-inducible shRNA system to investigate the function of DNMT3B, one of the de novo methyltransferases, in undifferentiated and retinoic acid (RA)-induced differentiating human embryonal carcinoma cells NTERA2/D1.
We have found that knockdown of DNMT3B significantly affects the expression of stage-specific embryonic antigens including increasing of SSEA1 and decreasing of SSEA3 and TRA-1-60. In addition, cell cloning efficiency is remarkably reduced upon DNMT3B knockdown. However, expression of the two key pluripotency specific transcription factors OCT4 and NANOG have not been changed by DNMT3B knockdown. These evidences indicate that DNMT3B might contribute to the stability of stem cell state.
NTERA2/D1 cells are capable of differentiating into a post-mitotic phenotype with features of mature neurons upon exposure to 10μM RA. In order to investigate the role of DNMT3B during differentiation, we induced the cells with 10μM RA for three weeks after DNMT3B was knocked down. Interestingly, knockdown of DNMT3B dramatically enhances the yield of TUJ1 positive neurons. By knocking down DNMT3B at different time points during RA induced differentiation, we have also identified that neuronal differentiation has only been enhanced when the knockdown was carried out at the early stage of differentiation, indicating that DNMT3B might involved in the initiation of neuronal lineage commitment.

This work has been supported by funding for the ESTOOLS consortium under the Sixth Research Framework Programme of the European Union contract LSHG-CT-2006-018739

Poster ID – 6

Heterogeneity and distribution of tumour stem-like cells in human glioblastoma

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In the last years the idea that tumours arise from a sub-population of cells endowed with "stem cell" features completely revolutionized the field of Oncology. This hypothesis found its initial confirmation by studies on non-solid tumours and then has been extended also to solid tumours. It is a common believe that the so called "cancer stem cells" concept could also provide new insight into the cellular and molecular mechanism of tumour growth and in the future to the development of new therapeutic strategies for the treatment of incurable cancers.
The most lethal tumour of the Central Nervous System is Glioblastoma Multiforme (GBM) which accounts for over 60% of brain tumours. Median life expectancy in optimally managed patients is only 12-14 months with only 25% surviving 24 months. The current clinical management of patients diagnosed with a GBM involves a combination of surgery, radiotherapy and chemotherapy. Radiotherapy has been the principle therapeutic modality since the late seventies and additional targeted chemotherapy is of only modest benefit. Thus the need for new treatments is an unmet clinical need.
It is hypothesized that "cancer stem cells" are a significant factor in gliomagenesis and in the emergence of treatment resistant clones in GBM. However, it is not known how these cells contribute to the heterogeneity of this process.
Here we report the isolation and genetic characterization of distinct "cancer stem cell" populations from different areas of the same GBM specimens. These populations retain stem cell features, such as clonogenicity and multipotency. However, only the cells derived from the core mass appear to possess long-term self-renewal and tumour-initiating capacity. Most importantly, these populations are genetically different, displaying patterns of karyotypic alterations that overlap only partially.
In conclusion, our findings show that distinct regions of the same GBM specimens contain "cancer stem cells" which are endowed with different tumorigenicity and independent genomic evolution. This approach will enable us to describe the molecular heterogeneity of GBM for every individual tumour and in the future to develop individualised therapeutic regimens.

Poster ID – 8

Stochastic elimination of cancer stem cells in patients with chronic myeloid leukemia

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Tyrosine kinase inhibitors (TKI) such as imatinib have revolutionized the therapy of chronic myeloid leukemia (CML). However, since they do not affect the leukemic stem cells, TKI are not considered curative for this disease regardless of the significant reduction of disease burden during treatment. This view however does not take into consideration the stochastic nature of hematopoiesis. We have combined the known biological features of CML with a stochastic model of hematopoiesis and serial quantitative data from patients treated with imatinib to study the evolutionary dynamics of leukemic stem cells in this disease. Our results show that in contrast to widely held belief, TKI therapy can cure this disease even in the absence of a specific impact on the leukemic stem cells. We find that in the overwhelming majority of patients the LSC population undergoes stochastic extinction even before disease diagnosis. Hence it is the leukemic progenitors, susceptible to TKI attack, that are the natural target for CML treatment. We predict that early diagnosis together with administration of TKI opens the path to CML eradication, leading to the wash out of the aberrant progenitor cells, ameliorating the patient's condition and lowering the risk of blast transformation and drug resistance. These results provide a paradigm shift in the study of acquired hematopoietic stem cell disorders and have direct impact on other HSC derived diseases.

Poster ID – 9

Generation of functional human melanocytes from pluripotent stem cells and reconstruction of 3D melanized epidermis.

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Melanocytes are highly differentiated cells deriving from the neural crest which are responsible for skin pigmentation. This mechanism is a key adaptive process playing a crucial role against the noxious effects of UV radiations from the solar light that cause DNA damages and skin cancers. A well-characterized differentiation program allows melanocytes to produce melanin within melanosomes that are transported to the dendrite tips of melanocytes before being transferred to keratinocytes, where they will protect the DNA.Impairment of melanocyte's functions and/or development results in various types of congenital pigmentary disorders.

Although melanocyte's functions are well characterized, little is known on the molecular mechanisms involved in melanocyte development and differentiation from human embryonic precursors. Human embryonic stem cells (hESCs) are pluripotent cells derived from the blastocyst inner cell mass and have become an attractive tool for human developmental modeling. Recently, human induced pluripotent stem cells (hiPSC) have been derived from somatic cells and present optimistic opportunities for patient specific regenerative medicine.

Based on their intrinsic capacities to be pluripotent and to self renew, hESC and hiPS can virtually be expanded unlimitedly and can generate any cell types of the human body. Several cell types have already been generated from pluripotent stem cells such as neurons, cardiomyocytes, insulin-producing cells or keratinocytes.

Here, we describe for the first time a new directed 2D protocol of differentiation of pluripotent stem cells (hESC or hiPS) into a pure and functional population of melanocytes. We show the expression of all major markers of melanocytes as well as their capacity to synthesize melanin and transfer melanosomes into keratinocytes. Moreover, we were able to generate 3D reconstructed melanized epidermis. These data bring substancial evidences for setting up pigmentation disease modeling using patient derived iPS and will help to improve current treatments for those pathologies.

Poster ID – 10

Genetic Changes in Cultured Human Embryonic Stem Cells as a Model for Germ Cell Tumor Development.

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Teratocarcinomas contain malignant stem cells (EC cells), providing a paradigm for the stem cell concept of cancer. Human embryonic stem (hES) cells, resembling EC cells, undergo culture adaptation on prolonged passage, marked by amplification of chromosomes typically amplified in EC cells, suggesting common underlying causes for genetic instability. Thus, elucidation of mechanisms of genetic instability in cultured hES cells can provide a model for exploring mechanisms of progression of stem cell based tumours. To investigate potential mechanisms underlying the development of chromosomal instability in hES cells, we determined the integrity of responses triggered by replication fork stress and DNA damage. Our data suggest that some of these responses are altered in hES cells relative to responses obtained with somatic cells, and that an alternative mechanism appears to be involved for the maintenance of genetic stability during replication stress in hES cells.

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Poster ID – 11

Expression profiles defined by eight novel monoclonal antibodies on stem cell and cancer cell lines

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One of the many challenges facing research into human embryonic stem (ES) cells and the differentiation of these cells into the three germ layers, is the availability of markers that identify the stem cell compartment and when cells depart this compartment to initiate differentiation. Here we describe the characteristics of eight novel antibodies to human ES cells.

The staining profiles of these antibodies: AA11, AG10, BE12, BF4, CC9, CH8, DA9 and EF12 were analysed on differentiated and undifferentiated human ES and embryocarcinoma (EC) cells, as well as various and cancer cell lines, by flow cytometry, immunohistochemistry and SDS-PAGE western blot.

The eight antibodies are immuno-reactive with human ES cell lines and human EC cell lines. Following differentiation by all-trans-retinoic acid in human EC and ES cells staining decreased significantly by 7 days and 14 days. These data are consistent with these antibodies being immuno-reactive with stem cells. All these antibodies appear to detect epitopes distinct from the existing widely used markers of human ES cells, and may distinguish distinct substates in the stem cell compartment. For example, the marker BE12 is unique in also staining the yolk-sac carcinoma 1411H and the choriocarcinoma cell lines Bewo, Jar and Jeg3 as well as stem cells. We speculate that BE12 can identify stem cells undergoing early differentiation into definitive endoderm and trophoblast lineage. Another antibody, BF4, appears to distinguish stem cells according to expression of DLK1, a member of the Delta/Jagged family of Notch ligands that may play a role in controlling self renewal of human ES cells.

Poster ID – 12

Forced expression of Lmx1b enhances differentiation of mouse ES cells into serotonergic neurons

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The LIM homeodomain transcription factor Lmx1b is involved in the early specification of midbrain development and in differentiation, as well as in maintenance of dopaminergic and serotonergic neurons.
Here, we explored the capacity of Lmx1b to direct differentiation of mouse Embryonic Stem Cells (mESC) into both dopaminergic and serotonergic neurons.
In the first approach, ectopic expression of Lmx1b was achieved by means of lentiviral vector infection. For efficient expression during all steps of differentiation, human Lmx1b cDNA was driven by the ubiquitous CAG promoter. During neural differentiation, the level of Lmx1b expression was found to strongly influence the capacity of mES cells to accomplish neuronal differentiation. High Lmx1b expressors showed enhanced cell death and poor neuronal differentiation. By contrast, low expressors exhibited higher neuronal density, longer neuronal outgrowths, and significantly increased levels of pan-neuronal marker expression. Analysis of lineage-specific differentiation markers showed an increase in serotonergic markers (serotonin transporter: 8-fold, Tph2: 9-fold, Pet1: 2-fold) after normalization to Map2 expression. Production of serotonin as measured by ELISA was augmented up to 20-fold.
In the second approach, Lmx1b was overexpressed in mESC-derived neural precursors by means of a conditional expression of the vector targeted into ROSA26 locus. Transcription of Lmx1b cDNA in undifferentiated mESC is blocked by floxed transcription termination signals. It is irreversibly activated by tamoxifen-induced Cre activity during formation and expansion of neuroepithelial colonies. After induction, Lmx1b is stably expressed throughout neuronal differentiation. Activation of Lmx1b expression in neuroepithelial colonies resulted in augmented serotonergic differentiation as evidenced by an elevated expression of serotonergic markers (SERT: 2.5-fold, Tph2: 1.6-fold, Pet1: 1.4-fold). Treatment of neural precursors with Shh and FGF4, two factors inducing dorsoventralization in the developing neuroepithelium, further enhanced differentiation of Lmx1b-overexpressing neural precursors into serotonergic neurons (SERT: 57-fold, Tph2: 31-fold, Pet1: 15-fold).
Together, our results highlight the capacity of Lmx1b to coax mESC-derived neural precursor differentiation towards a serotonergic fate.

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Poster ID – 13

From ES cells to Neurons: The role of Notch in Neural Progenitors

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Research into mammalian neural development as well as neurodegenerative diseases represent a field of study that could benefit from the use of embryonic stem (ES) cells. However, a better understanding of the basic mechanisms governing stem cell differentiation is required before effective new therapies emerge. In order to contribute to this, we have used an efficient and reproducible in vitro ES cell-derived neuroepithelial rosette culture system, which faithfully reproduces embryonic neural tube development, to start dissecting the molecular mechanisms of mammalian neural development.
The full transcriptome of cells at different differentiation points (day 0, day 1, day 3 and day 8) was determined by microarray analysis (Mouse Genome 430 Version 2 Array, Affymetrix). Using the transcriptional profiling data, we could identify five synexpression groups of genes whose activity reflect the successive cellular states that emerge along the path to neural differentiation starting from ES cells [1]. The analysis of these groups allowed us to additionally uncover the molecular pathways associated with the transitions between the different stages of neural progenitor (NPs) development. One of these pathways is the Notch signalling pathway. In order to address Notch function in regulating NP transitions, these cells were treated with a highly specific gamma-secretase inhibitor (LY411575). This resulted in massive neuronal differentiation, showing that Notch activity is necessary to prevent uncontrolled commitment of NPs to differentiation. To provide a global view of the molecular alterations underlying Notch activity during NP development, full transcriptome analysis was additionally performed on NPs treated with LY411575 and compared with untreated NPs. This resulted in the characterization of a Notch synexpression group, comprising a network of genes that are involved in implementing Notch activity during neural development. Further studies to address the role of the identified genes in the Notch pathway and their function during neural development will enhance our basic understanding of neural development, which is a critical issue for the development of efficient cell-based therapeutic strategies for neurodegenerative diseases.

[1] Abranches et al, Neural Differentiation of Embryonic Stem Cells: A Road Map to Neurogenesis in the Embryo, PLoS ONE 4(7): e6286 (2009)
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Poster ID – 14

Genetic evolution during factor induced reprogramming

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Somatic cell reprogramming to induced pluripotent stem cells (iPS) is achieved by forced expression of a defined set of transcription factors. The low induction efficiency associated with reprogramming has been attributed to p53 induced cell cycle arrest, apoptosis and senescence. However, it remains unknown if the reprogramming process itself induces DNA damage that can compromise genomic integrity and the efficiency of establishing iPS lines. Using a high resolution SNP array, we compared single nucleotide and copy number alterations of early and intermediate passage human iPS cells (hiPSc) to their respective parental fibroblast cell origins. We show that a high level of mutations is detected during early compared to intermediate passage numbers, with enrichment in regions of genomic fragility, suggesting that replication dependent DNA damage occurs during reprogramming. We also identified a subset of mutations that place the affected early passage hiPSc at a selective disadvantage. These results indicate that the reprogramming process is associated with high mutation levels. Remarkably, during culture, hiPSc undergo normalization through a selection process which brings the genetic stability of hiPSc equivalent to that of the human Embryonic Stem cells.

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Poster ID – 15

DNA demethylating agents and conditioned medium from neuronal cell lines enhance the expression of neuronal antigens in cord blood mononuclear cells.

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Cord blood is nowadays recognised as a readily available and often banked source of stem cells. The plasticity of adult stem cells (of which cord blood forms part) is being ever more clearly recognised, resulting in a growing interest in the possibility of trans-differentiation opening up new therapeutic possibilities.
Cord blood was obtained from term placental deliveries, after having obtained informed consent. The mononuclear cell portion was isolated using density centrifugation techniques and was cryopreserved until further use. Upon rethawing and pooling, cells were divided into aliquots and exposed for a week to combinations of chemical modifiers including retinoic acid, DNA demethylating agents, histone deacetylase inhibitors, insect cell extracts and/or medium conditioned by neuronal cells. p200 neuronal filament expression was then detected by fluorescent antibody staining and flow cytometry.
DNA demethylating agent 5 aza cytidine and neuronal cell conditioned medium enhanced neuronal filament expression whilst histone deacetylase inhibitors agents and insect cell extracts reduced the expression of these neuronal antigens.

Poster ID – 16

Differentiating human ES cells into chondrocyte-like cells: the role of AP-1 (Fos/Jun)

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Cartilage is essential for skeletal development during embryogenesis and juvenile growth. Later in life, loss of cartilage can lead to debilitating diseases, e.g. osteoarthritis. Finally, life-threatening chondrosarcomas can develop from chondrocytes.
While the molecular mechanisms controlling chondrocyte development have been intensively studied in mice, comparatively little is known for humans. We are interested in the control of human chondrocyte proliferation and differentiation, especially the function of the transcription factor AP-1. It was previously shown in mouse models that AP-1 plays an important role in chondrocyte/bone cell biology. To investigate this in human cells, we have developed a protocol to differentiate human ES cells (hESCs) into chondrocyte-like cells under serum-free conditions. Analyses of cells obtained at several intermediate time points using stains for extracellular matrix proteins of chondrocyte/bone cells, revealed an increasing differentiation efficiency over time with a maximum of up to 60-70% of the culture area. Moreover, bone nodule formation was observed. The characterization of these chondrocyte-like cells and intermediate developmental stages by RT-PCR shows a strong increase of mesodermal gene expression, such as Flk1 and Brachyury, at early time points and an increase of chondrocyte gene expression, such as Aggrecan, type II collagen and Sox9 at later differentiation stages. We are in the process of characterizing the cells by western blot and FACS analyses as well as kidney capsule transplant experiments. The latter should give us insights into their in vivo behavior, especially their differentiation potential. Since AP-1 proteins are expressed in chondrocyte/bone cells, we will employ gene knock-down and over-expression of Fos, Fra2 and Jun to obtain important information on the role of AP-1 in human bone cells. As cartilage has a very limited repair capability in vivo we will use our differentiation protocol to also differentiate human iPS cells into chondrocyte-like cells and analyze their properties with a view to establishing a protocol to derive chondrocytes for cell replacement therapies.

Poster ID – 17

Nanomaterials to drive the differentiation of stem cells into neuronal cells

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Several bioactive factors have the ability to differentiate stem cells towards desired lineage-specific precursors. However, the administration of some of these bioactive factors in vitro or in vivo presents a significant challenge because of their poor water solubility, short half-life, and potentially undesired side effects (1, 2). One example is retinoic acid (RA) that is able to induce in vitro differentiation of stem cells, particularly, into the neuronal lineage (3). Nanoparticles (NPs) can be an excellent platform to ensure protection and intracellular transport of RA. Herein we present a novel strategy to differentiate subventricular zone (SVZ) stem/progenitor cells from neonatal mice into neuronal cells using NPs able to release intracellularly RA. NPs prepared by complex coacervation of dextran sulfate with polyethylenimine had an average diameter between 80 and 90 nm, a RA payload of 12.3±3.4 microgram per mg of nanoparticles, and a positive net charge (+15 mV). Confocal microscopy studies indicate that SVZ cells were able to internalize the nanomaterials. Incubation of SVZ cells with RA-containing nanoparticles at concentrations ranging between 0.1 and 1 microgram/mL (corresponding to a RA payload of 1.2-12 ng/mL) induced an increase of NeuN+ cells (neurons) as well as the percentage of functional neuronal cells showing increases of intracellular calcium variations following KCl but not histamine stimuli. Furthermore, these RA-containing nanoparticles do not exert a cytotoxic effect as evaluated by TUNEL, propidium iodide uptake and active caspase 3 immunodetection. Of note, nanoparticle-conditioned medium is unable to promote the differentiation of stem cells indicating that neurodifferentiation is only mediated by internalization of the nanoparticles Taken together, we developed a novel strategy to efficiently drive the differentiation of stem cells into neuronal cells with minimal impact on cell viability.

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Poster ID – 18

Reconstruction of a pluristratified epidermis using human induced pluripotent stem cells.

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Human epidermis has been produced in vitro for decades using adult epidermal stem cells from donors to provide cell therapy and industrial use in pharmaceutical and cosmetic applications. We previously demonstrate that a fully pluristratified epidermis can be obtained in vitro and in vivo from human embryonic stem cells (Guenou et al). Now, we have demonstrated that induced pluripotent stem cells (iPSC) should represent a new unlimited source of keratinocytes. As a proof of concept, we demonstrate in the present study that both lentiviral or retroviral generated iPSC lines can be used to derive a homogenous and pure population of human keratinocytes. Then we show their capability to form a pluristratified epidermis exhibiting normal human characteristics in vitro. Use of wild type iPSC and iPSC lines carrying mutation for genodermatoses as epidermolysis bullosa represents a promising way of investigation of new treatments to improve patients' lives.

Poster ID – 19

Steering human induced pluripotent state cells towards pancreatic lineages.

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Human induced pluripotent state (hiPS) cells offer the possibility of generating large numbers of patient-derived pancreatic cells suitable for cell replacement therapy. Differentiating hiPS cells in vitro also provides a model system in which the molecular mechanisms that govern cell fate may be studied.

A protocol for the efficient differentiation of human embryonic stem cells to pancreatic cells has been reported. We used this protocol in an attempt to efficiently generate pancreatic cells from hiPS cell lines generated within our laboratory. We found the ability of hiPS cell lines to give rise to pancreatic cells using this protocol to be highly variable and inefficient.

We have developed fully-defined serum-free and feeder-free protocols to efficiently differentiate mouse embryonic stem (mES) cells to definitive endoderm and pancreatic progenitor cells. We are using our increased understanding of pancreatic differentiation from our mouse ES cell work to develop a protocol for efficient and robust production of pancreatic cells from hiPS cells.

Here we present data demonstrating that the addition of a small molecule inhibitor molecule used in our mES cell differentiation protocol significantly improved the efficiency of endodermal cell production in all of the hiPS cell lines tested. In addition, culturing the hiPS cell lines in conditions similar to those used for mES cell culture for 48 hours prior to differentiation significantly improved cell survival and increased endodermal cell production. We have incorporated these improvements into our novel step-wise hiPS cell differentiation protocol and have significantly increased the production of Pdx1+ pancreatic progenitor and Ngn3+ endocrine progenitor cell populations.

We are also developing tools for the identification and isolation of pancreatic progenitors from differentiating hiPS cell lines.

Poster ID – 20

The role of *Ccbe1* in cardiac differentiation of mouse embryonic stem cells

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Embryonic stem cells (ESCs) represent a unique experimental model to investigate the basic principles of mammalian cell differentiation and development. Indeed, the expression patterns of several key cardiac genes during ESC differentiation have been shown to closely reflect their endogenous expression during cardiogenesis *in vivo*.

Collagen and calcium-binding EGF-like domain 1 (*Ccbe1)* was firstly identified by comparing the transcriptome of chick heart precursor cells to non-cardiogenic control cells through a differential screening using the Affymetrix GeneChip system. *Ccbe1* has since been described to be involved in angiogenic sprouting and lymphangiogenic budding in zebrafish and associated with lymphatic dysplasia and hypertrophic cardiomyopathy in humans. Related with this, EGF family molecules have also been shown to be important for cardiac development as gene targeting approaches revealed that EGF signalling is required for proper cardiac development and function and also for proliferation and differentiation in mammals. In mouse, *Ccbe1* is expressed at very early stages in cardiac mesoderm precursor cells. Whole mount *in situ* hybridization and histology techniques have shown that *Ccbe1* is expressed specifically on the cardiac progenitors regions at E7.5 (intra-embryonic mesoderm) and E7.75 (cardiac plate) and in the myocardial tissue forming the primitive heart tube at E8.5.

Next, we intend to use mouse ESCs to assess the role of *Ccbe1* on cardiac differentiation. For this end, overexpression and knockdown of *Ccbe1* gene will be carried out in mouse ESCs and the expression of stage-specific markers of cardiac development assessed at the protein and transcript levels. Concurrently, using the ESC technology, we are generating a null allele of this gene in the mouse in order to determine its role in cardiogenesis.

Poster ID – 21

Towards a molecular blueprint for cardiac stem/progenitor cells response to heart injury

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The concept of the heart as a terminally differentiated organ was recently challenged with the identification of myocardium-resident stem cells. Putative cardiac stem cells (CSC) display the hallmarks of stemness and differentiate clonally into cardiomyocytes, endothelial and smooth-muscle cells. However, general lack of definitive molecular markers to identify CSCs raises the question as to whether they have their origin confined to the heart or are instead a result of continuous replenishment from other organs. Remarkably, the antigenic profile of cardiac cells displaying stem/progenitor activity coincides with that of bone-marrow stem cells and thus no reliable features exist for discriminating blood-borne from cardiac-resident stem cells. Other major constrain to a full understanding of the adult CSC compartment relates to the scarcity of these cells within the myocardium. Not surprisingly, CSC associated proteins activated upon heart-injury have only been depicted from crude tissue extracts. Our approaches were designed to bring forward data to: (i) prospectively identify CSC, (ii) disclose CSC injury-response targets. We report ongoing procedures to enable characterization of the CSC proteome under normalcy vs. pathological conditions. Briefly, an improvement on cell recovery of the target subset, i.e. Sca-1+ cells, isolated using a recently released Tissue-Dissociator, is reported. Transcriptomic and antigenic evaluation of the heart isolated Sca-1+ cells confirmed the display of early cardiac-affiliation transcription factors as well as stemness markers and the lack of markers of cardiac differentiation. However, a majority of Sca-1 purified cells was found to co-express CD31. To analyze defined subsets of the heart Sca-1 population a protocol was established for the separation of Sca-1+CD31+ and Sca-1+CD31- fractions. Preliminary evaluation for the *in vitro* differentiation potential and the transcriptional profiling for both subsets will be presented. Furthermore, aiming at the identification of molecules putatively involved in cardiac stem-cell stress-response signaling-pathway(s), and hypothesizing the involvement of Sca-1, the expression of this gene has been evaluated by real-time PCR in the population of cardiac Sca-1+ cells isolated from (i) non-manipulated, (ii) sham-operated and (iii) mice subjected to myocardial infarction (MI). Our data constitute a first step towards a molecular profiling of the CSC-response to myocardial-infarction.

Poster ID – 22

Characterisation of the pre-iPS cell state

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Induced pluripotent stem (iPS) cells are generated from somatic cells upon the introduction of reprogramming transcription factors. Reprogramming occurs with low efficiency over many days, hindering analysis of process and mechanism. We have previously described the generation and capture of incompletely reprogrammed intermediates known as pre-iPS cells. These partially reprogrammed cells are derived and maintained in normal ES cell culture conditions containing serum and LIF, do not express Nanog or Rex1 and are not competent to contribute to chimaeras upon injection into a blastocyst. The intermediates appear to be impeded in the process of reprogramming from progressing to pluripotency. We therefore applied molecularly defined conditions for the derivation and propagation of authentic pluripotent stem cells from embryos. We combined dual inhibition (2i) of mitogen-activated protein kinase signalling and glycogen synthase kinase-3 (GSK3) with the self-renewal cytokine leukaemia inhibitory factor (LIF). The 2i/LIF condition releases the intermediate cells from this state and allows them to attain full pluripotency as demonstrated by the expression of definitive markers of pluripotency and their competence for somatic and germline chimaerism. The switchable nature of this conversion of pre-iPS cell to pluripotency may allow us to begin to dissect the underlying mechanisms of induced pluripotency. We are now investigating the nature of these intermediate cells and their apparent impediment in the reprogramming process.

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Poster ID – 23

Directed Differentiation of hESCs towards a Ventral Midbrain Dopaminergic Fate

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The ability to direct differentiation of human embryonic stem cells (hESCs) into midbrain dopaminergic (mDA) neurons will prove invaluable for drug discovery and cell replacement therapies in Parkinson's disease. Controlling the fate of the hESCs involves considerations into the extrinsic signalling cues and the intrinsic transcription factors that bring about the correct signalling cascades to delineate mDA differentiation. With this in mind, our work focuses on the in vitro monolayer differentiation of hESCs and the conditions that the cells are subject to. Using the dual SMAD-inhibition, neural-inductive protocol, we seek to maintain an anterior phenotype whilst conferring a ventral character via optimising signalling factors and the timing of patterning cues and bring about a correct positional identity to the cell population. Additionally, overexpression of candidate transcription factors can promote a similar outcome and we aim to characterise one such candidate in hESC differentiation via gain of function studies. We make use of a tamoxifen-inducible transgene to stimulate an activation of Dmrt5. We expect to see a number of effects that have been seem in the mESC paradigm that ultimately lead to a more homogenous cell population.

Furthermore, Dmrt5 is expressed in a relevant spatiotemporal location that overlaps with the presumptive mDA neurons. This tightly regulated expression domain can be utilised to produce expression cassettes for in vivo studies and also reporter cells both for in vitro differentiation and in vivo cell tracking. We aim to characterise the regulatory landscape of Dmrt5 via pronuclear injections and define a midbrain specific enhancer. This enhancer will also hint towards the signalling network in these dopamine precursor cells and further characterise the understanding of mDA development.

Overall, our work aims makes use of the Dmrt5 gene of interest to further optimise the production of true mDA neurons in human tissue culture. This work will investigate intrinsic and extrinsic factors and will aim augment our ability to reproduce mDA neuron development.

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Poster ID – 24

From epidermis to pluripotency

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Human induced pluripotent stem cells are most commonly established by the use of oct4, sox2, klf4 and c-myc transgenes transduced into adult dermal fibroblast cells. The efficiency of fibroblast reprogramming is very low. The low reprogramming efficiency suggests that fibroblasts might not be the optimal cell type for reprogramming disease or patient specific cells. The primary fibroblast cell population is heterogeneous, which makes detailed analysis of molecular changes and processes of reprogramming difficult from these cells. The cells of heterogeneous fibroblast populations can accumulate multiple genetic abnormalities before or during the reprogramming process, so the iPS cells derived cannot provide a reliable genetic background for disease modelling.
Neonatal and adult epidermal keratinocytes can provide a better source of somatic cells for reprogramming, while these cells are clonogenic; can be propagated feeder and serum free for 25-30 population doublings. Human keratinocyte derived IPS cells were generated via transduction with oct4, sox2, klf4 transgenes. Both neonatal and adult keratinocytes generate human ES-like colonies quicker and significantly more efficiently compared to neonatal fibroblast cells. The generated IPS lines resemble the characteristics of human embryonic stem cells and show downregulation of keratinocyte specific genes and reprogramming transgenes. Our results suggest that epidermal keratinocytes could be used for the generation of patient and disease specific transgene free IPS cells.

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Poster ID – 25

Reprogramming human forebrain neural stem cells with transgene removal

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Pioneering studies in mice have established that somatic cells can acquire pluripotent properties by transcription factor induced reprogramming. Subsequently, human induced pluripotent stem cells have been generated from fibroblast and keratinocyte cell populations by transduction with reprogramming factors. In mice it has been established that brain derived neural stem cells can be reprogrammed with only one or two factors. Here we investigated reprogramming requirements for human neural stem cells derived from foetal forebrain. We will show that these cells can convert to undifferentiated status after either amphotropic retroviral transduction or piggyBac transposon delivery of Oct4 and Klf4 or Oct4 alone. The induced phenotype is obtained without selection and is stable upon continuous passaging. Converted cells exhibit a molecular profile similar to human embryo derived stem cells. They can differentiate in vitro and can form multidifferentiated teratomas. Transgenes can be excised by Cre recombination. Reliable creation of human induced pluripotent stem cells with minimal genetic modifications will facilitate development of this technology for biomedical applications in disease modelling, drug discovery and regenerative medicine.

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Poster ID – 26

Sox2 early deletion in mouse leads to severe defects in embryonic brain development and neural stem cells

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Sox2 encodes a transcription factor essential for pluripotent embryonic stem cells, and active also in neural stem cells (NSC). We investigated Sox2 function in neural development and NSC by a conditional inactivation strategy. We initially deleted Sox2 in mouse embryonic brain from day 11.5 of development (E11.5) with a NestinCre transgene. At birth, Sox2-deleted (Sox2flox/-;NestinCre) mice showed minor brain defects, but shortly afterwards NSC and neurogenesis were completely lost in the hippocampus, leading to dentate gyrus hypoplasia; in vitro NSC also became exhausted. In Sox2-deleted hippocampal primordium and NSC Shh was severely reduced, and chromatin immunoprecipitation (ChIP), gel shift and transfection assays identified Shh as a Sox2 target. A SHH pharmacological agonist (Shh-Ag) partially rescued NSCs and growth of the hippocampus. In vitro NSCs were partially rescued by Shh, and almost fully by medium conditioned by wild type cells. These experiments identified a previously unrecognized autocrine signalling mechanism for NSC maintenance, involving the regulation of Shh by Sox2.
In subsequent experiments, we found that Sox2 brain-specific deletion at earlier stages (E8.5) leads to severe ventral brain defects arising already during embryogenesis, pointing to unique stage- and region-specific roles of Sox2 in brain development. Sox2flox/flox;Foxg1Cre mice had reduced telencephalic vesicles and, most prominently, severe loss of ventral telencephalic structures, resembling that seen in early (Foxg1Cre-deleted) Shh conditional mutants, and in holoprosencephaly, a clinically relevant disease caused by mutations in SHH in humans and mice. Remarkably, Shh-Ag significantly rescued the ventral brain defect. At early stages (E10.5-E12.5) the most striking phenotype was a loss of ventral telencephalic markers, accompanied by expansion of dorsal markers, while cell proliferation appeared unaltered. Correspondingly, NSC in vitro showed altered regional specification, with loss of ventral gene-expressing NSC. Significantly, impaired ventral markers include transcription factors known to be important regulators of ventral brain development and, particularly, known transcriptional activators of Shh: Nkx2.1 and Six3 (a gene also mutated in holoprosencephaly). ChIP experiments indicate they are directly bound by Sox2. We propose that their transcriptional activation represents a critical, stage-specific function of Sox2 in early brain development.

Poster ID – 27

Identification and characterization of a transient cell population during early differentiation of human embryonic stem cells

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The establishment of human embryonic stem cell (hESC) lines not only opened a new avenue for the potential clinical and biomedical applications but also provide a valuable cell source for the study of early human development. We have previously established a neural differentiation protocol by which hESCs can be efficiently differentiated into neural progenitor cells in an adherent culture condition, which enable us to investigate more closely the changes during the neural differentiation. In this study, by carefully examining the expression of hESC surface antigens, we have identified a transient stage of cells during early differentiation of hESCs prior to the formation of neuroepithelim. These cells sustained expression of hESC antigen SSEA4, though at a lower level than that in hESCs, but lost expression of Tra-1-60/81. Gene expression analysis showed that the SSEA4+/Tra-1- cells expressed pluripotent hESC markers, Oct4 and nanog, as well as neural progenitor markers, Pax6 and Sox1. Nevertheless, the expression levels of Oct4 and nanog in these cells were lower than that in undifferentiated hESCs and Pax6 and Sox1 were lower than that of neural progenitors. In addition, the cells express primitive ectoderm marker FGF5. FACS sorted SSEA4+/Tra-1- cells were able to produce cells of three germ layers via embryoid body formation. However, these cells could not be reversed back to undifferentiated state when re-introduced back into the hESC self-renewal culture condition. These results suggest that these cells may be an intermediate stage of cells between hESCs and neural progenitors and still capable of differentiating into other lineages.

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Poster ID – 28

Paracrine signals restrict neuronal migration from human neural precursor cell containing grafts.

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Human pluripotent stem cells represent a promising source for cell-based therapies in brain injury and disease. Established techniques for the generation of stable well-characterized human neural stem cells provide prospects to generate large numbers of donor cells in high purity for neural repair. A critical issue in neural replacement therapy is the question how grafted cells can be directed to particular targets within the adult CNS. Transplants of hESC-derived neural precursors (hESNP) into an adult host brain have been shown to form dense clusters with limited neuronal migration and integration. In contrast, pure populations of immature hESNP-derived neurons exhibit enhanced migration potential in vitro and in vivo. Addition of undifferentiated hESNP to purified neurons suppresses their migration, suggesting that one mechanism underlying cluster formation is an interaction between neural precursors and their differentiated neuronal progeny. FGF2 and VEGF appear to play an important role in this interaction as both factors are expressed by hESNP and stimulate chemotaxis of hESNP-derived neurons in a Dunn-chamber assay. Neurons treated with the indolinone derivative BIBF1120, which is blocking VEGF receptor (VEGFR) and FGFR kinase activity, loose their chemotactic response to FGF2 and VEGF in vitro. In addition, BIBF 1120 treatment enhances tissue integration of grafted neurons from hESNP-containing transplants in hippocampal slice cultures. Our results suggest that disruption of this newly discovered "auto-attraction" between neural precursors and their neuronal progeny may provide a route to improve migration and integration of human neurons emanating from neural precursor grafts.

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Poster ID – 29

Inhibition of Notch Signaling in Human ES Cell-Derived Neural Stem Cells Delays G1/S Phase Transition and Accelerates Neuronal Differentiation

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The controlled in vitro differentiation of human embryonic stem cells (hESCs) and other pluripotent stem cells provides interesting prospects for generating large numbers of human neurons for a variety of biomedical applications. A major bottleneck associated with this approach is the long time required for hESC-derived neural cells to give rise to mature neuronal progeny. In the developing vertebrate nervous system Notch signaling represents a key regulator of neural stem cell maintenance. Here we set out to explore whether this signaling pathway can be exploited to modulate the differentiation of hESC-derived neural stem cells (hESNSCs). We assessed the expression of Notch pathway components in hESNSCs and demonstrate that Notch signaling is active under self-renewing culture conditions. Inhibition of Notch activity by the gamma-secretase inhibitor (GSI) DAPT in hESNSCs affects the expression of human homologues of known targets of Notch and of several cell cycle regulators. Furthermore, DAPT-mediated Notch inhibition delays G1/S phase transition and commits hESNSCs to neurogenesis. Combined with growth factor withdrawal, inhibition of Notch signaling results in a marked acceleration of differentiation, thereby shortening the time required for the generation of electrophysiologically active hESNSC-derived neurons. This effect can be exploited for neural cell transplantation, where transient Notch inhibition prior to grafting suffices to promote the onset of neuronal differentiation of hESNSCs in the host tissue. Thus, interference with Notch signaling provides a tool for controlling human neural stem cell differentiation both in vitro and in vivo.

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Poster ID – 30

Sox2 and Serrate1 cooperate to specify sensory competence of otic neural progenitors

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Hair cells of the inner ear sensory organs originate from sensory progenitors, which locate at specific domains of the otic vesicle: the prosensory patches. The transcription factor Sox2 and the Notch ligand Serrate1 (Ser1) are expressed in prosensory domains and they are necessary for normal sensory development. However, their specific functions and interactions during this process remain mostly unexplored. Here, we have studied the function of Ser1 and its interactions with Sox2 by in ovo electroporation of hJagged1 (hJag1) and cSox2 expression constructs in the chick otic vesicle. First, we show that Sox2 and Ser1 are co-expressed since early stages of otic development, but Sox2 is initially expressed in a broader domain which then restricts within the borders of Ser1, as prosensory patches are specified. Secondly, ectopic expression of hJag1 is sufficient to maintain Sox2 expression in those broader domains, provided Notch signalling is active. In parallel, hJag1 induces the expression of endogenous Ser1 and of Hey1, Hey2 and Hairy1, but not of Hes5, Notch target genes, suggesting a function through the mechanism of lateral induction. Ectopically extended Sox2 expression domains are maintained until late stages of sensory development, when hair cells differentiate, and they result in enlarged sensory organs. Finally, we show that Sox2 alone is sufficient to ectopically induce hair cell fate in the otocyst. We suggest that during otic sensory development Ser1 functions to maintain Sox2 expression, and thus sensory potential, within restricted domains, specifying prosensory patch size and location in the otocyst.

Poster ID – 32

Hypoxia Enhances Proliferation of Mouse Embryonic Stem Cell-derived Neural Stem Cells

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In vitro expansion and differentiation of neural stem (NS) cells provide a source of material with potential applications for neural drug testing, developmental studies as well as for novel cellular therapies for neurodegenerative diseases, which affect millions of people.

In this work, the continuous ex-vivo expansion of mouse embryonic stem (ES) cell-derived NS cells was characterized and optimized under serum-free conditions. In the presence of epidermal growth factor and basic fibroblast growth factor these cells can be expanded in adherent conditions, which may be advantageous compared to the usual NS cell culture as neurospheres.

The optimization of the adherent culture of mES cell-derived NS cells started with parameters such as initial plating density and oxygen concentration. The results showed that 2-5% oxygen, which is the physiological oxygen concentration range in the brain, leads to fold increase values in total cell number about twice higher than the ones observed under 20% oxygen (20 vs 10 fold, respectively). When using an optimal initial cell density of 104 cells/cm2, the maximal growth rates were 1.9 day-1 under hypoxia versus 1.7 day-1 under normoxia. The final lactate concentrations reached under hypoxia and normoxia were lower than the potentially inhibitory values for this toxic metabolic end-product and no significant differences were observed concerning the specific lactate production rate. Importantly, the expansion of CGR8-NS cells under hypoxia does not affect their multipotency.

To verify if the positive effect of hypoxia was due to a positive effect on proliferation or to a reduction in cell death, cell divisional history, cell cycle and apoptosis/necrosis studies were performed for both culture conditions. Although the number of apoptotic and necrotic cells was comparable under both oxygen tensions (less than 10%), cells divide faster under hypoxic conditions, as determined with the membrane dye PKH67. However, a larger population in a quiescent G0 phase was observed in normoxic conditions compared to 2% O2.

Poster ID – 33

Transcriptomic study of fetal porcine neural precursors and their in vitro differentiation

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Pigs and miniature pigs are gaining importance as large animal models in the field of regenerative medicine, including stem cell research. With their size, organ capacity, and physiology resembling in several aspects that of humans, pigs are well suited for preclinical experiments and long-term safety studies. Several somatic stem cell populations, including neural precursors were successfully isolated in miniature pigs. The lack of stable embryonic stem cell lines could be probably overcome by recent advances in induced pluripotent stem (iPS) cell generation. As fetal neural stem and progenitor cells in other species have some favorable properties for iPS generation (e.g. endogenous Sox2 expression), we have decided to characterize transcriptome of neural progenitors, isolated from 40 days old minipig fetuses and their in vitro differentiated progenies. Using microarray chip containing 20201 S. scrofa spotted genes (Pig protein annotated oligonucleotide array, The University of Arizona), we have analyzed 6 independent biological replicates of porcine neural progenitors and cells induced for differentiation by 5 day treatment with 1 μM all trans retinoic acid. We have identified 6800 differentially expressed genes, 3800 genes up-regulated in differentiated cells and 3000 up-regulated in neural precursors. To verify the results from microarray analysis we have performed real-time RT-PCR for 3 genes up-regulated in differentiated cells (α-B-crystallin; Hemeoxygenase 2; Glial fibrillary acidic protein) and 10 genes up-regulated in neural precursors (hnRNPB1; Sox2; vimentin; Galectin 1; CXCR4; hnRNPH; β-III tubulin; FGFR1; LIFR and BMP4). Two genes highly but constantly expressed in both analyzed stages were used as housekeeping genes (S23, AP-TNAP). With the exception of Hemeoxygenase 2, results of our real-time RT-PCR study confirmed differences in gene expression observed on microarrays. Confirmation of high expression of Sox2 in porcine neural precursors together with observed expression of LIF receptor and BMP4 suggests that porcine neural precursor cells could be used as starting population for reprogramming into iPS cells.

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Poster ID – 34

Activation of type 1 cannabinoid receptor (CB1R) induce neurogenesis in the murine subventricular zone

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Stem/progenitor cells present in the subventricular zone (SVZ) niche produce new neurons important for normal brain function and potentially useful for brain repair. In the present work we aim to further support the role of endocannabinoids on neurogenesis in SVZ stem/progenitor cells. For that purpose, 7 days SVZ neurospheres (P0-3 C57BL/6 mice) were adhered to poly-D-lysine coated coverslips and treated for 7 days with 100 nM, 300 nM, 1 µM and 3 µM of CB1R agonist (R)-(+)-Methanandamide. The presence of CB1R in SVZ cells was assessed by immunocytochemistry and western blotting. Immunoreactivity to CB1R was detected in nestin- and in GFAP- positive cells cells but not in polysialated neural cell adhesion molecule (PSA-NCAM), doublecortin and microtubule associated protein (MAP-2) positive cells. To functionally evaluate neuronal differentiation, we analysed the variations of intracellular calcium levels ([Ca2+]i) following 50 mM KCl and 100 microM histamine stimulations. Analysis of the response of cells to histamine, when compared to KCl (Hist/KCl ratio), allowed us to evaluate different cell populations present in culture. Exposure of SVZ cells to increasing concentrations of (R)-(+)-Methanandamide resulted in increased differentiation of cells displaying a neuronal-like profile of [Ca2+]i responses, as compared with the predominant profile of immature cells observed in control cultures. In fact, in control cultures about 5-10% of cells display a neuronal-like profile while (R)-(+)-Methanandamide exposure induced about 40% of cells showing a neuronal-like profile. Taken together these results suggest that CB1R activation induce neuronal differentiation from neonatal subventricular zone cell cultures of mice.

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Poster ID – 35

Artificial Delivery of Notch intracellular domain into Neural Stem Cells induces Proliferation and impairs Neuronal Differentiation

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Embryonic stem (ES) cells have become a major focus of scientific interest as a potential source of transplantable cells for regenerative medicine. However, the controlled derivation of tissue-specific stem and progenitor cells and their controlled differentiation still pose major challenges. Notch receptors participate in a wide variety of biological processes, including self-renewal and differentiation of neural stem cells (NSC). They are able to respond to cell-cell interactions via ligands expressed on the surface of neighboring cells. Our study explores the potential of protein transduction to directly deliver recombinant Notch 1 intracellular domain (NICD) into mammalian cells in order to accomplish transgene-free Notch activation. We engineered a cell-permeant version of NICD and evaluated its cellular function on mouse and human NSC. NICD protein delivery induced a 10-fold upregulation of the Notch target gene HEY1. Conversely, NICD transduction antagonized gamma-secretase inhibitor-mediated down-regulation of HEY1. We further found that transducible full-length NICD, but not a truncated control protein induces three-dimensional growth of NIH 3T3 and enhances proliferation of NS cells. Furthermore NICD treatment restored DAPT-mediated decreased proliferation back to control levels as determined by BrdU incorporation. On the other hand, NICD delivery impairs neuronal differentiation in the presence and absence of DAPT. Remarkably, activation of Notch signaling by cell-permeant NICD suffices to maintain more than 90% of NS cells in a nestin-positive state even in the absence of growth factors. NICD protein transduction allows precise control over dosage and time of Notch activation and completely circumvents genetic manipulation of the target cell. These observations depict NICD protein transduction as a novel tool to modulate cell functions in a wide variety of target cells.

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Poster ID – 36

Cellular Distribution of p53 is Regulated by JMJD3 in Mouse Neural Stem Cell Neurogenesis

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The histone H3 lysine 27-specific demethylase JMJD3 plays an important role in activating specific components of the neurogenic program. In addition, p53 appears to be pivotal during neurogenesis, and is required for neurite outgrowth in primary neurons as well as axonal regeneration in mice. Finally, JMJD3 induces ARF expression in mouse embryonic fibroblasts; demethylases may also fulfill p53 activity and its interaction with co-activators by acting on non-histone proteins. The aim of this study was to explore the potential cross-talk between p53 and JMJD3 during mouse neural stem cell (MNSC) differentiation. Our results showed that total levels of JMJD3 mRNA increased approximately 30% at 3 days of MNSC differentiation. Immunocytochemistry analysis revealed that neuronal precursors are initially detected at this stage of differentiation. In addition, p53 DNA binding activity was significantly increased at 3 and 8 days of differentiation, while siRNA-mediated silencing of p53 significantly reduced the number of β-III tubulin-positive cells at 3 days. Importantly, overpresssion of both Wt and C-terminal mutant JMJD3 in MNSCs resulted in increased total p53 protein, coincident with augmented total ARF mRNA expression. Interestingly, the demethylase activity of JMJD3 appeared to be crucial in regulating p53 cellular distribution, but not the induction of ARF expression. Notably, a significant decrease in nuclear p53 was observed in cells transfected with the mutant form of JMJD3, without the C-terminal region associated with demethylase activity. In fact, in these conditions, a marked accumulation of p53 was detected in cytosol. Finally, immunoprecipation assays using both SH-SY5Y cells and MNSC revealed a direct interaction between p53 and JMJD3, in a JMJD3 C-terminal region-independent manner. These data suggest that p53 cellular localization is regulated by direct JMJD3-dependent demethylation of p53 during neurogenesis. In conclusion, our results demonstrate that JMJD3 and p53 may act in a common pathway to regulate neural differentiation. A better understanding of this potential cross-talk will be crucial to control neural stem cell fate, possibly contributing to the development of novel therapeutic approaches that make stem cell lines suitable for neuro-replacement therapy.

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Poster ID – 38

Modelling eye specification in human embryonic stem cells

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Eye specification results from three steps of embryonic inductive interactions. First, the neuroectoderm is induced in the dorsal embryonic region. Subsequently, antero-posterior patterning of the neuroectoderm specifies the forebrain at its rostral end, while progressively posterior regions are specified as midbrain, hindbrain and spinal cord. Finally, a subset of the forebrain is specified as eye-forming region (eye field), while neighbouring regions take on different forebrain fates, such as telencephalon or diencephalon. A network of eye field transcription factors (EFTFs), including Pax6, Six3, Lhx2, Six6 and Rax, becomes active in the eye field and controls its specification to eye fates. However, the mechanisms controlling expression of this EFTF network remain unclear. By employing human embryonic stem cells (hESCs) as a human developmental model system, we show that the first two steps leading to eye specification, namely neuroectoderm induction and forebrain specification, can be successfully reconstructed in hESCs grown in chemically defined, feeder-free, adherent conditions by means of Nodal, Wnt and BMP antagonism in the absence of exogenous FGF. However, these conditions appear to be insufficient for robust Rax expression and full eye fate specification. Experiments are underway in order to elucidate the molecular signals controlling Rax expression within the anterior neuroectoderm.

Poster ID – 39

Characterization of murine ESC-derived neural precursor cells

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Background:
Self-renewing and multipotent neural stem cells (NSCs) are considered as a very promising tool in the field of regenerative medicine. However, a disturbed function of endogenous NSCs could also contribute to the development of neurological diseases as well as to brain tumour formation. Therefore, there is increasing interest for understanding the biological properties of NSCs and to completely characterize these cells. However, progress in this field id hampered by the lack of specific NSC markers and by the low numbers of NSCs in the brain tissue.

Methodology:
In this context, several laboratories have established different approaches to differentiate in vitro embryonic stem cells (ESCs) towards the neural lineage. Here, in this study we have compared different protocols to induce neural differentiation in adherent monolayer of ESCs and investigated the generation of neural precursors. Using immunostaing with antibodies to differentiation stage specific markers we have characterized the neural differentiation at various times after induction of differentiation. To specifically investigate the generation of NSCs we have used clonal neurosphere assays and to follow the developmental of the generated NSCs we have analysed the expression of EGFR, which is absent in early development NSCs but is upregulated from late development onwards.

Outlook:
Taken together, our data indicate that in particular differentiation conditions ESCs sequentially generate NSCs displaying characteristics of early and late development NSCs. In addition, they also show that EGFR expression can be used as a marker for the isolation of ESC-derived NSCs.

Poster ID – 40

Consistent generation of expandable Neuroepithelial-like stem cells from human pluripotent stem cells

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Consistency amongst human stem cell lines is frequently low compared to mouse stem cell lines derived from inbred stains. Here we show consistent derivation of human long-term neuroepithelial-like stem (lt-NES) cell lines from five different human induced pluripotent stem (iPS) cell lines and two human embryonic stem (ES) cell lines. These lt-NES cell lines of different origins are nearly identical in respect to morphology, growth properties, gene expression, and differentiation potential. They grow with an organised rosette-like morphology and express neural rosette markers as Dach1 and PLZF. In the present study we have compared expression levels of 190 neural stem cell genes between seven lt-NES cell lines and three human foetal neural stem (NS) cell lines clearly showing a close relationship between lt-NES cells from different origins and their distinction from foetal NS cells. Upon growth factor removal lt-NES cells differentiate to a high percentage of mature mostly GABAergic neurons some which displaying functionality. Lt-NES cells have a caudal positional identity, however with a potential to re-pattern in response to extrinsic factors to acquire a ventral midbrain identity. iPS cell derived lt-NES cells have the potential to provide a powerful tool for comparing neural stem cell self-renewal and differentiation behaviour between different human genetic backgrounds, for example disease modelling using patient and non-patient cells.

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Poster ID – 41

Redox profile during neural precursor cell proliferation and differentiation

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Oxidative stress, either associated with normal metabolism or disease, affects many cellular activities. Reactive oxygen species (ROS) generated within the cells may act as signaling molecules that regulate a wide range of cellular processes depending on cell types, such as cellular defense, differentiation and proliferation. However, there are few data about how the unique properties of neural precursor cells (NPC) are affected by oxidative stress. Recently, it was demonstrated that ABC transporters regulate NPC proliferation and differentiation, but the role of the multidrug resistance-associated protein-1 (Mrp1), that maintains glutathione homeostasis is still unclear. Thus, we aim to explore the redox status and the expression of Mrp1 in both proliferating and differentiating NPC. NPC were obtained from E15 mouse brains, growing as neurospheres in the presence of EGF and bFGF. Differentiation was first induced by plating NPC onto poly-D-lysine-coated plates in the presence of low concentrations of bFGF. Cells were cultured in the absence of bFGF for 4, 8 and 18 DIV. Cell viability was determined by trypan blue dye, total glutathione (GSt) by an enzymatic assay, protein oxidation and lipid peroxidation by slot-blot analysis and Mrp1 by western blot, in both NPC and differentiating cells. NPC differentiated into astrocytes and neurons, with the first representing 55% at 4 DIV and 64% at 18 DIV. Loss of cell viability increased 6-fold ($p<0.01$) from proliferating NPC to 4, 8 and 18 DIV differentiating cells. The results obtained in NPC point to higher levels of intracellular GSt (3-fold, $p<0.05$), as well as of protein oxidation (1.6-fold, $p<0.01$) and lipid peroxidation (2.5-fold, $p<0.01$) as compared to the lower level obtained in differentiating cells. Moreover, we observed, for the first time, that Mrp1 is highly expressed in NPC. Immature cells (4 DIV) presented the lowest levels of Mrp1 and during nerve cell maturation this protein slightly increased, although never reaching the expression levels of NPC (0.7-fold for 4 and 8 DIV, $p<0.05$; 0.9-fold for 18 DIV; versus NPC). These preliminary results show that NPC proliferation is associated with increased levels of lipid and protein oxidation. Thus, we may hypothesize that the higher concentrations of GSt and Mrp1 expression observed in NPC constitute self-defense mechanisms, which will prevent oxidative injury in this proliferating state.

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Poster ID – 42

Activin induces the differentiation of ES derived neuronal precursors towards LGE progenitors

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The cerebral cortex functions in memory and higher-order cognitive processing. Two classes of neurons carry out these roles, the excitatory projection neurons and the inhibitory interneurons. The interneurons, which comprise 20 –30% of cortical neurons, serve a vital role in modulating cortical output (Krimer and Goldman-Rakic, 2001). The inhibitory interneurons originate during embryonic development due to a dorso-ventral regionalization of the forebrain, caused by the ventralizing effects of Shh and the dorsal specification action of Wnts (Sur and Rubenstein, 2005). This morphogen gradient drives the formation of the two main populations of cortical neurons, the glutamatergic excitatory neurons in the dorsal region and the inhibitory interneurons in the ventral region. The ventral region of the forebrain is subsequently sub-divided into two regions, the middle ganglionic eminence (MGE) from which Parvalbumin and Somatostatin interneurons originate, and the lateral ganglionic eminence (LGE), that gives rise to calretinin interneurons the stiatum and part of the olfactory bulb (Marín and Rubenstein, 2003; Waclaw et al 2009). The mechanism for the sub-division of the ventral forebrain is still poorly understood.

The “in vitro” differentiation of embryonic stem (ES) cells provides a powerful tool to understand cortical development. The serum free monolayer differentiation protocol allows to reproducibly obtain relatively pure populations of forebrain neural progenitor cells that respond to forebrain morphogens (Shh and Wnt3a) in the same way as occurs during cortical development (Ying et al. 2003, Watanabe et al. 2005).

Here, we utilise ES derived neural precursors to identify novel morphogens that pattern the forebrain. We have found that Activin, a member of the TGB super-family, is a potent neurotrophic factor as it induces the differentiation of neural precursors derived from ES cells into postmitotic neurons in a fast and efficient way. A detailed analysis of the mechanism by which Activin promotes the neuronal differentiation of ES cells found that it did so coordinating two of the main steps involved in the generation of differentiated neurons, the exit from the cell cycle, via the inhibition of he Shh pathway, and the induction of differentiated neural identity, trough the enhancement of the response to RA signalling.

We also have found that Activin stimulates the expression of LGE markers. In accordance with this, we find that Activin greatly enhances the proportion of GABAergic and Calretinin subtype neurons. Given the relevance of striatal neurons to neurodegenerative disorders such as Huntington's disease, our ability to generate LGE precursors is potentially of great interest to regenerative medicine. Together our results show that by using ES cell based approaches we have potentially identified a novel morphogen that provides regional identity to the forebrain.

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Poster ID – 43

Patient-specific induced pluripotent stem cells to model Dravet Syndrome in vitro

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Dravet Syndrome is a form of epilepsy known as severe myoclonic epilepsy in infancy (SMEI) usually starting with seizures within the first year of life and finally leading to a status epilepticus. Further characteristics are psychomotoric delay, ataxia and cognitive impairment. Unfortunately, common antiepileptic drug therapy is ineffective. The molecular cause for Dravet Syndrome is a variety of loss-of-function mutations in one allele of the SCN1a gene (including missense, nonsense and splice-site mutations). SCN1a codes for the pore-forming alpha subunit of voltage-gated sodium channel 1.1 (Nav 1.1), which is the major sodium channel of GABAergic interneurons localized in diverse brain regions including prefrontal cortex, hippocampus and spinal cord. In the mouse, Nav 1.1 haploinsufficiency causes reduced sodium currents and impaired action potential firing. In humans, the study of Dravet Syndrome is hampered by poor access to patient-specific primary neurons. To overcome this limitation, we aim at the derivation of primary Dravet-specific neurons from human induced pluripotent stem cells (hiPSCs). Fibroblasts of Dravet patients co-transduced with OCT4, SOX2, KLF4 and c-MYC gave rise to hiPSC lines with typical hESC-like morphology and pluripotency marker expression. Pluripotency of the cells was further confirmed by multilineage differentiation in vitro as well as teratoma formation in vivo. Since prominent Nav 1.1 expression has been observed in GABAergic neurons, we used an in vitro differentiation protocol, which yields a long-term self-renewing neuroepithelial stem cell (lt-NESC) population with a strong preponderance for GABAergic differentiation. Dravet-specific lt-NESCs express the neural stem cell markers PAX6, SOX2 and NESTIN, exhibit a rosette-like morphology and can be proliferated extensively without losing their neurogenic potential. Differentiated lt-NESCs derived from patient-specific and control hiPSCs showed robust expression of mutated and wild-type SCN1a alleles, respectively. These lt-NESCs should provide an unlimited source of Dravet-specific neurons and thus facilitate the standardization of downstream analyses, including electrophysiological studies focusing at the functional consequences of the various Nav 1.1 mutations associated with this disease.

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Poster ID – 44

Generation of Inner Ear Sensory Cells from Human Bone Marrow-derived Mesenchymal Stem Cells

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Worldwide, 500 million people are estimated to be affected by some form of hearing loss, making hearing impairment the most common sensory disorder in humans. In the majority of the cases, the cause is directly or indirectly linked to degeneration and death of the so-called hair cells of the cochlea in the inner ear, and their associated neurons. The former perform the essential conversion of mechanical stimuli to neural signals and their loss may result from aging (around 50% of people over 65 years of age suffer some kind of hearing disorder), excessive exposure to loud stimuli, bacterial and viral infections, or ototoxic drugs. In mammals, unlike in birds and lower vertebrates, loss of hair cells of the auditory sensory epithelium (the organ of Corti) is irreversible because regeneration does not take place.
Different avenues have been explored in order to tackle the problem of hearing loss, such as gene therapy, exogenous delivery of neurotrophic factors, and, recently, the differentiation of stem cells into sensory cell types (i.e. hair cells and sensory neurons) that could be used for future transplantation cell therapy approaches. Embryonic stem cells, as well as mouse mesenchymal stem cells (MSCs), have already been shown to differentiate into inner ear sensory cells. Nevertheless, no reports have yet been made on the differentiation of human MSCs into hair cells or sensory neurons. We are growing human MSCs under various culture conditions and monitoring the expression of markers for inner ear sensory cells via QRT-PCR and immunocytochemistry. We have established conditions under which we obtain a significant increase in the expression of Atoh-1, considered a master gene in the hair cell lineage, as well as that of myosin VIIA and myosin VI, expressed by hair cells upon differentiation. The availability of an *in vitro* system of human hair cells and sensory neurons would not only allow us to pursue a further use of these cells for transplantation into animal models but it would also give us a model to study the cell biology of these cells and their gene expression profiles, as well as their manipulation.

Poster ID – 45

Excitation of SCA3 iPS cell-derived neurons induces calpain-mediated ataxin-3 cleavage and aggregation

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Spinocerebellar ataxia type-3 (SCA3, Machado-Joseph Disease) is an inherited neurodegenerative disorder caused by polyglutamin-encoding CAG repeat expansions in the ataxin-3 gene product. Proteolytic cleavage of ataxin-3 is believed to be causative for the formation of ataxin-3 containing aggregates, an initial mediator of the disease. The mechanism that triggers this process, however, has remained elusive. Here we report the generation of induced pluripotent stem (iPS) cells from a patient with SCA3 and his non-affected sibling and their differentiation into patient-specific neuronal cultures. We demonstrate that glutamate-induced excitation of SCA3-neurons is sufficient to initiate cleavage of ataxin-3 and the formation of formic acid soluble aggregates. This process is can be efficiently blocked by the calpain inhibitors AC-Leu leu Nie-al (ALLN) and calpeptin but not by caspase inhibition, indicating that excitation-induced activation of calpains is a crucial step in the initiation of the disease. Thus, our study illustrates the powerful potential of iPSC-derived cellular models to identify fundamental pathophysiological processes in human degenerative disorders.

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Poster ID – 46

Identification of Dmrt5 in specifying ventral midbrain neural progenitor fate and midbrain dopamine neuron differentiation in embryonic stem cells

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Understanding the control of cell fate choices during ESC differentiation is crucial for harnessing strategies for efficient production of desired cell types for pharmaceutical drug screens and cell transplantation. Dmrt5 is a transcription factor we isolated from an expression screen. It exhibits restricted expression in the developing ventral mesencephalic neuroepithelium that gives rise to dopamine neurons (mesDA). Conditional gain-of-function of Dmrt5 in ES cells promoted the generation of Foxa2+Lmx1a+ neural progenitors, and subsequently dopamine neurons expressing key midbrain characteristics. This was accompanied by the suppression of the transcription profile associated with alternative neural progenitor fates, notably, those that reside in the ventrolateral mesencephalon (eg. Nkx2.2, Helt, Isl1, Brn3a, and Meis2). In contrast, conditional Dmrt5 knock-down by shRNA and loss of Dmrt5 via consecutive gene targeting in ES cells resulted in a defect in mesDA gene expression. These data indicate that Dmrt5 is an important player in mesDA neuron differentiation in ES cells and a strong candidate regulator of midbrain dopamine neuron development. Current works focuses on investigations of signalling pathways that may mediate the observed Dmrt5 effect.

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Poster ID – 47

Generation and characterization of iPS cells from HD patients

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Huntington's disease (HD) is a devastating autosomal dominant neurodegenerative disorder caused by the expansion of a CAG trinucleotide repeat in the gene encoding for huntingtin. Here we aim at generating and characterizing iPS cells from fibroblasts obtained from HD patients (HD-iPS) and from healthy individuals (WT-iPS) and to further analyze them for the appearance of molecular and cellular phenotypes typical of HD. For the infection we used a polycistronic lentivirus coding for Yamanaka's reprogramming factors (with or w/o c-Myc). We report that the mutation does not affect the reprogramming process nor its efficiency and that HD-iPS cells carry the typical expression profile expected for pluripotent cells. Cells immunopositive for markers of the three germ layers are found when HD-iPS are differentiated via EB formation. We also show that the CAG expansion is stable over multiple passages. Finally, our results demonstrate that both WT- and HD-iPS are able to differentiate into mature neurons after exposure to a monolayer protocol based on dual inhibition of SMAD signalling (Chambers et al 2009) or by culturing iPS cells in suspension and exposing them to a neuralizing medium (Li et al., 2009).

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Poster ID – 48

Disease modelling of spinocerebellar ataxia type 3 by using neurons derived from patient-specific iPS cells

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Polyglutamine (polyQ) repeat diseases are hereditary neurodegenerative disorders caused by expansion of a polyglutamine tract in the respective disease protein. Spinocerebellar ataxia 3 (SCA3) is linked to an expansion of the polyQ repeat in the C-terminus of Ataxin-3 (ATXN3). From the spinocerebellar ataxias SCA3 is the most frequent form in Europe, Japan and the United States. Studying the pathology of SCA3 is restricted to artificial disease modelling systems like animal models or transgene overexpression in neuroblastoma cell lines. Reprogramming of patient-specific cells to derive induced pluripotent (iPS) of SCA3 could overcome these limitations by offering the opportunity to generate cells from the central nervous system (CNS). Recently, we established a protocol for the derivation of long-term neuroepithelial stem (lt-NES) cells from human embryonic stem cells. lt-NES cells express the neural stem cell markers PAX6, SOX2 and Nestin, exhibit a rosette-like morphology and can be cultivated indefinitely without losing their neurogenic potential. Thus, iPS-derived lt-NES cells may represent an attractive donor source for the generation of patient-specific neurons. To test this hypothesis we generated iPS cells from a SCA3-patient and a sibling as control. Two clonal lines per specimen matching the pluripotency criteria were chosen to generate lt-NES cells. All four cell lines exhibit specific characteristics for lt-NES cells and give rise to homogenous cultures of neurons upon growth factor withdrawal. Expression of the expanded polyQ form of ATXN3 was confirmed in the patient-specific lt-NES cells derived neurons. First data suggest that cell culture conditions impacting on ATXN3 cleavage promote a selective vulnerability and enhanced propensity for ATXN3 deposition in SCA3 lt-NES-derived neurons. We expect this in vitro model to provide further insight into the cellular and molecular pathology of SCA3 and to serve as useful in vitro system for compound screening.

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Poster ID – 49

Histamine-releasing PLGA microparticles as a tool to impel brain repair

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In the adult mammalian brain, stem cells from the subventricular zone (SVZ) have an important role for normal brain function and repair strategies. Neurogenesis at this niche is modulated by a broad range of factors, among which is histamine, a signaling molecule involved on a wide spectrum of physiological and pathological events. However, its role on neurogenesis is elusive. Thus, the aim of this work was to elucidate the role of histamine on SVZ neurogenesis.
Exposure of P1-3 C57BL/6 mice SVZ neurospheres to 100μM histamine for 7 days increased the percentage of NeuN-neurons as compared to control. Neuronal functional identification was also performed, analysing the variations of intracellular calcium levels ([Ca2+]i) following KCl and histamine stimulations. Single Cell Calcium Imaging technique allowed us to monitor the increased differentiation of cells displaying a neuronal-like profile of [Ca2+]i responses in histamine treated cultures, when compared with predominant immature profile observed in control. This proneurogenic effect was mediated via activation of histamine 1 receptor. Moreover, histamine increased phospho-JNK-labeling in growing axons, suggesting a role in axonogenesis.
To promote a more efficient delivery of histamine in this neurogenic niche, poly(lactic acid-co-glycolic acid) (PLGA) microparticles were engineered to release histamine in a controlled way. Exposure of neurospheres to these microparticles also increases the percentage of NeuN-neurons as compared to control. An ex vivo approach was also developed by grafting GFP SVZ neurospheres, previously treated with histamine-releasing microparticles, on the dentate gyrus of organotypic hipocampal slice cultures. After 1 week of co-culturing, 1μg/ml of histamine-releasing microparticles promoted an increase of doublecortin(DCX)/GFP positive cells, as compared with unloaded microparticles co-cultures. To provide evidences of the in vivo histamine effect on neurogenesis, GFP SVZ neurospheres, previously treated with histamine-releasing microparticles, were injected on the hippocampus of adult mice. Three weeks after injection, animals that received histamine treated neurospheres showed few DCX/GFP positive cells at the granular cell layer.
Taken together these results suggest that histamine, either in a one-pulse or in a controlled release fashion, induces neuronal differentiation in SVZ cell cultures. Histamine may thus help to identify new pathways to promote brain repair.

Poster ID – 50

Neurogenic iPS cells from HD mice

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Huntington's disease (HD) is a devastating autosomal dominant neurodegenerative disorder which causes progressive motor, psychiatric and cognitive dysfunctions. The HD mutation is an excessive repetition of a CAG trinucleotide repeat in the gene encoding for huntingtin which causes the production of a protein bearing a polyglutamine expanded tract in its N-terminus (HDRCG, 1993).

Transgenic mice (R6/2 strain) have been developed to model human Huntington's disease (HD) by expression of a portion of the human HD gene under human gene promoter elements (1 kb of 5 UTR sequence and exon 1 together with ~140 CAG repeats).

Here we present the generation and the characterization of several clones of induced pluripotent stem cells (iPS) from fibroblasts derived from R6/2 HD mice and from wild type mouse.

We report that the CAG expansion does not affect the reprogramming efficiency. mHD-iPS clones are similar in term of expression of pluripotency markers to mouse embryonic stem cells and can differentiate into cells of the three germ layers. 6 clones of mHD-iPS and 6 clones of mWT-iPS are under further characterization to study the effect of the mutation on cell proliferation, mitochondrial and lysosomial activity and neural differentiation potential.

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Poster ID – 51

Induction of Pluripotency for in-vitro Modelling of Long QT Syndrome

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Congenital Long QT syndrome (LQTS) is a genetically heterogenous disorder caused by a variety of monogenetic mutations in potassium and sodium channels which lead to a prolongation of action potential duration in cardiomyocytes and an increased risk of syncope and sudden death.

Although the ion channel defects associated with the condition are well described, the precise molecular mechanisms by which individual mutations lead to disease remain unclear. In particular, the characteristic incomplete penetrance and variable expression disease-causing mutations suggests a role for modifier genes, identification of which could lead to improved risk stratification and management of LQTS patients.

Efforts to elucidate the molecular mechanisms underlying LQTS will be aided by detailed in-vitro molecular and electrophysiological analysis of patient cardiomyocytes. Unfortunately however, primary human cardiomyocytes are generally unavailable due to their short life-span in culture and the obvious clinical risks associated with their acquisition.

iPS cells derived from patients with LQTS are a promising tool for investigating the relationship between LQTS genotype, modifier gene expression, and electrophysiological phenotype as they are genetically identical to the individuals they originate from, are derived from clinically accessible tissues and can provide an unlimited source of cardiomyocytes by directed differentiation.

We hypothesise that cardiomyocytes derived from patient-specific iPS cells will have characteristic electrophysiological phenotypes attributable to their LQTS genotype. In order to test this, we are establishing primary keratinocyte cell lines from skin biopsies of normal adults and patients with genotype confirmed LQTS and deriving iPS cells by forced expression of Oct 4, KLF 4 and Sox 2. These cells will be differentiated into cardiomyocytes for electrophysiological studies. Our initial aim is to demonstrate that cardiomyocytes derived from LQTS patient-specific iPS cells possess an electrophysiological phenotype related to their LQTS genotype.

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Poster ID – 52

Stem cell derived neural progenitors and inflammatory response in a mouse model of cerebral stroke

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Cerebral stroke is the third leading cause of death worldwide and the current treatment is restricted to the tPA-therapy in 3 hour window and rehabilitation afterwards. Because inflammation and apoptosis are late contributors to the ischemic lesion, novel neurorestorative therapies capable of halting or compensating these processes are needed.
Human embryonic pluripotent stem cells (hESC) are able to divide indefinitely and differentiate into any kind of cells. Studies have shown that transplanted ESC-derived neural progenitor cells (NPCs) survive and enhance functional recovery in rodents with cerebral stroke, yet the mechanism is obscure. While direct neuronal replacement may not play a prominent role in the recovery, it has been suggested that modulation of inflammation is mostly behind the beneficial outcome.
In this study, we transplanted 200 000 hESC-derived, either ultra small superparamagnetic iron-oxide (USPIO) or green fluorescent protein (GFP)-labeled NPCs into the ipsilateral striatum of aged middle cerebral artery occluded Balb/c mice. MRI showed that USPIO-labeled NPCs migrated towards the ischemic site in stroke animals already 3 days after transplantation. 12 wks after transplantation, tape test revealed a significant recovery ($p=0.005$) in treated vs. untreated animals. Preliminary immunohistochemistry suggested that transplanted cells in ischemic brain may enhance endogenous neurogenesis and the NPCs might also be responsible for the late recruitment of protective microglia to the lesion site.

Poster ID – 53

Myogenic potential of adherent fraction of umbilical cord blood cells and umbilical cord mesenchymal stem cells.

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Isolated form umbilical cord umibilical cord blood cells and mesenchymal cells of Wharton's jelly have been shown to be able to differentiate in various cell types. Thus, as a cells that are readily available and do not rise any ethical issues are considered to be a potential source of material that can be use in regenerative medicine. In our previous study we tested the potential of non-adherent fraction of human umbilical cord blood cells and showed that they are able to participate in the regeneration of injured skeletal muscle. In the current study we focused at the adherent fraction of umbilical cord blood cells and umbilical cord mesenchymal stem cells isolated from Wharton's jelly. We documented that despite both types of cells express pluripotential and myogenic markers only umbilical cord mesenchymal stem cells are characterized by their unlimited potential to be in vitro expanded. These mesenchymal stem cells are able to undergo myogenic differentiation in vitro, and also to colonize injured skeletal muscle, and with low frequency participate in the formation of new skeletal muscle fibers. Moreover, transplantation of umbilical cord derived cells enhance the regeneration of skeletal muscle.

Poster ID – 54

TissueFAXS: A Novel tool for hESC characterisation

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The UK Stem Cell Bank (UKSCB) strives to provide the stem cell community with quality controlled stem cell lines. As part of our quality control programme we seek to identify and utilise tools that will enhance the characterisation of these cell lines.

The characterisation of the human embryonic stem cell (hESC) lines includes immunofluorescence analysis of cells both in situ, using standard fluorescence microscopy, and as single cells using flow cytometry. These technologies allow us to quantify the number of cells that we classify as pluripotent stem cells using a panel of canonical antibodies.

The UKSCB are currently qualifying a quantitative microscope system:TissueFAXS (TissueGnostics). Using a number of hESC lines we hypothesise that this instrument is a viable tool for hESC characterisation. The TissueFAXS will be used to investigate the expression of 6 pluripotency markers: Oct4, Nanog, SSEA3, SSEA4, Tra-1-60 and Tra-1-81 on these hESC lines. The TissueFAXS uses image acquisition software to provide quantitative analysis of in situ cell staining following immunofluorescence. The results from the TissueFAXS will be compared to those obtained using the Guava flow cytometer (Millipore) to determine the efficacy of the TissueFAXS as an hESC characterisation tool for use in field of stem cell biology.

Poster ID – 55

ESTOOLS Database for Integrative and Comparative Stem Cell Data Analysis

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We have developed ESTOOLSDB, a database for peer-reviewed, continually updated embryonic stem cell measurement data, as part of the FP6 ESTOOLS project. The data in ESTOOLSDB is collected from ESTOOLS members and from public sources. It contains data from more than 900 samples of pluripotent stem cells, different multipotent progenitors as well as of differentiated cells from a variety of tissues. Data types range from gene expression, exon expression, SNP, ChIP-chip, and ChIP-seq to copy number variation (CNV) data. All the data within ESTOOLSDB has been preprocessed and normalized utilizing similar algorithms systematically across each data type and platform in question. Thus, the comparison of the data measured by different partners and also other integrative studies of the stem cell data are possible. With various advanced data analysis options present in ESTOOLSDB, different data values can be fused and mined together, for example to detect differentially or similarly expressed genes, to cluster the data, or to quickly find the highly expressed genes within several datasets. In addition, the enriched Gene Ontologies (GO) and KEGG pathways can be detected for a given set of genes. Furthermore, comprehensive biological annotations for all the samples in the database have been manually curated and included to provide end users with convenient ESTOOLSDB querying options. The annotations allow for example to retrieve gene expression data based on differentiation state or epigenetic characteristics of stems cells and many other cell types. The goal of ESTOOLSDB is to continuously provide high quality, up-to-date stem cell data coupled with easy-to-use analysis tools for the stem cell research community.

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Poster ID – 56

Bone marrow mesenchymal cells differentially modulate PBX-1, Bmi-1, and adhesion molecules expression in human cord blood HSC co-cultures.

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Cytokine-expanded hematopoietic stem cells (HSC) lose their homing and engraftment properties during culture. Evidence shows that human mesenchymal cells (hMSC) are able to maintain HSC self-renewal ability and to enhance HSC short term engraftment capability, by mechanisms still to be deciphered. The aim of this study was to evaluate the MSC ability to maintain primitive immunophenotype and to modulate expression of adhesion molecules and self-renewal associated molecules in HSC. Human bone marrow (hBM)-MSC were isolated and characterized by morphologic and immunophenotypic criteria and by their ability to differentiate into osteoblasts, chondrocytes and adipocytes. Cord blood (CB)-HSC were isolated by positive CD34 immunomagnetic selection and cultured in the presence of SCF, TPO and Flt-3L alone or in co-culture with BM-MSC (with or without cytokines). CD34+ cells were stained with CFSE to differentiate them from BM-MSC and to follow cellular divisions. After different periods of culture, surface adhesion molecules and gene expression were assessed by flow cytometry and qRT-PCR. We found consistent differences in proliferation of HSC cultivated just with cytokines or in co-culture with BM-MSC with cytokines. The ability to maintain HSC primitive immunophenotype (CD34+CD38-CD33-) was significantly higher in the co-cultures. The adhesion molecule CD54 was downregulated in cytokine-expanded CD34+ cells and upregulated in cytokine-expanded CD34+ cells in presence of MSC. We found that CXCR4 was downregulated in the presence of cytokines and BM-MSC and not in the cytokine treated cells. PBX-1, Bmi-1, and HOX-B4 were differentially expressed during the culture period when co-culture with MSC in the absence or presence of cytokines. CD54 expression has been correlated with time to engraftment in patients undergoing transplant. Our results on proliferation and CXCR4 downregulation in co-cultured HSC are consistent with a recent finding that shows that CXCR4 negative cells tend to have a proliferative phenotype. A differential regulation of PBX-1, Bmi-1, and HOX-B4 in the HSC co-cultivated with mesenchymal stem cells. These differences in adhesion molecule surface expression and self-renewal associated molecules could explain molecularly the better expansion and engraftment properties of HSC expanded in co-culture with MSC.

Poster ID – 57

Regulation of the stem cell epigenome by REST

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The transcriptional repressor RE1-silencing transcription factor (REST) plays important and distinct regulatory roles in embryonic (ESC) and neural(NSC) stem cells. In murine ESCs, REST binds to over 2400 regulatory sites
and is part of the pluripotency regulatory gene network and shares many target genes with Sox2 , Oct4 and Nanog. Despite this silencing of REST produces little change in the transcriptome in ESCs. In NSCs, REST occupies a subset of about 1400 of these target genes and silencing of REST leads to an upregulation of many more of these genes. However, in NSCs, we have shown that silencing of REST produces changes in the local epigenetic signature of several target genes, even though very little accompanying change in transcription is observed. This has led us to propose that REST is responsible for maintaining a local repressive chromatin structure by
maintaining low levels of H3K9ac and H4ac. This may be a means of maintaining these genes in a pre-repressed state prior to recruitment of activators. In this manner, we propose that REST may be considered as much
an epigenetic regulator as a transcriptional regulator. Here we investigate this idea further by carrying out genome-wide analyses of histone acetylation in ESCs and NSCs where REST has been silenced.

Poster ID – 58

Chromaffin Progenitor Cells from Adrenal Medulla: Characterization and Dopaminergic Differentiation

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Chromaffin cells and sympathetic neurons derive from a common sympathoadrenal progenitor cell. Unlike sympathetic neurons, chromaffin cells are able to proliferate throughout life. Here, we describe the isolation and in vitro characterization of proliferative-competent progenitor cells from the bovine and human adrenal medulla. Similar to neuronal progenitors that form neurospheres, isolated chromaffin cells formed spheroid clusters called chromospheres (CS). Such clusters are heterogeneous structures that support enrichment of progenitors and their proliferation in a low-attachment environment. Our data establish enrichment of progenitor cells within CS that were nestin+/PNMT- indicating their pro-neural but not pro-chromaffin properties. They expressed several progenitor markers such as nestin, Musashi1, Sox1, Sox10, and vimentin.

In this study, we investigated two major hall marks of stemness: self-renewal and differentiation. Clonal growth of cells from primary spheres resulted in secondary spheres directly providing evidence of progenitor's self-renewal. Moreover, sphere initiation capacity that depends on seeding density slightly decreased after 4 weeks of culturing.

Under differentiation conditions, chromaffin precursor cells were capable to derive neurons positive for tyrosine hydroxylase (TH), dopamine β hydroxylase, and dopa decarboxylase. Shift towards neuronal differentiation was accompanied with simultaneous downregulation of neural progenitor markers such as Hes1, Hes5, nestin and Notch2 and upregulation of neural markers such as β III tubulin, MAP2 and Pax6. Moreover, frequency of generated dopaminergic neurons positive for TH was significantly elevated after treatment with retinoic acid (RA) and ascorbic acid (AA). Compared to neurons derived by standard procedure, stimulation of neurons derived after RA and AA treatment revealed increased dopamine production with a tendency to lower nor- and epinephrine synthesis. In addition, typical neuronal excitability and existence of voltage-dependant channels was found after patch-clamping confirming functional gain of generated neurons.

In summary, our data establish the presence of progenitor/stem cells with pronounced ability to differentiate into dopaminergic neurons. Hence, chromaffin precursors might be a promising source in the treatment of neurodegenerative diseases such as Parkinson's disease.

Poster ID – 59

A lentiviral-based approach for inducible and reversible loss-of-function of Polycomb proteins (PcGs) in hESC

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Human Embryonic Stem Cells (hESC) self-renew in vitro and are predicted to differentiate toward several fates. However, our ability to study molecular mechanisms involved in cell fate choices is limited by the current lack of standardized procedures to perform functional assays like RNAi. Here, we have set up a lentiviral-based approach for inducible and reversible loss-of-function of Polycomb proteins (PcGs) in hESC. PcGs form two distinct and interacting complexes PRC1 and PRC2, which bind and repress developmental regulators in hES chromatin. In a proof-of-principle analysis, we have found that Bmi1 (PRC1) ablation slightly impairs hESC proliferation whereas no evident alterations were observed during differentiation as gauged by gene expression for candidate markers. We are currently applying the same procedure to other PcGs, including Cbx7 and Ezh2, which belong to the PRC1 and PRC2 respectively.

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Poster ID – 60

Laser assisted Cell Printing for biofabrication of tissue grafts

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Computer assisted biofabrication of fully functional living tissue for regenerative medicine involves generation of complex three-dimensional constructs consisting of living cells and biomaterials. Laser assisted BioPrinting (LaBP) based on laser-induced forward transfer provides unique possibilities for the deposition of different living cells and biomaterials in a well-defined 3D structure. LaBP can be applied to generate scaffold-free 3D cell systems through a layer-by-layer technique.
By combining benefits of LaBP with the abilities of mesenchymal stem cells (MSCs) for self renewal and differentiation capacity into different lineages, scaffold-free tissue grafts can be generated in a computer-controlled manner.
We demonstrate that: (1) laser printing does not cause any cell damage; (2) laser printed MSCs can be differentiated towards bone, fat, and cartilage; (3) LaBP allows printing of cell densities high enough for the promotion of chondrogenesis.
Thus, LaBP is demonstrated as a promising tool for the generation of ex vivo tissue replacements.

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Poster ID – 61

Massively Parallel Synthesis of Oligonucleotides Enables Accurate and Precise High Resolution Fluorescence Imaging of Chromosomes

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Fluorescence imaging of chromosomes is a powerful means for studying chromosome structure, organization, dynamics, and stability. Fluorescence in situ hybridization (FISH) and karyotyping are the frequently preferred methods, since they afford single cell analysis of chromosome biology. However, these methods to date were not sufficiently robust or flexible to be routinely applied to the full spectrum of applications that can benefit from single cell chromosome analyses.

We have leveraged the ability to chemically synthesize oligonucleotides in massively parallel reactions, thereby de novo constructing complex libraries of DNA sequences for generating fluorescent DNA probes. The oligonucleotide sequences that comprise these libraries are in silico selected using empirically determined selection criteria to target only the most informative elements in the genomic region of interest. Our downstream workflow closely mimics traditional FISH, and we are able to detect regions as small as 1.8 kb and as large as whole chromosomes robustly and reliably. Co-detection of proteins by immunocytochemistry and fluorescent imaging of chromosome domains has been readily achieved.

Because of the inherent flexibility in our probe design methods, we readily visualized regions rich in repeats and/or GC content and discriminated highly homologous mouse pericentromeric regions. Through simultaneous hybridization of probes labeled with up to three different fluorophores for multicolor visualization, copy number changes in a small subset of cells within mouse intestinal tissue sections could be readily visualized alongside the pericentromeric chromosome enumeration probes.

In addition, we have generated whole chromosome painting probes that may be used in combination with structured illumination microscopy; these probes are being applied to the investigation of sub-nuclear space occupied by chromosome domains in human ES and iPS cells.

Because the use of complex oligonucleotide libraries provides an unprecedented flexibility for visualizing a wide variety of genomic DNA sequences, we are continuing to explore applications that can be enabled by oligonucleotide-based fluorescence imaging methodology to further the understanding of molecular mechanisms involving chromosome structure and dynamics.

Poster ID – 62

Challenges of making iPS with piggyBac

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Background:
The piggyBac transposon-transposase system represents a valide alternative for gene delivery into mammalian cells. It consistently shows precise excision upon element transposition and an exclusive use of TTAA target sites during both excision and integration. This enables to remove the reprogramming factors from iPS permanently without a trace, thus avoiding stable genomic integration, risk of insertional mutagenesis, and lack of silencing in pluripotent state. In this study we compare different methodology for iPS induction with piggyBacs.

Methods:
To test plasmids, FuGeneHD was used to transfect cells with a mixture of piggyBac transposons (the four "Yamanaka" factors separately or MKOS), reverse tetracycline trans activator (rtTA), and piggyBac Transposase at a FuGene:DNA ratio of 8µl:2µg. The medium was supplemented with doxycycline 24 hours after transfection. iPS colonies were monitored by morphology and AP staining. FuGENE HD Transfection Reagent, BioRad Electroporator system, and Amaxa Nucleofector system were used to transfect HFF, AF, and Myoblasts. The PB-Factor constructs and PB-Transposase were either transfected simultaneously with rtTA or after establishing rtTA-puro lines. A pCAG-EGFP-IRES-puro plasmid was also used.

Results:
rtTA-puro Myoblasts and HFF lines were established with FuGene:DNA ratio of 8µl:2µg. AF were not transfected and no production of iPS colonies was observed. rtTA-puro Myoblasts/HFF/AF lines were established by electoporation and confirmed by a second transfection with tetO-dsRED. However, PB-Oct4 and Transposase were transfected with extremely low efficacy. No production of iPS colonies was observed with either BioRad electroporator or Amaxa Nucleofector. More promising preliminary results have been obtained with the Neon Transfection system using a mixture of PB transposons (four factors separately or MKOS), rtTA, and PB Transposase, as well as shorter versions of MKOS and Transposase, both with CAG promoter. Also small molecules (e.g. Sodium Butyrate) are used to enhance the reprogramming efficacy.

Conclusions:
rtTA-puro Myoblasts and HFF lines were obtained by electroporation or using FuGENE, indicating that plasmids of ≈ 10 Kb can be transfected. No iPS were produced independent of the method used, of the total amount of plasmid, and of the PB-MKOS:Transposase ratio. A highly efficient transfection system is essential for iPS production from neonatal and adult cells using piggyBacs.

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Poster ID – 63

AAV vectors for iPS induction

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Introduction:
Differentiated cells can be reprogrammed into pluripotent state by forced expression of defined transcription factors. Conventional methods rely on retroviral or lentiviral gene delivery for efficient induction of pluripotency. The downside of these vectors is the random integration of transgenes into the host genome which could affect the cells later on. To overcome this issue transient episomal expression systems have been developed but so far most of them have proven to be inefficient. Adeno associated virus (AAV) based vectors can be used to achieve transient episomal expression of transgenes and subsequent loss of episome during cell divisions. AAVs can be produced in different serotypes with different cell specificities. The aim was to study the feasibility of using AAV based vectors for iPS induction.

Methods:
Transcription factors OCT4, SOX2, KLF4 and c-myc were cloned into pSubCMV-WPRE or pSubCAG-WPRE. Viruses were produced in 293T cells using helper plasmid and purified from cell lysates by iodixanol gradient ultracentrifugation. Virus quality and quantity were determined by qPCR and transduction was verified by infecting 293T cells and analyzing transgenic protein expression. AAV serotypes 2 and 9 were tested by transducing human foreskin fibroblasts (HFF) with GFP-expressing control AAVs. For pluripotency induction tests HFFs were infected 1 to 4 times with the four factors. Cells were split onto either MEFs or Matrigel at day 3-5 after the first infection. After splitting culture media was changed to hES-media on MEFs or MEF conditioned media on Matrigel.

Results:
Based on GFP expression AAV serotype 2 was found to infect fibroblasts efficiently. RT-PCR of infected 293T cells showed viral mRNA expression and immunocytochemical staining of infected cells detected proteins of all factors. Western blot showed correct sized proteins for OCT4, SOX2 and KLF4. AAV mediated transduction of fibroblasts with reprogramming factors using single or multiple rounds of infection resulted in formation of colonies morphologically closely resembling hESC colonies, albeit at low frequency. However, until now, these colonies have not maintained stable undifferentiated morphology upon further passaging, prohibiting their detailed analysis. RT-PCR analysis of propagated colonies showed retained expression of all or a subset of transgenic factors.

Conclusions:
AAV vectors have so far not proven to be efficient in inducing pluripotency in fibroblasts.

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Poster ID – 64

Vitrification of human embryonic stem cell with a closed embryo straw and thin layer of cryoprotectant.

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Ultra rapid cooling of cells can be achieved by direct contact of liquid nitrogen (LN2) and cryoprotectant to avoid ice formation. Cryoprotectants such as propylene glycol (PROH), ethylene glycol, glycerol and dimethylsulphoxide (DMSO) are small molecules which penetrate through cell membranes (permeating) to form hydrogen bonds with intercellular water thereby preventing ice crystallization during vitrification. Direct contact between LN2 and cryoprotectant has a cooling rate of 24,000°C/min while closed unmodified 0.25mm embryo straw with a column of cryoprotectant have a cooling rate of 2000°C/min. Here we have successfully vitrified hESC single colonies as 4-6 cell clumps at lower passage in a thin layer of cryoprotectant inside unmodified closed 0.25mm embryo straw. The hESC colonies were immersed for 1 minute in Vitrification solution 1 (containing 10% DMSO, 10% ethylene glycol and 20% FCS in DMEM) and briefly in Vitrification Solution 2 (containing 20% DMSO, 20% ethylene glycol, 0.5M Sucrose in DMEM and 20% FCS) before loaded into embryo straws using Cook-IVF-Flexipet denuding pipette (170 um) releasing only small amount (3-5ul) of cryoprotectant with cell clumps to form a thin layer inside the embryo straw. The straw was sealed at both ends and later plunged directly into LN2. Thawing was performed at room temperature in air for 30 seconds. The plugs at both ends were removed and the straw attached to 1 ml syringe. ES medium was aspirated into the straw until the hESC clumps detached from the inner wall of the straw and gently flushed out and washed twice before transferred into culture dish. Very good survival and cell proliferation was obtained of the clumps (< 80%). This simple reliable method allows cell lines to be cryopreserved after minimal proliferation (as insurance) and can be adapted for storing potential iPS cell colonies for later analysis.

Poster ID – 65

Self-assembled polymeric 3D scaffold for mouse embryonic stem cell culture

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Embryonic stem (ES) cell research has emerged as a vibrant area in biomedical breakthrough. This is evidently because ES cells have the ability to proliferate indefinitely in an undifferentiated state (self-renewal) and the potential to differentiate into various cell types depending on specific signals (pluripotency).
Conventionally, ES cells have been typically cultured in 2D systems, which differ drastically from the 3D environment of a whole organism and consequently, cells isolated from higher organisms frequently alter their metabolism, morphology and gene expression profile. In addition, the existing 3D culture systems formed from animal-derived biomaterials pose problems for replacement therapies and contain residual growth factors and undefined constituents.
The present work brings us a new artificial 3D nanofiber scaffold formed from self-assembly of amphiphilic biodegradable peptide-copolymers that was designed for ES cell culture. This newly developed synthetic system allows elimination of animal-derived products and provides a complex network of nanofibers in a scale similar to the native extracellular matrices.
Undifferentiated mouse embryonic stem (mES) cells were cultured using the 3D nanofiber scaffold in order to evaluate the potential of the scaffold for applications in ES cell research. It was assessed the morphology, proliferation, survival rate, self-renewal and pluripotency of mES cells. In this preliminary study, the results show that the 3D nanofiber scaffold promoted mES cells growth and seem to retain their undifferentiated state. Testing pluripotency and self-renewal of mES cells cultured with the 3D nanofiber scaffold and others already available culture systems will allow us to evaluate the potential of the new 3D scaffold for future applications in ES cell research and regenerative therapies.

Keywords: 3D scaffold, embryonic stem cells, pluripotency, differentiation.

Poster ID – 66

Efficient adherent neural differentiation from mouse epiblast-derived stem cells (EpiSCs)

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Neural cells derived from pluripotent stem cell lines like embryonic stem (ES) cells or epiblast-derived stem cells (EpiSC) could be an ideal source for cell-based disease modelling, drug screening and cell replacement therapies. Pluripotent cell lines are an attractive cell source because they can be grown indefinitely as pure populations without the need for feeder cells using chemically defined media. Furthermore, they are amenable to gene knock-out, knock-down and gain-of-function strategies which facilitates the analysis of candidate genes. Here we describe an adherent, feeder-free neural differentiation protocol from various mouse EpiSC lines using chemically defined media. We can show that mouse EpiSCs differentiate in our conditions more rapidly and homogenously than mouse ES cells. Already after three days of differentiation pluripotency markers have been down-regulated and we can identify neuroectoderm precursors that are dependent on the cell-cell contact signal Notch. Neurons and astrocytes/oligodendrocytes can be identified from day 5 and 21, respectively. At the moment we are identifying the regional identity of the neural precursors and neurons, and evaluate the effects of Retinoic Acid (RA), Wnt and Sonic Hedgehog (Shh) signalling on the anterior-posterior and dorsal-ventral patterning of these cells. Our findings might help determine the optimal differentiation cues for human ES cells which self-renewal conditions are similar to mouse EpiSCs.

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Poster ID – 67

Viable mice generated from gene corrected disease-specific induced pluripotent stem cells

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Direct reprogramming of somatic cells into induced pluripotent stem (iPS) cells by retrovirus-mediated expression of pluripotency-associated genes in mice and humans is a promising approach for the derivation of disease-specific iPS cells. In the clinical setting, patient-specific iPS cells must undergo repair prior to autologous cell transplantation, if the underlying condition was caused by a genetic disorder.
In this study, we focused on a mouse model of tyrosinemia type 1 (fumarylacetoacetate hydrolase deficiency; $FAH^{-/-}$ mice) as a paradigm for a genetic metabolic liver disorder that can be treated by an iPS cell-based appraoch, and analyzed the phenotype of disease specific- and gene-corrected iPS cells in vitro and in vivo. To this end, fetal fibroblasts (13.5 dpc.) were used for retroviral expression of Oct4, Sox2, Klf4, and c-Myc. Embryonic stem cell-like colonies emerging 3 weeks after transduction were subcloned based on morphological selection and maintained as individual iPS cell lines. RT-PCR and immunofluorescence analyses showed expression of pluripotency markers (Oct4, Nanog, and Sox2) in iPS cells with levels similar to those of wild-type embryonic stem (ES) cells, and teratoma formation demonstrated the pluripotency of the iPS cell lines. To provide proof for the full pluripotency of the generated $FAH^{-/-}$-iPS cells, we performed tetraploid embryo aggregation experiments and were able to obtain viable mice, from $FAH^{-/-}$-iPS cells, which mimic the diseased phenotype of "normal" $FAH^{-/-}$-mice. In another experiment, we rescued the diseased phenotype by lentiviral transduction of an FAH transgene, which resulted in constitutive expression of FAH in the gene-corrected cells (FAH^{gc}-iPSC). We then applied in-vitro differentiation protocols and embryo aggregation experiments to these FAH^{gc}-iPS cells and analyzed their capability to form functional hepatic cells. Interestingly, we obtained healthy mice from FAH^{gc}-iPS cells, which show FAH expression in the liver and did not exhibit any pathological abnormality.
In conclusion, we demonstrate that metabolic liver disease-specific iPS cells can be gene-corrected without loss of the pluripotent phenotype, and we provide strong evidence that these gene-corrected iPS cells do not acquire functional restrictions. Hence, derivatives of gene-corrected iPS cells may be obtained and applied towards cell-based therapeutic applications.

Poster ID – 69

Engineering cell-permeant FLP recombinase for tightly controlled inducible and reversible overexpression in human embryonic stem cells

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Combined application of DNA recombinases Cre and FLP enables tightly controlled independent and/or sequential gene regulations. However, in practice such dual recombinase strategies are hampered by the comparably low efficiency of the FLP recombinase. Here we present the engineering of a recombinant cell-permeant FLP protein (TAT-FLP) that induces recombination in more than 75 % of fibroblasts and mouse as well as human embryonic stem cells (hESCs). We show that TAT-FLP ideally complements the strength of cell-permeant Cre recombinase for genetic engineering as exemplified by FLP-ON-Cre-OFF, an inducible transgene expression cassette that enables tightly controlled expression in a reversible manner. We exemplify this concept by conditional overexpression of LacZ and the caudal-related homeobox transcription factor CDX2. We expect our FLP transduction system to become widely useful for numerous genetic interventions addressing complex biological questions and the generation of transgene-free therapeutically applicable ES cell-derived cells.

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Poster ID – 70

Osteogenic Differentiation of Human Dental Pulp Stem Cells in 3D Scaffolds

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The aim of this study was to characterize the in vitro osteogenic differentiation of dental pulp stem cells (DPSCs) in 2D and 3D cultures. DPSCs were separated from dental pulp by enzymatic digestion and then isolated by magnetical cell sorting using antibodies against c-Kit, CD34 and STRO-1 surface antigens. After sorting, in a first phase, cells were differentiated toward osteogenic lineage on 2D surface of culture flask by using an osteogenic medium. Differentiated cells express specific bone proteins like Runx-2, Osx, OPN and OCN with a sequential expression analogous to those occurring during osteoblast differentiation and produce extracellular calcium deposits. In a second phase DPSCs were cultured in MatrigelTM and Collagen 3D scaffolds in order to differentiate cells in a 3D space that mimes the physiological environment. Cells cultured on these scaffolds show an improved osteogenic differentiation and produce a mineralized extracellular matrix. In MatrigelTM we observed cells differentiated with osteoblast/osteocyte characteristics and calcified nodules containing cells connected by gap junction constituting a 3D intercellular network. On the other hand DPSCs differentiated in collagen sponge actively secrete human type I collagen micro-fibrils and form calcified fibres assembling in trabecular-like structures. These neo-formed DPSCs-scaffold devices may be used in regenerative surgical applications in order to resolve pathologies and traumas characterized by critical size bone defects.

Poster ID – 71

Modulating SOX2 level in human ES cells by Cre-mediated overexpression and knock-down

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The high mobility group transcription factor SOX2 is involved in induction and maintenance of pluripotency as well as in neural determination. While its role has been extensively studied in mouse embryonic stem cells (ESC) there are only few data from human ESCs. We established inducible gain- and loss-of-function models to assess the role of SOX2 in pluripotent human ESCs and neural progeny derived thereof. We used the Cre-loxP-regulated RNAi vector pSico system, which permits conditional activation of short hairpin RNA (shRNA) transcripts (Ventura et al. 2004). In this system, Cre-mediated recombination induces the transcription of shRNA directed against the target transcript. Sox2-specific shRNAs were identified using the psico-oligomaker software. Employing these new sequences, along with a published sequence, pSico lentiviruses also carrying GFP were produced and used for infection of both, mouse and human, ESCs. FACSsorting of GFP-positive cells enabled enrichment of the transduced cells to a purity of ≥95%. Application of cell-permeant Cre protein resulted in high recombination rates as monitored by the loss of GFP fluorescence. Compared to control cells, Cre-treated cultures harboring the Cre-inducible shSOX2 construct showed a higher percentage of differentiated cells. In the Cre-inducible gain-of-function study, SOX2 overexpressing human and mouse ESCs maintained a pluripotent phenotype and gave rise to more colonies in a low-density colony forming assay as compared to non-induced wildtype cells. Under differentiation conditions Cre-induced SOX2 overexpression had a strong neural induction effect as monitored by neural rosette formation and by the marker expression of PAX6. Thus, Cre-induced overexpression and knock-down of SOX2 provides a powerful means to investigate the role of SOX2 in hESCs.

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Poster ID – 72

Differentiation of human embryonic stem cells into smooth muscle like cells: effect of original cell population, inductive signals and 3D environment

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Vascular smooth muscle cells (VSMCs) are specialized cells able to contract. Some studies have reported the therapeutic effect of these cells in the neovascularization of ischemic tissues, in the improvement of the contractile properties of the heart after infarction, and in the preparation of tissue-engineered blood vessels (1, 2). Unfortunately, one major limitation in the use of VSMCs in regenerative medicine has been finding a reliable source of VSMCs. Human embryonic stem cells represent a potential unlimited source of VSMCs. Recently, we reported the isolation of vascular progenitor cells (VPCs) that had the ability to differentiate into endothelial and smooth muscle cells (3). We further demonstrated that VSMCs transplanted subcutaneously in an animal model were able to contribute for the formation of functional blood microvessels. Despite these advances, it is poorly understood the (i) mechanism and bioactive molecules involved in the differentiation process, (ii) whether VSMCs can be obtained from other sources than VPCs, (iii) the contraction mechanism, and (iv) the response of the cells when encapsulated in 3D scaffolds.
In this work we examined the ability of three hESC populations (CD34+, CD34+KDR-, CD34-) isolated from EBs at day 10, and cultured as single cells in basal media supplemented with inductive to differentiate into SMCs. We report that from all inductive signals tested in this study, PDGFBB is the most effective in guiding the differentiation of hESC-derived CD34+ cells into SMLCs, based in gene and protein analysis, contractility response to depolarization agents and vasoactive peptides, secretion of cytokines and 3-D culture behavior. The contractility reponse in hESC-derived SMLCs is mediated by Ca2+, and involves the activation of Rho A/Rho kinase- and Ca2+/calmodulin (CaM) /myosin light chain kinase (MLCK)-dependent pathways. We show that these hESC-derived SMLCs can be encapsulated in fibrin gel scaffolds while exhibiting similar gene expression and functional properties as encapsulated differentiated VSMCs. Collectively, our data indicate that hESC-derived SMLCs might be an alternative source of SMCs for tissue engineering and regenerative medicine.

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Poster ID – 73

Scaffold-driven stem cell regenerative therapy for the spinal cord injury.Biomimeting neurogenesis in the CNS

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One of the most promising strategies for the treatment of spinal cord injuries is the transplantation of neural stem cells (NSCs). The combination of NSCs with biodegradable polymeric matrices has several advantages over transplantation of simple cell suspensions. A 3D scaffold can serve as a vehicle for the cells, while contributing to retain the transplanted cells in the site of the lesion and to sequester neurite growth promoting factors providing a favorable environment for NSC survival, proliferation and differentiation. In this context, we have developed a chitosan porous hollowed scaffold to be used for NSC transplantation, and subsequently seeded it with endothelial cells (ECs). Besides contributing to angiogenesis in vivo, ECs are expected to contribute to NSC survival and neuronal differentiation, by mimicking the NSC niches found in the adult brain where NSCs are closely opposed to the laminin-rich ECM surrounding ECs within the so-called vascular niche.

Here we report the behavior of NSCs in fibrin (Fb), and the optimization of the 3D culture conditions. NSCs (NS-5 cell line derived from the 46 C ES cell line) were suspended in a Fb gel in the form of neurospheres, and cultured in RHB-A/Neurobasal media for 14 days to induce neuronal differentiation. During the first 4 days of culture, bFGF was added, to promote NSC survival. Cell morphology was followed by microscopy while cell viability and phenotype were assessed using Calcein AM/PI dual staining and immunofluorescence against Nestin (NSC marker), bIII tubulin (early neuronal), GFAP (astrocytes), and O4 (oligodendrocytes), respectively.

Results showed abundant cell sprouting from the neurospheres during the incubation period, with retention of cell viability. Few PI+ cells were found, mostly in the centre of the neurospheres. At day 14 of culture, besides Nestin+ cells a large number of cells expressing bIII Tubulin were observed (with a neuron like morphology), which suggests that NSCs in Fb retain the ability to differentiate into neurons. Throughout the cell culture, immunoreactivity against GFAP and O4 was not observed.
The NSC behavior in Fb gels in the presence of the endothelized scaffolds or their conditioned media will be subsequently assessed. These studies will be key to give an insight into the EC-NSC cross-talk in a 3D environment, as well as into the outcome of co-transplantation of ECs with NSCs.

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Poster ID – 74

Multiple mechanisms underly the role of Rex-1 in preimplantation development and pluripotency in mouse ES cells

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Expression of the Zinc Finger protein Rex-1 (Zfp42) is restricted to preimplantation embryos, and pluripotent (stem) cells both in vivo and in culture. Despite the coincidence of its expression with pluripotency, Rex-1 appears dispensable for selfrenewal of embryonic stem cells in culture (ESC). We speculate that it may be necessary for the establishment of pluripotency, and as a consequence for derivation of ESC or iPS (induced pluripotent cells). We have generated a high affinity serum to investigate expression during preimplantation mouse development, and association to target genes. We found that Rex-1 protein is present at high levels in unfertilized oocytes. Levels of Rex-1 protein diminished gradually during development, and became undetectable in late blastocysts cultured in vitro. Throughout preimplantation development, Rex-1 was dynamically localized between the cytoplasm, in perinuclear conformation and in the nucleus. At various stages Rex-1 co-localized with peri-centromeric heterochromatin and HP1 proteins, thought to contribute to the assembly of chromatin.

We have demonstrated expression of Rex-1 in both trophectoderm and ICM of the blastocyst. Similarly, we show expression of Rex-1 both in ESC and in TS. Rex-1 localized to chromatin in ESC and interacted with epigenetic regulators both in yeast dihybrid assays and in tissue culture cells. To understand the contribution of Rex-1 to the generation and maintenance of pluripotent cells, we identified Rex-1 target genes in ESC. We found association of Rex-1 to several genes identified previously as either co-expressed in Rex-1 expressing subpopulations of ES cells or induced in subpopulations devoid of Rex-1. We hypothesized that Rex-1 may contribute to PcG function, considering its homology to YY1, a gene identified genetically as Polycomb in D. Melanogaster. Indeed, we found association of Rex-1 to several genes described as regulators of differentiation and repressed by PcG in mouse and human ESC. We conclude that Rex1 might contribute to the generation of selfrenewing pluripotent cells via its contribution to Polycomb-mediated gene regulation.

Rex-1 colocalization with and interaction with proteins that contribute to the assembly and regulation of chromatin, and the high expression of Rex-1 during early development while pluripotency is maintained, are compatible with a role for Rex-1 in organizing the chromatin structure favorable for pluripotency.

Poster ID – 75

Development of tools for genetic engineering of hES cells.

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Efficient methods for genetic modification are required to exploit fully the potential of human embryonic stem (hES) cells as a tool for basic research and clinical investigations. Here we report our results of the efficient transgenesis (including BACs) and targeting in hES cells.
Previously, we described the generation of H7s6 hESC reporter clones for the main pluripotency genes (hOCT4-mCherry, hOCT4-GFP, Nanog-GFP and Nanog-mCherry) by random insertion of large DNA plasmid constructs sized around 20kb. In all cases, transgene silencing was observed upon subsequent passaging of the cells.
To avoid the silencing effect we attempted to transfer BACs that contain the GFP reporters for hOCT4 and Nanog genes using nucleofection. Nevertheless, we observed silencing of the transgene and rapid loss of GFP. We analyzed the copy number of the BAC in the resistant clones by qPCR on the genomic DNA. We found that only the part of the BACs containing the promoter driving the transgene expression was integrated in all of the analyzed clones. This shows that nucleofection leads to the breakage of the BAC and incomplete integration of the construct resulting in unstable reporter expression. We are currently developing alternative methods for BAC transfer to achieve the intact BAC integration.
An alternative method to the random gene insertion is a knock-in of the reporter cassette into the specific locus via homologous recombination. We have targeted GFP-IRES neo cassette into the C-terminal part of hOCT4 locus in H7s6 and H9 lines. We have obtained G418-resistant clones expressing fusion Oct4-GFP protein with nuclear localization. The efficiency of the targeting was 67-70% for H7s6 and 7-12% for H9 cells according to Southern blot analysis.
We demonstrated stable expression of the targeted reporter in the undifferentiated hES cells without signs of silencing during long-term culturing even without selection pressure. As expected, the expression of GFP was completely downregulated upon differentiation of the cells showing the functionality of the targeted hOct4 reporter. We performed chromatin immunoprecipitation (ChIP) using GFP antibody and showed more than 20-fold enrichment of the Oct4-binding sequences.
Altogether, the transgene silencing is a significant problem for the stable transfection of hES cells and can interfere with the efforts to produce the genetically modified lines. Gene targeting can serve as a solution although it is often difficult to achieve.

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Poster ID – 76

Improving expansion of pluripotent human embryonic stem cells in perfused bioreactors

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Human embryonic stem cells (hESCs) with their ability for extensive proliferation and multi-lineage differentiation can serve as a renewable source of cellular material in regenerative medicine, drug screening and in vitro toxicology. However, the successful transfer of hESC technology and cellular products into clinical and industrial applications needs to address issues of automation, standardization and generation of relevant cell numbers of high quality. In this study, we demonstrated that 3D microcarrier technology and controlled stirred bioreactors in perfusion mode, where scalability, straightforward operation and tight control of the culture environment are combined, can be used to improve the expansion of pluripotent hESCs.

Firstly, different microcarrier matrixes and inoculum concentrations were tested in spinner flasks. The best conditions to promote hESCs attachment and growth were selected by evaluating cell concentration, viability and microcarrier colonization during time.

We next developed a bioreactor protocol by evaluating different operating conditions. Bioreactor cultures were monitored in terms of cell concentration, viability and metabolic performance (specific rates of glucose and oxygen consumption and lactate production were determined), undifferentiated phenotype and pluripotency. The results obtained showed a significant improvement in hESC metabolism, cell growth and viability without compromising their stem cell characteristics.

In conclusion, our findings show that tight controlled conditions assured by bioreactors allow the production of high quality hESCs. When compared to the standard 2D colony culture, our 3D strategy improves by 12-fold the final yield of pluripotent hESCs, providing a potential bioprocess to be translated to clinical and industrial applications.

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Poster ID – 77

Using developmental biology for studying stem cell differentiation and tissue engineering of kidney structures

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Embryonic stem cells are cells isolated from the inner cell mass of an embryo and have the potential to differentiate to any tissue of the body. In the past decade they have attracted a lot of interest from the scientific community as they have demonstrated a great potential for cell-based regenerative therapies. Strategies have successfully been devised for the differentiation of ESCs to many lineages, for example, neural, pancreatic, hepatic, hematopoietic cells as well as cardiomyocytes and the first clinical trials, which involve embryonic stem cells, have been approved. Even though some methods for differentiation have proven quite successful there have been very few studies, which have reported the differentiation of ESCs to a renal lineage (reference).

We used a disaggregation-reaggregation method to place ESCs in the environment of a developing kidney and asses their potential to differentiate into kidney cells. As our results suggested that the influence of the niche is not sufficient for successful ESC differentiation, we speculated that identifying the earliest tissue with a nephrogenic capacity will pinpoint the earliest stage that embryonic stem cells must be taken to in order to become competent to contribute to kidney structures. We have therefore tried to determine at what developmental stage is the earliest ancestral to the kidney tissue found in the mouse embryo by using the same method to assess the competency of tissue from different developmental stages to integrate in the structure of forming nephrons and collecting duct precursors.

In embryonic development, the cell of the inner cell mass progress first to mesoderm and then to intermediate mesoderm to independently give rise to the two structures, which initiate metanephric kidney development – the ureteric bud (or UB - gives rise to the collecting system) and the metanephrogenic mesenchyme (or MM - gives rise to the nephrons). Reports in the literature have identified the MM as the first tissue restricted to form nephrons. Our results challenge the existing model and suggest that earlier lineages are competent to give rise to kidney structures. This finding also suggests that fewer steps might be needed for the successful differentiation of ESC to renal cells.

Poster ID – 78

Gene targeting efficiencies in human ES cells using BAC-derived vectors

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We report here the results of a comprehensive series of gene targeting experiments conducted in the human ES cell line SHEF 4. Vectors designed for promoterless targeting selection and positive-negative targeting selection were constructed by recombineering utilising existing BAC genomic resources to provide homology arm sequences. The set of target loci comprised both expressed and non-expressed genes in human ES cells. Processing of drug resistant hES cell clones on a 96 well plate format allowed large numbers (>300) to be analysed from each experiment for definitive identification of targeted events. The accumulated data shows that gene targeting efficiency using BAC-derived vectors is highly variable between loci.

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Poster ID – 79

Derivation and characterization of rabbit embryonic stem cell lines.

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Rabbit embryos differ in many respects from rodent embryos, not least the epiblast, which forms a disc at the yolk sac surface - as opposed to an egg-cylinder in the rodents - and gastrulation starting before implantation. Owing to the availability of embryos in large numbers, rabbit is a particularly attractive species to explore the capacity of epiblast to produce chimaera-competent pluripotent stem cells in non murine species.
Thirty embryos were dissected at embryonic day 6.5 and the epiblasts plated, either onto murine embryonic fibroblasts (MEF), or onto fibronectin-coated dishes in medium supplemented with foetal calf serum, FGF2 and activin. Although most epiblast explants initially formed outgrowths, most cells became differentiated after dissociation and replating. By contrast, when inner cell masses (ICMs) were isolated from rabbit blastocysts by immunosurgery, and plated onto MEF in medium supplemented with FGF2 and activin, 50% were able to form secondary outgrowths and 16% produced a population of highly proliferating cells that could be regularly passaged. Like mouse and primate ES cells, they express the pluripotency markers Oct4, Nanog, Klf4, TRA-1-60, and TRA-1-81. They also express both SSEA-1 cell surface antigen characteristic of mouse ES cells, and SSEA-4 antigen characteristic of primate ES cells. Upon infection with EOS - a lentiviral vector expressing the Green Fluorescent Protein (GFP) under the control of the distal enhancer of the mouse Pou5f1 (Oct4) gene - only mouse ES cells showed extensive fluorescence (EOS+), whereas rabbit and primate ES cells did not (EOS-). To eliminate the possibility that the Pou5f1 distal enhancer is not active in the rabbit, early cleavage stage rabbit embryos were infected with EOS, and subsequently cultured until the blastocyst stage. Confocal microscopy analysis revealed the presence of fluorescent cells within the ICM. Furthermore, after ICM isolation, infection with EOS, and subsequent plating, GFP-positive cells were visible in the resulting outgrowths, but fluorescence disappeared after 48 hours. Therefore, we conclude that the Pou5f1 distal enhancer is active in rabbit embryonic stem cells in vivo, but its activity is rapidly lost upon in vitro culture. Taken together, these results indicate that rabbit ES cells display both mouse (SSEA-1+) and primate (SSEA-4+, EOS-) characteristics. The capacity of rabbit ES cells to colonize the preimplantation embryo is currently being investigated.

Poster ID – 80

Support of undifferentiated Human Embryonic Stem cell growth by mouse and human feeder cells.

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Human embryonic stem cells (HESC) have huge potential for the treatment of patients. Much research has been conducted to develop robust protocols for the differentiation of HESC towards various lineages, representative of the three germ layers. At the UK Stem Cell Bank (UKSCB) cell lines are expanded to produce banks of undifferentiated HESC to be used for research, as seed stock for basic cell biology and differentiation studies. To prevent phenotypic and genotypic changes to the cells, it is important that the banking of HESC cells be carried out with minimal expansion and manipulation of the cell lines.

The UKSCB would like to standardise the way that undifferentiated cell lines are expanded as the Bank moves towards the scale-out and scale-up of these lines to meet the demand of the Stem cell community. In general, the HESC at the UKSCB are expanded on inactivated mouse feeders or human feeders and passaged either using manual dissection or using enzymatic treatment to dissociate the cells. Manual dissection of HESC is both labour intensive and time consuming. Although enzymatic treatment using Trypsin has been used for extensively for the expansion of stem cells it is thought to have caused genetic abnormalitites in cell lines through prolonged use. On the other hand TrypLE express (Gibco) a recombinant stable enzyme with trypsin-like properties has been shown to dissociate cells in with a more gentle action than trypsin.

The UKSCB is currently running a programme of work to look at the optimisation and standardisation of the cell banking process .This is being achieved by comparing the growth of four different HESC on both human and mouse feeders over 20 passages, using TrypLE express. During the 20 passages, the HESC will be examined for morphological changes using microscopy and changes in the expression of cell surface markers using flow cytometry and quantitative microscopy. Comparison of gene expression markers will be examined using the human pluripotency panel low density array cards (Applied Biosystems) and low resolution aCGH (Perkin Elmer) will be used to monitor any karyological changes.

Preliminary results from this study will be presented on the poster.

Poster ID – 81

Immunoregulatory function of mesenchymal stem cells in EAE depends on differentiation state and secretion of PGE2

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Stem cells (SC) have been shown to modulate immune response by influencing immune cell function. The mechanisms of SC-induced immunoregulation are largely unknown but are likely to be mediated by soluble factors. We have found that the immunoregulatory effect of bone marrow mesenchymal SC (BMSC) in experimental autoimmune encephalomyelitis (EAE) depends on secretion of prostaglandin E2 (PGE2). BMSC-induced amelioration of EAE was diminished with neuronal differentiantion of BMSC (nBMSC) in that nBMSC showed reduced efficacy to inhibit EAE compared to freshly isolated BMSC (fBMSC) enriched for the pluripotent marker, Sca1. Transwell system experiments showed that the secreted soluble fraction of fBMSC mediated inhibition of autoreactive cell proliferation in response to PLP139-151. fBMSC secreted large amounts of PGE2 compared to nBMSC which displayed a significantly lower efficacy to inhibit EAE. The PGE2 inhibitor, meloxicam, abolished secretion of PGE2 by fBMSC and given to EAE mice transferred with fBMSC, it reduced serum levels of PGE2 and decreased the inhibitory effect of fBMSC. High level PGE2 secretion by fBMSC correlated with high expression of indoleamine-2,3-dioxygenase (IDO) and meloxicam accordingly blocked IDO expression in fBMSC transferred mice. The current findings indicate PGE2 involvement in BMSC-induced inhibition of EAE and provides a mechanistic link between BMSC-derived PGE2 and IDO-dependent immunoregulation of this autoimmune condition.

Poster ID – 82

The Trans-differentiation Of Pancreatic Progenitor Cell Into Hepatocytes Is Mediated By A Suppression of WNT signalling

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The AR42J-B-13 (B-13) pancreatic progenitor cell trans-differentiates into functional hepatocytes in response to glucocorticoid. Since nuclear receptors are able to directly interact with elements of the WNT signalling pathway that regulates tissue differentiation and development, the role of the WNT signalling pathway - specifically the activity of the proximal WNT regulated Tcf/Lef transcription factors - in the B-13 response to glucocorticoid was examined.
B-13 cells and cells treated with 10nM dexamethasone for 14 days to promote hepatic phenotype (B-13/H) were transfected with the topflash Tcf/Lef luciferase reporter construct (or foplash, an identical vector minus the Tcf/Lef binding response element) with a renilla construct for normalisation. 24 hours after transfection, cells were treated with known modulators of WNT signalling activity - quercetin (Q) or 2-amino-4-(3,4-(methylenedioxy)benzylamino)-6-(3-methoxyphenyl)pyrimidine (WA) or DMSO vehicle. After a further 24 hours, cells were harvested and luciferase and renilla expression determined using a Dual-Luc kit and luminometer.
WNT signalling activity was high in B-13 cells on the basis of high Tcf/Lef transcriptional activity, and activity was increased by WA (a Tcf/Lef activator) and inhibited by quercetin (a Tcf/Lef inhibitor). WNT signalling activity was suppressed in B-13/H cells relative to B-13 cells.
To test the regulatory role of the WNT signalling pathway in trans-differentiation, B-13 cells were transfected with a β-catenin siRNA which specifically suppressed β-catenin protein levels. β-catenin suppression alone induced C/EBP-β, induced liver-specific gene expression and promoted hepatocyte phenotype. Over-expression of a non-phosphorylatable β-catenin protein inhibited glucocorticoid-dependent B-13 trans-differentiation. Inhibition of Tcf/Lef transcriptional activity with Q did not drive trans-differentiation although its addition with glucocorticoid further promoted trans-differentiation, suggesting β-catenin acts via both Tcf/Lef-dependent and independent mechanisms. Treatment with WA blocked glucocorticoid-dependent trans-differentiation, indicating a dominant negative role for Tcf/Lef activity and hepatic differentiation.
These data therefore demonstrate that the WNT signalling pathway plays a critical role in B-13 cell plasticity and that suppression is a critical mechanism controlling trans-differentiation into hepatocyte-like cells.

Poster ID – 83

VPU102, a novel animal-free matrix supports undifferentiated growth of hES and hiPS cells

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Culturing of human embryonic stem cell (hESC) and induced pluripotent cells (hiPSC) requires an extracellular matrix coating. Matrigel, a mixture from murine matrix is the current golden standard but is not acceptable for potential clinical applications. We here report culturing of hESCs and hiPS on a novel matrix molecule VPU102.

We have demonstrated using 4 hESC cell lines and 4 local hiPSC lines that VPU102 is compatible for culturing these cells. Undifferentiation and pluripotency were demonstrated by the expression of relevant surface and mRNA markers as well as by using an in vitro embryoid body assay and in vivo teratoma assay. The cellular response for VPU102 and Matrigel was compared by studying the mRNA expression profiles of about 90 genes related to differentiation.

Based on the results the VPU102 system enables fully-defined, animal-free culture system for human pluripotent stem cells.

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Poster ID – 84

SNP genotype effects on gene and transcript expression variation in human embryonic stem cells

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Motivation: Several cis-variants in genome are known to affect both gene expression levels and transcript isoforms. Associations have been studied, inter alia, in lymphoblastoid cells and human osteoblasts [1-2]. However, genome wide studies on cis-variations of human embryonic stem cells have not been previously reported and thus we performed such an analysis with nine different human stem cell samples [3].

Methods: We carried out a cis-association study of SNPs and gene, transcript and exon expression values. Both variation and expression values were measured with microarrays and correlation was determined with regression analysis where genotype variation is used as an explanatory variable to the observed expression values.

Results: Almost 100 000 SNPs included in the study were found in the gene or promoter regions of the genes studied. Less than half percent of these SNPs clearly correlated with gene, transcript or exon expression. Such SNPs that influenced gene or transcript expression were usually located within the sequences whose expression they afftected. On the contrary, if the expression of an exon was affected, the SNP was located within the introns or the other exons of the gene.

Conclusion: We have performed a genome-wide study of variation effects on gene and transcript expression. We identified a set of SNPs that have a potential to cause gene expression and alternative splicing variations between different human embryonic stem cell lines. An interesting future direction of our research is to study how our findings correlate with the diversity of stem cell lines such as the variation in their differentiation potential.

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Poster ID – 85

Novel regulators of stem cell fates identified by a high content screen of small compounds on human embryonic stem cell colonies

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Human embryonic stem (hES) cell fate decision is governed by complex mechanisms. In order to probe these mechanisms we undertook a cell-based phenotype screen of the effect of small compounds. We have established a high-content assay using the IN Cell Analyzer 1000 automated microscopy-based imaging system. Our assay was designed to detect changes in the phenotype of hESC colonies upon treatment with specific compounds by quantifying multiple parameters, including the number of cells in a colony, colony area and shape, intensity of Hoechst 33342 nuclear staining, the percentage of cells in the colony that express a marker of pluripotency (TRA-1-60), as well as the number of colonies per well. A screen of 1040 pharmacologically diverse compounds uncovered novel hits that affected hES cell survival and pluripotency. Some of the hits identified were further validated and studied for their effects on hES cells providing insight into both known and novel pathways of hES cell self-renewal and differentiation. Our results show that high-content screening can be applied to investigating signalling networks in hES cells and the compounds identified in our screen provide mechanistic tools for exploring hES cell biology.

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Poster ID – 86

Toward understanding the role of FGF-2 in maintenance of pluripotency: importance of energy homeostasis

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FGF-2 is an essential component of media for maintenance of pluripotency of human embryonic stem cells in cultures. FGF-2 is also important for survival of cancer stem cells which share their features of pluripotency with hESC. It is however currently unclear how FGF-2 promotes stemness. Yet, it is known that pluripotent cells possess low number of under-developed mitochondria, and therefore must depend mostly on glycolysis to receive their energy. We hypothesize that FGF-2 fulfills its role in maintenance of stemness by regulating essential metabolic pathways, most probably by promoting glycolysis.

We aimed at elucidating the FGF-2 driven molecular mechanisms of maintenance of pluripotency by using system biology approach. For that we designed a strategy with a combination of i) metabolic profiling in pluripotent cells by metabolic flux analysis; ii) elucidation of recruitment of FGF-2 and components of FGF-2 protein complexes to chromatin by ChIP-Seq; and iii) characterization of FGF-2 regulated transcription by digital gene expression analysis.

At an initial stage of our project we aimed at elaborating culture and lysis conditions for measurement of metabolic parameters, as well as at establishing a comprehensive tool kit for studies on metabolic pathways in hESC. We found that lysis conditions with 40 mM NaOH are the most appropriate for simultaneous measurements of a number of metabolic parameters in hESC. Our results show that intracellular ATP is substantially lower in pluripotent hESC in comparison to their differentiated counterparts. Our data also suggest that lactate is actively produced in hESC cultured on mouse embryonic fibroblast feeders. Moreover, the NAD+/NADH metabolic ratio is substantially higher in pluripotent hESC when compared to their differentiated counterparts. These data support the assumption that glycolysis is used to cover energy requirements of hESC in cultures.

We suppose that feeder cells, in addition to growth factors, supply hESC also with essential metabolites, as well as take up metabolic products released by hESC. The same type of relationship might also be valid for cancer stem cells which get metabolic support from surrounding differentiated cancer cells. These studies add to both understanding the development potential of stem cells and establishing xeno-free culturing conditions for medical grade hESC; as well as to understanding the mechanism of resistance of cancer stem cells against therapeutic interventions.

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Poster ID – 87

NANOG reporter cell lines by gene targeting in human embryonic stem cells

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Pluripotency of mammalian stem cells in vitro and during early embryogenesis is controlled by the pluripotent cell-specific transcription factor Nanog. Thereby, Nanog is both a determinant and an indicator for true pluripotency, although the regulation of Nanog and its role in pluripotency require further characterization. In this study, we created tools to visualize Nanog expression in living cells the regulation and mechanism of Nanog-induced pluripotency. To achieve this, we created fluorescent NANOG reporter cell lines by gene targeting in human embryonic stem cells (hESCs). In these reporter lines, expression of a fluorescent reporter gene closely reflected expression of endogenous NANOG and enabled the fractioning of hESCs based on NANOG expression levels.

Poster ID – 88

Cited2 controls somatic and embryonic stem cell fates

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KRK and JB contributed equally to this work

The common regulators necessary for the maintenance of somatic and embryonic stem (ES) cells remain poorly defined. We report a requirement for the p300/CBP-binding transcriptional co-activator Cited2 in adult haematopoietic stem cell (HSC) and ES cell maintenance. Conditional deletion of Cited2 in the adult mouse resulted in loss of HSCs causing multilineage bone marrow failure. Additional deletion of Ink4a/Arf (encoding p16Ink4a and p19Arf) or Trp53 (encoding p53, a downstream target of p19Arf) in Cited2-deficient mice rescued HSC activity indicating that Cited2 maintains adult HSCs, at least in part, via Ink4a/Arf and Trp53. Moreover, Cited2 depletion in both mouse and human ES cells resulted in loss of stem cell functions. Cited2 directly activated the expression of Nanog suggesting a novel Cited2-Nanog pathway in the maintenance of ES cell pluripotency. Taken together, our studies indicate that Cited2 is a conserved master regulator of stem cell fates and controls diverse genetic pathways to orchestrate somatic and embryonic stem cell functions.

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Poster ID – 89

Single-cell gene expression during BMP-induced germ cell differentiation

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Ten to fifteen percent of couples are infertile, and the most common causes are linked to a lower production of sperm and oocytes. However, due to inaccessibility of biological material from early developmental stages, our understanding of human germ cell development is poor. By stimulating human embryonic stem (hES) cells and human induced pluripotent stem (hiPS) cells with members of the transforming growth factor β family (i.e. Bone Morphogenic Protein 4, 7, 8b) we have successfully obtained primordial germ cells (PGCs). In order to study the mechanism in further details, we have now monitored the gene expression at different time points after stimulation using the Fluidigm (BioMark) platform that allows the simultaneous detection of 48 genes in 48 single-cells. We found interesting correlations between specific BMPs and an increased expression of differentiation marker genes like GATA6 and CDX2. At the same time, the single-cell gene expression data is queried with respect to the variability in gene expression. These results will be compared with gene expression data from mouse germ cell development to determine the extent to which BMPs induced hES cells start differentiate into the germ cell lineage and to look for common or species-specific gene expression. Understanding how to induce the differentiation of human embryonic stem (hES) cells into germ cells is important both for research and for rising societal health issues, for example infertility.

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Poster ID – 90

Characterization of the Differentiation and Proliferation Potential of Ectomesenchymal Stem Cells from spongious and cortical bone

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The increasing number of elderly people creates a strong need for tooth reconstruction. For successful and longtime implant anchorage a sufficient vertical and transversal bone level is necessary. Particulated, non-vascularized bone autografts are discussed for the reconstruction of the jaw bone for implant anchorage. Such bone particles are produced during the implant-bed preparation or are created by bone ablation with surgical round-drills and can be collected with a bone filter integrated into surgical suction pipe. However, contradictory statements have been made about this material until now. One problematic aspect is that bone tissue cannot be obtained in a sterile manner from the oral cavity. Nevertheless, this material is a natural bone substitute and is expected to contain living cells that might improve the implant anchorage. Additionally, this material is obtained as a side-product during surgery without any additional invasive procedures. In this study, bone chips from 85 donors were qualitatively and quantitatively investigated, in order to detect the properties of the contained cells. In particular their ability to proliferate out of the material has been determined. Here we show that spongious bone chips in comparison to cortical bone chips give rise to living cells more often. Most of the samples showed cell outgrowth between one and two weeks. These cells were characterised using RT-PCR and FACS to determine their lineage specific commitment. No significant difference in the plasticity of the isolated cells could be observed. The differentiation capacity of the obtained cells was independent of the quantity and quality of the present microorganisms, which were examined in detail. The microbial populations consisted mainly of Streptococceae and Staphylococceae. We were able to show that the bacterial contaminations could be reduced via perioperative antibiotic treatment. Taken together, bacterial contaminations have been found in all samples, but could be reduced. The quality of the obtained cells depends on the precise place of extraction but not on the quantity and quality of the present microorganisms, making this material a useful tool in regenerative dental medicine.

Poster ID – 91

Oxygen-regulated transcriptional networks controlling Human Embryonic stem cell pluripotency

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The pluripotent human embryonic stem cells (hESC) are isolated from the inner cell mass (ICM) of blastocyst stage embryos. These embryos receive metabolites and oxygen from the uterine fluid containing 2% - 5% of dissolved oxygen. Early embryo develops in low oxygen environment suggesting that hypoxic growth conditions would be physiological for hESC cultures. Indeed, hypoxic growth conditions (1% - 4% O2) reduce spontaneous differentiation and enhance hESC self-renewal indicating that the processes mediating spontaneous differentiation are suppressed under low oxygen. However, how hypoxia contributes to pluripotency and self-renewal is not completely understood. To study the mechanisms involved in oxygen-regulated pluripotency and self-renewal, three different hES cell lines (HS401, H9 and HS360) were plated on Matrigel and cultured in 4% (hypoxia) or in ambient oxygen concentration for fixed time intervals. Consistent with the hypothesis of hypoxia supporting pluripotency, the data from Western and Flow Cytometer analyses revealed that in response to low oxygen environment, hES cells activate canonical hypoxia responses and inhibit the downregulation of the pluripotency markers. To identify the hES cell specific transcriptional programs regulated by oxygen, RNA was extracted from all the time points for which microarray analysis, using the Affymetrix Human Exon 1.0 ST array platform. Data analysis revealed that hypoxic stress induced differential expression of over 200 genes, affecting various biological processes regulating hESC pluripotency and differentiation.

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Poster ID – 92

hESCs with decreased level of APE endonuclease show slower release of IR induced double strand breaks

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Human embryonic stem cells (hESCs) might play an important role in future cell therapy of human diseases. As a part of the future possible hESCs' application in clinical medicine, the cells must be produced in culture. Prolonged cultivation of hESCs, however, leads to adaptation to culture conditions, including changes, such as mutations and aneuploidy. Such changes lead to adaptations which give the cells the advantage of faster growth or higher cloning efficiency demonstrated by shorter passaging period. We have screened several genome maintenance mechanisms most often causing such changes. Changes were found neither in telomere maintenance, nor in nonhomologous end joining efficiency. We have nevertheless shown that prolonged passaging induced culture adaptation is accompanied by decreased expression of APE endonuclease. Such decrease might in turn suppress the efficiency of base excision repair (BER) machinery, one of the most utilized repair mechanisms in stem cells. Indeed we have shown decreased short patch BER efficiency in late passage hESCs. We have than decided to find evidence supporting the idea of increasing the genome instability, due to the decreased BER efficiency. We have established siRNA technology decreasing APE endonuclease expression in hESC to the level similar to other somatic cells (feeder cells). Oxidative DNA damage, prevalent damage after exposure to ionizing radiation, is converted to double strand breaks (DSBs) via base repair of clustered damage. Thus the efficiency of BER should be limiting factor in the conversion of base damage to DSBs. Indeed we have shown that the dynamics of release of DSBs in hESC treated with IR differs in cells with decreased expression of APE by siRNA technology. Single cell agarose electrophoresis under non denaturing conditions followed by comet analysis showed slower DSBs release after IR treatment in early passage hESC treated also with siRNA against APE endonuclease, demonstrating direct link leading from base damage to toxic double strand breaks with participation of BER enzymes. The mechanism of increased mutagenesis in cells with lower APE expression might thus lead through either slower repair dynamics enabling establishing the mutation or less error proof mechanisms to take over the repair or perhaps via rendering the cells more resistant to IR because of less DSBs are released after the treatment thus not triggering the apoptosis.

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Poster ID – 93

Different Stages of Pluripotency Determine Distinct Patterns of Proliferation, Metabolism and Lineage Commitment of Embryonic Stem Cells under Hypoxia

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We have studied the effect of low oxygen levels (2% O2), or hypoxia, in the expansion and neural commitment of mouse embryonic stem (ES) cells. In the presence of leukemia inhibitory factor (LIF), cell proliferation was reduced under hypoxia and a simultaneous reduction in cell viability was also observed. Specific growth rate was also reduced in hypoxia (0.43 ± 0.03 day-1), as compared to 20% O2 (0.84 ± 0.11 day-1). Slight morphological changes were observed suggesting some early differentiation for hypoxic conditions, even though cells retained high levels of pluripotency marker expression. However, when cells were maintained in a ground state of pluripotency, by inhibition of autocrine FGF4/ERK signaling, hypoxia did not affect cell proliferation, and did not induce early differentiation. This resulted in identical growth rate values for both conditions tested (0.82 ± 0.04 day-1 for 20% O2 and 0.79 ± 0.02 day-1 for 2% O2). In addition, over 95% of cells were positive for Oct-4 and Nanog, either at low or at atmospheric oxygen tensions, as quantified by flow cytometry.

During neural commitment, low oxygen tension resulted in a faster commitment of ground state ES cells towards neural progenitors. The use of a green fluorescent protein (GFP) knock-in reporter ES cell line (46C) allowed the examination of the process by which ES cells acquire neural identity since the open reading frame of the Sox1 gene was replaced with the coding sequence of GFP. This revealed that the maximum expression of Sox1-GFP was reached faster at 2% O2 than at 20% O2, and by day 4 cells maintained under low oxygen levels were already over 80% Sox1-positive (83.8 ± 3.1%) whereas under 20% O2 the percentage of Sox1-positive cells was only 65.6 ± 7.3%. In addition, by day 8, flow cytometry quantification of neuronal class III β-Tubulin (Tuj1) also revealed a slightly higher percentage of neuronal cells at low oxygen tension.

Overall our results demonstrate the need to specifically regulate the oxygen content, along with other culture conditions, when developing new strategies for ES cell expansion and/or controlled differentiation.

Poster ID – 94

Characteristic of Molecular Changes Coupled to Differentiation Pathways in Human Embryonic Stem Cells

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Human embryonic stem cells (hESCs) show specific cell cycle properties, such as short doubling time, abbreviated G1 phase, and stable expression of several cell cycle regulating molecules. Interestingly, all of these characteristics are rapidly changed with the onset of differentiation. Therefore it has been hypothesized, that cell cycle regulating molecules, which primarily function in regulation of cell cycle progression, can also participate in the process of differentiation as well as maintenance of particular differentiated phenotype.
We aimed to assess complex changes in configuration and activities of cell cycle regulators as well as cell cycle characteristics associated with molecular switch between self-renewing population of hESCs and proliferating neural progenitors/neural stem cells (NP/NSC) and neural crest precursor cells (NCPCs) derived from hESCs.
We show that particular cell cycle features are changed with the onset of differentiation while some of these changes might be specific only for the neural lineage commitment or even for particular NP/NSC or NCPC line. Interestingly, in addition to other changes, level of cyclin E was elevated prominently in differentiated NCPCs as well as NP/NSCs lines in comparison with hESCs. This effect might be specific for particular pathway of differentiation as such increase was, to our knowledge, observed in differentiation to neural lineage in Drosophila. We also show that stability of configuration of cell cycle regulating machinery in NP/NSCs line during longterm cultivation might be compromised as level of regulatory molecules (such as cyclins A and B) decreases during the prolonged cultivation of NP/NSCs.
Our data provide an insight into configuration and changes in molecular mechanisms that accompany process of differentiation of hESCs into specific cell type and point out the crucial role of molecules regulating cell cycle progression in differentiation.

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Poster ID – 95

Characterisation of CC-9, a novel antibody that defines the stem cell compartment.

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Introduction
Estools work package 1a.4.3 was undertaken to make a new generation of antibodies capable of defining the stem cell state. The derivation of these new antibodies was undertaken by the estools partner Axordia.
Antibody production
Antibodies were generated by immunising Balb/C mice with the human embryonic stem cell line Shef 1 which had been maintained in HES media on mouse feeders. Blood was taken from immunised mice and tested for reactivity against Shef-1 cell lysates. Immuno-reactive mice were then used to generate hybridoma cell lines by fusing splenocytes from the successfully immunised mouse with SP2/0 mouse myoloma cells. Eight new monoclonal antibodies were produced by this method and this study describes one of these eight antibodies; CC-9.
Antibody characterisation
CC-9 is a Kappa-chain IgM monoclonal antibody (determined by Isotype stripe from Roche). Western blot analysis of CC-9 reactivity along with enzymatic digestion demonstrated that the CC-9 antibody recognises a large sialated, non-ceramide antigen with a molecular weight of approximately 250kDa. Microscopic analysis shows that the CC-9 epitope is present on the cell surface of all twelve human pluripotent stem cell lines and two embryo-carcinoma cell lines examined to date.
Flow cytometric and microscopic analysis demonstrates that expression of the epitope recognised by the CC-9 antibody correlates strongly with the nuclear transcription factors Oct4a, Nanog and Sox-2; as well as with the cell surface pluripotency markers SSEA-3 and TRA-1-60r. Following fractionation by flow cytometry and electrostatic cell sorting, the expression of the antigen recognised by CC-9 is highly correlative with self-renewal as measured by clonogenic assay.
Conclusion
In conclusion, the monoclonal antibody CC-9 binds a cell surface epitope that defines cells within the stem cell compartment. CC-9 can be used to identify pluripotent stem cells from within a heterogeneous culture.

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Poster ID – 96

PBX1 regulates self-renewal of human embryonic stem cells through cell cycle control

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Self-renewal of human embryonic stem cells (hESC) is maintained by a core regulatory network involving transcription factors NANOG, OCT4 and SOX2. To understand the self-renewal mechanism of hESC, we have previously reported PBX1, a homeodomain transcription factor is a novel transcriptional activator of NANOG. In this study, we demonstrated that PBX1 is involved in hESC self-renewal. PBX1 is expressed in hESC and its knockdown by RNA interference alters the molecular and cellular phenotype of hESC. The PBX1 shRNA vector stably knocked down PBX1 protein expression by 90% in hESC. Gene expression analysis of both embryoid body differentiation and retinoic acid induced differentiation of PBX1 knockdown cells showed that there was no difference in three lineage markers expression. It suggests that PBX1 does not regulate early embryonic lineage commitment. However, PBX1 knockdown cells were growth impaired in undifferentiated state and grew more slowly than control cells. In these PBX1 stable knockdown cells, down-regulation of PBX1 in hESC decreases proliferation and increases apoptosis by using EdU incorporation assay and Annexin-V apoptosis assay. In cell cycle profile analysis, PBX1 knockdown cells were arrested at G1 phase, whereas control cells progressed to S phase. Reintroduction of PBX1 by stably overexpression in PBX1 knockdown cells rescued the cell cycle G1 arrested phenotype, allowing cells to progress toward S phase. Besides, changes in protein expression of Cyclins involved in G1 progression and cell cycle inhibitors were confirmed by Western Blot analysis. Our data indicate that PBX1 has a vital role in promoting cell cycle progression in hESC and controlling self-renewal of hESC.

Poster ID – 97

A comparison of germ cell differentiation in a male and female hESC line

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The early development of the mammalian germ cell is determined mainly by gonadal factors rather than the genetic sex of an individual. Previous studies have reported on the differentiation of human embryonic stem cells (hESCs) to primordial germ cells (PGC) and early gametes although this process is very inefficient. The aim here was to compare the differentiation of a male and female hESC line during germ cell development through meiosis. Shef hESC lines (Shef 2, XX; Shef 4 XY) were cultured on mouse embryonic feeders (MEFs) and allowed to undergo spontaneous differentiation in the presence of fetal calf serum or in medium supplemented with combinations of retinoic acid and bFGF. Differentiation to PGCs and germ cells was analysed using a variety of cell surface markers and mRNA expression by Q-PCR. The progression of cells through meiosis was assessed by expression of various meiosis-associated genes (MND1,DMC1, FANK1, SPANXC, MAK, SYCP3) correlated with in-situ hybridization of single cells (FISH). In XY Shef 4 cell line the differentiation to PGCs occurred after 4-7 days in culture with bFGF and retinoic. In comparison, XX Shef 2 cells showed greater differentiation to PGCs without bFGF and expression of meiosis marker earlier than Shef 4. Haploid cells were detected with FISH at 14 days of cultures with 2-3 times as many cells for Shef 2 than Shef4. Further investigation with different cell lines is now in progress to establish whether these differences are mainly cell line or sex specific.

Poster ID – 98

Dynamic epigenetic alterations reveal distinct pluripotent states within human embryonic stem cell cultures

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A growing array of mouse and human pluripotent stem cell lines has been derived from the early embryos as well as from adult cells reprogrammed by ectopic expression of transcription factors - i.e. induced pluripotent stem (iPS) cells. All of these cell lines share the expression of key pluripotency markers (Oct4, Nanog and Sox2). Their relationship to each other and whether they correspond to different pluripotent states with distinct affiliations in vivo remains unclear however. Determining the developmental status of different embryo-derived pluripotent cell lines is not only of intrinsic interest but is likely to further understanding of molecular reprogramming to pluripotency in vitro.

Here we take advantage of DNA replication-timing assay as a tractable approach to investigate how chromatin shutdown contributes to lineage restriction and cell fate identity through development. To address whether discrete pluripotent states can be reliably discriminated at the chromatin level, we examine the replication timing profiles of several human embryonic stem (hES) cell lines alongside mouse ES and epiblast stem (EpiS) cells - a pluripotent stem cell population derived from post-implantation embryos.

Remarkably, we show that hES (and hiPS) cells can transit from primed to naive (ICM-like) pluripotent states by changing growth culture conditions. In particular, extensive and dynamic shifts of replication timing (from late to early) are consistently observed at many silent, developmental genes in undifferentiated hES cells upon increased Smad2/3 and p300 histone acetyltransferase (HAT) activity. Importantly, these alterations are reversible and associated with differential gene expression profiles and functional properties of hES cells. Collectively, our data reveal the existence of distinct but interchangeable pluripotent hES cell states and propose a pivotal role for TGF-β/Activin signalling through Smad2/3 action and the HAT p300 in balancing naive versus primed pluripotent states within hES cell cultures.

Poster ID – 99

Human embryonic stem cells are capable of executing G1/S checkpoint activation

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Embryonic stem cells are unique by their fast progression through cell cycle providing limited space for some cell cycle regulatory circuits to fully pronounce their functionality, which is typical for somatic cells. Among such cell cycle-linked circuits, those that are involved in inhibition of progression of cell cycle upon the damage to DNA are of paramount importance as their deficiency may contribute to rather low genetic stability observed specifically in human embryonic stem cells (hESC). In this study, we exposed undifferentiated hESC to DNA damaging UVC light and followed their response in terms of progression through G1/S border, activity of major driver of cell cycle for this period - cyclin-dependent kinase 2 (CDK2), and functioning of molecules known to mediate inactivation of CDK2 in somatic cells. We show that when irradiated in G1 phase a large portion of hESC becomes entrapped in cell cycle before DNA synthesis and that such arrest coincides with low activity of CDK2. We also show that direct activator of CDK2, phosphatase Cdc25A, is down-regulated in irradiated hESC and this decrease is dependent on the action of checkpoint kinases Chk1 and/or Chk2. Importantly, the classical effector of p53-mediated pathway, protein p21, does not prove itself as regulator of G1/S progression. Taken together, our data unequivocally document that *in vitro* cultured undifferentiated hESC possess capacity to prevent entry into S phase of cell cycle (to activate G1/S checkpoint) when the damage to their genetic complement takes place.

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Poster ID – 100

TGFβ superfamily members in human embryonic stem cells

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Results from gene ablation studies in mice indicate that the Transforming growth factor β (TGFβ) superfamily has a direct effect on embryonic development not only by enhancing self-renewal, but also in directing their differentiation into the mesodermal lineage. The TGFβ superfamily probably has a major role in both self-renewal and differentiation in human and mouse ES cells.

TGFβ is a multipotent growth factor which transduces signals from the membrane to the nucleus by binding to a heteromeric complex of serine/threonine kinase receptors known as TGFβ type I (ALK) and type II receptors that propagate the phosphorylation signal to receptor-regulated R-Smads. Activated R-Smads form complexes with Smad4 and accumulate in the nucleus where they regulate transcriptional activity of their target genes.

The aim of the project is to study the role of the TGFβ superfamily on pluripotent human ES cells and their differentiation into cardiomyocytes.

In order to determine which genes and pathways are regulated by the main members of the TGFβ superfamily in human ES cells we have used gene expression analysis (4-plex expression arrays from Roche-NimbleGen). Results from data analysis will be presented.

Consistent with some reports, our results show that Bone Morphogenetic Protein (BMP4) induces human ES cells to differentiate into beating cardiomyocytes. This effect could be inhibited by the BMP inhibitor, Noggin. To dissect further the role of BMP4 we have adenovirally infected ES cells with different constitutively active TGFβ superfamily receptor (ALKs) constructs and other genes downstream of the TGFβ superfamily. The amount of beating cardiomyocytes was assessed and ES cells were analysed at different time points with regard to expression of mesodermal genes.

Poster ID – 101

Neuronal differentiation of human ES cells: Comparison with mouse ES cells

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Work with mouse embryonic stem (mES) cells has led to the development of a simple, robust protocol allowing the reproducible differentiation of mES cells into excitatory, glutamatergic neurons (Bibel, 2004; Bibel, 2007). This turned out to be a powerful discovery tool allowing unbiased investigations on the mechanisms of axonal degeneration (Plachta, 2007), new insights on the role of the transcription factor Pax6 (Nikoletopoulou, 2007) or of the amyloid precursor protein (Schenk-Siemens et al., 2008). The success of this protocol critically depends on the homogeneity of the mES cells kept pluripotent by growing them with leukemia inhibitory factor (LIF) and serum.
The main goal of our work with human ES (hES) cells is to establish a similarly robust protocol to generate defined populations of human neurons. Culture conditions were first standardized by trypsinizing hES cells (clones HS181 and Mel-1) received from ESTOOLS partners. hES cells are then grown on mouse feeder cells in the presence of human leukemia inhibitory factor (LIF), as opposed to basic fibroblast growth factor (bFGF). These hES cells were found to express the components of the LIF signalling pathway, including phosphorylation of STAT3. Like with mES cells, hES cells are then aggregated and treated with retinoic acid (RA) to generate neuronal progenitors and neurons. Both cell types show striking similarities with those generated from mES cells: glutamatergic neurons emerging as the main differentiated cell type from Pax6-positive, radial glial cell-like progenitors.
While this protocol has yet to reach the stability and reproducibility achieved with mES cells, our results with hES cells indicate striking similarities both with regard to the procedure and the results obtained. These ES cells, as well as retrovirally-generated human induced pluripotent stem (hiPS) cells, are now used to analyse progenitors and neurons carrying mutations in MeCP2 and a polymorphism identified in the brain derived neurotrophic factor gene.

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Poster ID – 102

Pramel7: A new determinant of pluripotency in embryonic stem cells

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A unique and complex network allows embryonic stem cells (ESCs) to undergo extended proliferation in vitro and to nevertheless maintain their capacity of multilineage differentiation. Genuine ESC identity can only be maintained when both self-renewal and suppression of differentiation are active and balanced. We recently have identified Pramel7 (preferentially expressed in melanoma like 7) as a new candidate gene, involved in the maintenance of pluripotency in ESCs. In situ, Pramel7 expression is exclusively restricted to the pluripotent pools of cells, namely the central part of the morula and the inner cell mass (ICM) of the blastocyst. Transgene-mediated overexpression Pramel7 relieves ESCs from their dependence on gp130/LIF stimulation even when cultivated for a prolonged period on a feeder layer -/- fibroblasts. Furthermore overexpressionof Pramel 7 suppresses the capacity to differentiate in vitro and in vivo. Following recombinase mediated excision of the transgene the recombined cells reverted to a LIF-dependent state and were able to participate in the formation chimeric mice. We also show that Pramel7 acts as a downstream effector of the LIF/Stat3 pathway and blocks differentiation by reversing the activation of Erk.
Taken together these findings emphasize the central and essential role of Pramel7 in the network of pluripotency regulation and selfrenewal
in ESCs.

Poster ID – 103

Investigating Oct4 and Sox2 fluctuation in human embryonic stem cells using reporter lines

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The transcription factors Oct4 and Sox2 are considered key regulators of pluripotency, whose expression is necessary to maintain embryonic stem (ES) cells within the stem cell state. However, investigating the impact of fluctuation in these proteins in live human ES cells is hampered by their nuclear localisation. To overcome such problems, reporter lines have been generated for both Oct4 and Sox2, with GFP expression acting as a read-out. We have confirmed the accuracy of these reporter lines and, based on clonogenic assays, assessed the influence that changing levels of Oct4 and Sox2 have on human ES cell self-renewal.

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Poster ID – 104

The role of Laminins -511 and -111 in human embryonic stem cells

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Recently, it has been shown that basement membranes not only provide mechanical support for tissues but also have unique roles in cell signaling. The most important group of biologically active signaling proteins in basement membranes is laminins (Lm), which are produced by cells in a tissue type-specific manner. We have shown that only two laminin (Lm) isoforms, Lm-511 and Lm-111, out of 16 known, were produced by hESC cultures. However, as culture matrices, they show opposing functions in supporting hESCs suggesting that laminin expression and interactions within the hESC extracellular matrix is tightly regulated. Lm-511 demonstrated strong support of hESC pluripotency and self-renewal, whereas, when placed on Lm-111, the cells show increased differentiation and migration of peripheral cells from the colonies. We suggest that the balance and production of extracellular matrix proteins by human embryonic stem cells (hESCs) are likely to play major roles in hESC maintenance and lineage commitment.
By comparing the synthesis of Lm-111 and Lm-511 in undifferentiated and differentiating hESCs, we are investigating the roles of these laminin isoforms in the early phases of lineage commitment in hESCs. Currently, we are using single-cell qPCR to investigate how the expression of laminin isoforms and pluripotency markers correlate in hESC colonies undergoing minor, spontaneous differentiation. To elucidate these observations, we will utilize RNAi technologies to inhibit specific laminin chains in hESCs. Using RNAi and other approaches, we hope to elucidate in detail the mechanisms of laminins in the self-renewal and lineage specification of hESCs. Taken together, our data uncovers new roles for these laminin isoforms in defining the fate of hESCs.

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Poster ID – 105

Regulation of cardiac progenitor cells proliferation and differentiation by microRNAs

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In recent years stem cells and progenitor cells have been identified in the heart, changing the paradigm that the heart is a post mitotic organ. These different cellular populations have the ability to proliferate and to differentiate into beating cardiomyocytes in vitro, and also into other cardiac cells, as endothelial cells and vascular smooth muscle. The paradigm shift that is emerging from these studies can change the understanding and therapeutic approach of heart diseases, opening new possibilities in the field of cell therapy. Several genes have been identified as regulators of stem cell function and cardiomyocyte differentiation. More recently, microRNAs (miRNAs) have been identified as master switches controlling proliferation and differentiation events and, in particular, to function as key regulators of cardiac development and response to stress. The goal of our project is to obtain insights into the lineage of origin of the minute stem cell population present in the adult heart and identify possible regulators of stemness, cell proliferation and differentiation that can be used to manipulate cell fate in vivo. miRNAs are ideal tools for this purpose, as they can modulate gene expression in the absence of genetic modification. In our current study we have established methods to purify total RNA from small numbers of c-kit positive and Sca-1 positive cells isolated from adult mouse hearts by Fluorescence Activated Cell Sorting (FACS) and quantify miRNA using a TaqMan based qRT-PCR approach. We have compared the expression profile of five miRNAs (miR-1, miR-100, miR-206, miR-208, miR-21) in cardiac progenitor cells, adult cardiomyocytes and bone marrow (BM) cells. The results reveal that cardiac progenitor cells have a miRNA expression profile more similar to cardiac cells than to BM cells. Moreover, we have identified a higher expression of miRNA 21 (miR-21) in cardiac progenitor cells, establishing a distinction between the three populations. Interestingly, miR-21 has been shown to be upregulated in stress responses in the heart and in anti-apoptotic pathways in cancer cells. These preliminary data suggest that cardiac progenitor cells are distinct from BM stem cells, and identify miR-21 as a possible regulator of stemness, cell proliferation and differentiation in the heart.

Poster ID – 106

Targeting the Pdx1 Locus in Human Embryonic Stem Cells

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Given that human embryonic stem cells have the potential to produce unlimited quantities of any human cell type considerable focus has been placed on their therapeutic potential. At present, the culture conditions required for efficient stem cell differentiation towards functional, insulin-producing β-cells remain unknown. Efforts in this process have been slowed down by the challenges associated with monitoring the appearance of specific cell types during the differentiation in culture. To efficiently identify and derive pure populations of specific cell types, genetic modification of human embryonic stem cells can be undertaken. Since all pancreatic cells arise from Pdx1-expressing precursors, we aim to establish a Pdx1 reporter cell line by introducing the gene for Enhanced Green Fluorescent Protein (EGFP) at the 5'UTR of the Pdx1 coding region, so that the Pdx1 promoter regulates the EGFP gene. This will be achieved by gene targeting of the Pdx1 locus in human embryonic stem cells.
Hence, using Bacterial Artificial Chromosome Recombineering we have designed a targeting construct that contains a promoterless EGFP-gene, a positive selection gene and 5' and 3' homologous arms for gene targeting. In addition, the plasmid vector backbone harbours a negative selection marker, the Thymidine Kinase gene, to eliminate human embryonic stem cell clones with random integration of the construct. After electroporation and successful gene targeting, the promoter/enhancer region of the endogenous Pdx1 locus is expected to drive expression of the EGFP gene.

Poster ID – 107

Culture Conditions and their Effects on Pluripotency and Differentiation Potential on hESC and iPSC

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Abstract Human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSC) are characterized by being pluripotent and by their ability to proliferate indefinitely, and differentiate. Culture conditions affect these fundamental features of the cells. Clear evidence for culture adaptation and accumulation of specific non-random chromosomal aberrations over time has been documented. This is not surprising, as the higher order chromatin conformation is similar in related cell types, leaving certain regions in a more fragile position, and, hence, constitutes easier targets for breakage. Here, we address the issue of epigenetic effects, in particular targeting histone acetylation in hESC and hiPSC, as a response to various culture techniques, and whether these are reversible. We are also analyzing histone acetylation of promoter regions as a mechanistic regulator of stemness-associated and cancer-related gene expression. In particular, we are focusing on culturing the two types of stem cells on human foreskin fibroblasts (hff) as feeder cells using Knockout DMEM with serum replacement, in comparison to culture of the same lines on matrigel in mTeSR1 medium (StemCell Technologies). We will also discuss how different techniques of cryopreservation of cells may influence their nature, and put these results in to the aspect of the potential of selective differentiation of stem cells towards various lineages. Finally, we will address the issue of the reversibility of the effects on culturing of stem cells. Grant acknowledgment We thank the Swedish research Council, ESTOOLS and Karolinska Institutet (KID) for supporting the project.

Poster ID – 108

Identification of a stem cell population within the adrenal cortex.

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Adrenal glands are small, but extremely important organs. They serve, through the production of a variety of hormones, several vital functions in the human body including the regulation of blood pressure, glucose metabolism, and the response to immunological stimuli and stress. The importance of studying adrenal development and homeostasis is highlighted by the existence of severe pathologies associated with hypo- (i.e. Addison disease) or hyper- (i.e. Cushing syndrome) adrenal function. As is the case in other organ systems, adrenal cells need to be replaced, when they are damaged or lost throughout life. To replace these cells the existence of a stem cell population that resides in the cortical zone of the adrenal gland has been postulated. Definitive experimental data and/or molecular markers that identify such a cell population are still missing.
The Wilms' tumor suppressor gene 1 (WT1) encodes a transcriptional regulator that plays a key role during development of several organs. To date, Wt1 function in adrenal glands was thought to be limited to the establishment of the adrenogonadal primordium (AGP), the common primordium between adrenals and gonads. Once the primordium has separated into gonad and adrenal, Wt1 is repressed in the latter.
In the present study we show that Wt1 expression is maintained throughout life in a small population of cells that reside in the adrenal capsule in the mouse. Using cell lineage tracing experiments in adult mice, we show that Wt1+ cells give rise to cells that reside in the adrenal cortex. To further test the involvement of Wt1 in adrenal development and homeostasis, we generated a mouse line that allows tissue specific activation of Wt1 using a Rosa26 knock-in approach. Ectopic activation of Wt1 within adrenocortical cells was achieved by crossing this strain with the Sf1:Cre line (Bingham et al., 2006). Interestingly, Wt1 expressing cells failed to differentiate into the steroidogenic cell lineage, which may suggest that Wt1 is not only a marker of adrenocortical stem cells, but might also be a determinant factor of stem cell fate. Isolation of Wt1 positive cells from the adrenal capsule is presently being performed and will allow us to ultimately prove their stem cell identity.

Poster ID – 109

Studying the epigenetic landscape of micro-RNA genes in Human Embryonic Stem Cells

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Epigenetics refer to the heritable regulation of gene expression independent of the primary DNA sequence. The epigenetic network consists of different regulatory mechanisms with more important being the DNA (CpG) methylation, the histone modifications and, lately, the micro-RNAs (miRNAs). A great discovery in the field of epigenetic research was the observation that during malignant transformation gene-promoters containing CpG islands are getting hyper-methylated and enriched in repressive histone marks, resulting in the post-transcriptional gene silencing (PTGS) of those genes. Critical developmental regulators are marked in early human embryonic cells (hESCs) with similar chromatin marks known as "bivalent domains", containing both positive and negative histone modifications. These marks maintain the genes at a low transcriptional rate, keeping them "poised for activation" at a later differentiation stage. A recent hypothesis supports the idea that these stem cell chromatin marks render critical genes (such as tumor suppressor genes) prone to aberrant CpG island DNA hyper-methylation and, thus, to PTGS. The miRNAs comprise a new class of small non-coding RNAs involved in the post-transcriptional regulation of many target genes. MiRNAs have been already implicated in hESCs self-renewal, pluripotency, and differentiation, and, dependent on their target-gene, they can function as oncogenes or tumor suppressors.This work focuses on the characterization of the epigenetic landscape of miRNA genes in hESCs. We selected a list of miRNA genes previously found to exhibit tumor suppressor features and employed bisulfate sequencing and chromatin-immunoprecipitation assays in order to compare the methylation status and the distribution of stem cell-chromatin marks within their promoter region, in a panel of undifferentiated, differentiated hESCs, teratoma, cancer cell lines and normal tissues. Taqman miRNA assays were used to assess the expression levels of those genes. The results of these studies are expected to shed light onto the mechanism (at the chromatin level) that might be responsible for the hyper-methylation and, subsequently, the silencing of miRNA genes in cancer. In addition, we will present data of the genome-wide analysis performed in order to compare the methylation profiles of the promoter-related CpG islands of miRNA genes in undifferentiated hESCs versus differentiated hESCs, cancer cell lines and normal tissues.

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Poster ID – 110

iPSC generation and characterization

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Induced pluripotent stem cells (iPSC) can be created in a variety of ways, but induction efficiency can be very low especially for non-viral induction methods. This is a specific issue relating mainly to future clinical applications; on one hand they should produce cells that are not stably gene modified, yet on the other hand allow reprogramming of adult primary cells.
However, integrating retroviral vector transduction is a gene transfer method that gives the highest iPSC yields. The subsequently produced cells seem to suffice for most research applications if specific parameters are watched. We have produced and characterized iPSCs using the Thomson factors stably introduced through lentiviral vectors. We would like to present some characterization data and discuss several issues to be aware of in context with other current reprogramming methods.

Poster ID – 111

Commitment to endoderm differentiation is reduced in culture-adapted human embryonic stem cells relative to their normal counterparts

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Cultured human embryonic stem (hES) cells that have acquired karyotypic abnormalities and an enhanced growth rate, a phenomenon referred to as culture adaptation, provide a unique model to investigate the stem cell landscape of pluripotency and differentiation. Previous observations have suggested that culture-adapted hES cells may possess an altered capacity for differentiation, and perhaps even an enhanced tumorigenic capacity. However, the differentiation capacity of adapted cells has not yet been examined directly. The working hypothesis is that culture-adapted hES cells display a reduced capacity for spontaneous endoderm differentiation. A variety of quantitative methodologies, including multiparametric assessment of cell surface antigens by flow cytometry and functional assays, were used to assess differences in propensities for endoderm lineage selection and differentiation between culture-adapted hES cells and their normal counterparts. Our results demonstrate that endoderm lineage priming and commitment are reduced in culture-adapted hES cells relative to their karyotypically normal counterparts. These results bring forward new evidence of consequences of culture-adaptation of hES cells on their lineage selection and commitment to exiting the stem cell compartment and subsequently differentiate, and could offer valuable insights into human development and possibly oncogenesis.

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Poster ID – 112

Haematopoiesis of mouse embryonic stem cells - the role of p38alpha kinase

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Embryonic stem (ES) cells are in vitro population of pluripotent cells derived from an inner cell mass of blastocyst. They are able to maintain their self-renewal and pluripotent potential without any limitation. Thus ES cells can be used for developmental studies and in case of human ES cells, also as a source for cells and tissue therapies and replacements. The aim of our work was to study the mechanisms and effectiveness of haematopoiesis in mouse ES cells. In first step, we confirmed the occurrence of haematopoiesis in our experimental model of spontaneous differentiation of ES cell by formation of embryoid bodies. Further step was the analysis of the role of p38α kinase in haematopoiesis. P38 kinases belong to the mitogen-activated protein kinase family (MAPK). Similarly to other members of MAPK family they participate in various cellular processes that influence the regulation of cell proliferation, differentiation, and apoptosis. Recently, it has been shown that p38 inhibitors inhibit differentiation of haematopoietic progenitors. Moreover recent hypothesis suggests that p38 kinases play role in the maintenance of somatic stem cells. In our first approach to this idea we presume that if p38α kinase participates on the maintenance of the haematopoietic stem and early progenitor cells, depletion of p38α kinase change the dynamics of haematopoiesis in p38α -/- when compared to the wt p38 ES cell line. This idea is tested in presented work.
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Poster ID – 113

BF4 - a novel human embryonic stem cell antibody isolates specific subsets within stem cell niche.

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Human embryonic stem cells colonies have an apparent heterogenic dynamic which appears to enhance their ability to maintain the stem cell state[1,2]. They transiently express a variety of surface markers giving rise to defined states whilst still maintaining their propensity to self renew[3]. Highlighted in this study is the proteoglycan BF4. Characterising various subsets BF4 high have an increased cloning efficiency where as BF4 low may demonstrate lineage priming to specific cell types within the stem cell compartment. Here dissect and examine the stem cell niche further and try to exemplify the mechanism by which sub-states confined inside the stem cell colony may act to assist in their maintenance.

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Poster ID – 114

Ikaros family members control neuronal differentiation of neural stem cells

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Neural progenitor cells that are located at concrete positions during brain development, give rise to different neuronal and glial cell types. Several pro-neural transcription factors that follow specific expression patterns regulate neurogenesis of discrete neuronal populations. Here we describe a novel role for Ikaros-family members in the regulation of striatal neurogenesis. Ikaros and Helios are expressed during striatal development starting at E14.5 until postnatal stages presenting a peak at E18.5. However, their pattern of expression is different. Helios is expressed in both the subventricular zone (SVZ) and the mantle zone (MZ) of the lateral ganglionic eminence (LGE) while Ikaros is exclusively expressed in the boundary between the SVZ/MZ. Within the MZ Ikaros and Helios do not localize in the same cells. The study of the role of Ikaros shows that its over-expression in neural stem cells (NSCs) induces cell cycle arrest by the up regulation of the cyclin-dependent kinase inhibitor (CDKi) p21Cip/Waf1. This effect is coupled with the neuronal differentiation of NSCs since the number of tubulin-b-III positive cells is increased. On the contrary, Ikaros induces a reduction in the number of GFAP positive astrocytes suggesting that it induces neurogenesis at the expenses of gliogenesis. In addition, over-expression of Ikaros-1 in primary embryonic striatal cultures increases the number of calbindin- and ENK-positive neurons. Similarly, Helios over-expression in NSCs induces neuronal differentiation but it does not have any effect on the number of GFAP positive cells. Furthermore, over-expression of Helios in striatal embryonic primary cultures increases the number of neurons that express the striatal markers calbindin, enkephalin and DARPP-32.
Thus Ikaros-family members present similar effects in the neuronal differentiation of NSCs. However, Helios cannot exert its neurogenic effect in neurospheres derived from Ikaros knockout mice. These findings suggest that Helios is expressed upstream of Ikaros and these factors together regulate striatal neurogenesis.
In conclusion, our results demonstrate that Ikaros and Helios constitute a new pro-neuronal transcription factor family that participates in striatal neuronal determination.

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Poster ID – 115

Regulation of stem cell fate by redox modifying agents

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Embryonic stem (ES) cells provide a powerful tool for studying embryogenesis as well as potent source of pluripotent cells and differentiated progeny for use in regenerative medicine. Besides the well-documented LIF induced STAT3 activation, self-renewal and maintenanace of mouse ES undifferentiated phenotype appears to be under control of PI3K/Akt signalling pathway. Differentiation seems to be promoted by Ras/Erk cascade. A number of intrinsic transcription factors have been identified so far, among them Nanog and Oct3/4 play pivotal role in pluripotency regulation. There is a growing body of evidence that unique characteristcs of stem cells are also closely conncted to redox regulation. Under physiological conditions in their niche, stem cells are kept in hypoxic microenvironment. Leaving their niche they are exposed to normal levels of oxygen. Thus reactive oxygen species (ROS) formation in stem cells due to hypoxic conditions could be considered as important regulator of stem cell fate. Here we analyzed effects of inhibitors of NADPH oxidases apocynin or diphenyleneiodonium (DPI) and a glutathione precursor N-acetyl-cysteine (NAC) on maintenance of mouse ES cells. Inhibitors of NADPH oxidases and NAC decreased proliferation of ES cells without significant effects on cell cycle distribution. Apocynin strongly inhibited Akt that was accompanied with dowregulation of Nanog and Oct3/4 expression. Apocynin also promoted changes in morphology of cells towards more differentiated phenotype. By contrast DPI enhanced Akt activation. NAC did not modified STAT3, Akt or Erk. Obtained results are compared with effects on mouse ES cells cultured in hypoxic condition. Hypoxic cultivation markedly reduced Erk phosphorylation, with no effect on Akt and STAT3. It could be concluded that apocynin significantly modulate intracellular signaling pathways involved in maintenance of undifferentiated phenotype of mouse ES cells. However, the mechanism of these effects through the apocynin induced inhibition of ROS formation is questioned and remains to be clarified, as well as different response to apocynin and DPI treatment.

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Poster ID – 116

Molecular effects of forced Lmx1a expression in differentiating human embryonic stem cells

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Parkinson's disease (PD) is defined by a localised neurodegeneration of the dopaminergic cell population in the substantia nigra pars compacta. The confined cell loss of PD characterises this disease as an optimal candidate for cell replacement therapy, and promising clinical results have been obtained from intra-striatal transplantation of fetal midbrain tissue. Midbrain dopaminergic (mDA) neurons may also be obtained from human pluripotent cell sources, but the efficiency of differentiation is low, as it is challenging to control the patterning of neuronal subtypes in vitro. During the past recent years, mouse models of brain development have provided valuable insight into the molecular mechanisms of midbrain specification, and several transcription factors (i.e. Lmx1a, FoxA2, Nurr1 and Msx1) have been identified as key determinants of the mDA cell fate. Although the roles of these factors have been studied in the mouse, little is known about their role in human mDA specification. In this study, we make use of a lentiviral system expressing Lmx1a under control of mir292, thereby ensuring transgene expression in differentiated cells and excluding any exogenous Lmx1a expression in the pluripotent hESC population. We show that neural induction of hESCs transduced with Lmx1a-mir292 induced massive upregulation of Lmx1b, Msx1, Otx2, Wnt1 and Mash1 when compared to GFP-transduced cells. Interestingly, early treatment with high levels of Shh initially antagonised the effects of Lmx1a expression through downregulation of Lmx1a mRNA, subsequently allowing for sustained Lmx1a expression only in the FoxA2 positive progenitor population. We are currently investigating these downstream effects of Lmx1a more closely in combination with various different growth factor treatments. In addition, it is being evaluated whether Lmx1a alone can drive dopaminergic differentiation in hESCs, or whether further patterning is needed for proper mDA specification in human cells.

Poster ID – 117

Antibody mediated modulation of Notch signalling

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The Notch signalling pathway is instrumental for cell fate diversification at multiple stages in development and in cell proliferation in both normal and diseased states. The Notch signalling pathway has been studied extensively in mammalian neural stem cells both in vivo and in vitro; however there is still much uncertainty about the specific functions of the individual Notch receptors and ligands. Notch signalling occurs via direct cell:cell contact and links the fate of cells to their neighbours. In mammals there are 5 ligands which promiscuously activate the 4 different Notch receptors (Notch 1-4). Despite this promiscuity, individual Notch family members have distinct roles in development and in certain tissues. Thus the ability to specifically block or activate individual Notch receptors would provide greater understanding of the role of the individual receptors and would permit a greater degree of control in model systems, e.g. stem cells.

From a scFv phage display antibody library we have selected and characterised antibodies specifically binding to Notch family members including receptors Notch 1-3 and the ligands Jagged and Delta. Further, we have identified blocking antibodies for Notch1 and Notch2 and Dll4. The potency of blocking antibodies have been determined in a co-culturing assay and antibodies targeting Notch 1 and 2 are silencing signalling completely. By qRT-PCR we have verified that downstream target genes of Notch are down regulated when blocking antibodies are added to neural stem cells in vitro. Functional antibodies provide a route to block or activate the individual Notch receptors. In contrast to other methods, this could be done reversible in genetically unmodified cells or animals and thus enable flexible regulation of signalling at multiple stages.

Poster ID – 118

Genetic stability control of Human pluripotent stem cells in I-STEM

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Due to their original properties, human pluripotent stem cells (hESC) and their progenies are highly valuable not only for regenerative medicine but also as tools to study development and pathologies or as cellular substrates to screen and test new drugs. However, ensuring their genomic integrity is one important prerequisite for both research and therapeutic applications. Indeed, chromosomal abnormalities such as gain of entire chromosomes (12, 17 and X), gain of chromosome arms (12p, 17q) or gain of part of a chromosome (20q11.21) occur non-randomly and quite commonly in hESC lines. There are various methods to assess karyotypic integrity which differ both in their sensitivity and resolution. Conventional cytogenetics allows the identification of abnormal chromosome number and structural changes of large part of chromosomes whereas molecular technologies such as array-based comparative genomic hybridization and Single Nucleotide Polymorphism array have a higher resolution but are less sensitive. In I-STEM we have developed a quality control platform using these methods as complementary approaches, which provide meaningful information when combined.

Poster ID – 119

ABCG2 multidrug transporter expression in human embryonic stem cells

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Human pluripotent stem cells provide new hopes for the clinical treatment of a number of diseases and, at the same time, they are excellent models for tissue development and physiological cell differentiation. The ABC-MDR proteins are primary active transporters as they utilize the energy of cellular ATP for performing a vectorial, transmembrane movement of drugs or xenobiotics. In humans, the three major types of MDR-ABC proteins (ABCB, ABCC and the ABCG subfamily) may form a special network of chemo-defensive mechanism. ABCG2 is physiologically expressed in relatively high levels in the canalicular membrane of the liver, in the epithelia of small intestine, colon, lung, kidney, adrenal and sweat glands, as well as in the endothelia of veins and capillaries, including the capillary endothelial cells of the blood-brain barrier, and in stem cells. The presence of this transporter in human embryonic stem cells may significantly contribute to stem cell defense mechanisms. Data regarding ABCG2 protein expression in human embryonic stem cells is controversial in the literature; therefore we examined the expression of ABCG2 in various human embryonic stem cell lines (HUES1, HUES4, HUES9 and BG01V). We used quantitative real-time PCR to detect the mRNA level of ABCG2 in four different ES lines. Real time PCR data showed that the endogenous mRNA level of ABCG2 was relatively high in all undifferentiated HUES lines. We determined the co-expression of the undifferentiated stem cell marker SSEA4, and the cell surface expression of the ABCG2 by flow cytometry. We also showed plasma membrane localization of the ABCG2 protein by confocal microscopy. Furthermore, we demonstrated the function of the ABCG2 protein by measurements of fluorescent dye transport. We found that the endogenous expression level of the ABCG2 transporter was high in undifferentiated hESCs and significantly decreased during their differentiation into hematopoietic ($CD34^+$) and mesenchymal-like cells. These measurements show that a functional ABCG2 protein is present in undifferentiated human ES cells and we propose that ABCG2 has an important role in the protection of human embryonic stem cells against damage by toxins, xenobiotics and hypoxia.

Poster ID – 120

GABA signaling during early embryonic development: common features of the GABAergic cells

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In addition to being the principal inhibitory neurotransmitter in the central nervous system (CNS) of vertebrates, GABA is thought to act as a paracrine trophic factor during embryonic development. In the CNS, GABA is synthesized by the enzyme glutamic acid decarboxylase (GAD) and released at inhibitory synapses from synaptic vesicles of the GABAergic inhibitory neurons. Thereafter it activates GABAA (GABAAR) and/or GABAB (GABABR) receptors exerting membrane hyperpolarization and neuronal inhibition. In immature neurons GABA can be released by a reversal of the membrane GABA transporter (GAT); acting on embryonic GABAR it induces depolarization and rise of intracellular Ca2+ [Ca2+)i thereby affecting cell proliferation, differentiation, migration and synaptogenesis.
In our previous studies we have shown that GABAAR and GABABR modulate differentially the proliferation of mouse embryonic stem (mES) cells acting through distinct [Ca2+]i signalling and second messenger pathways.
Here we have studied the expression of different components of the GABA signaling pathway in non-differentiated mES cells and during initial stages of mES cell differentiation. We have found, that both adult GAD forms- GAD65 and GAD67 and enzymatically inactive embryonic GAD25 are expressed, albeit at low levels, at early passages and are upregulated at later passages and/or prolonged culturing with or without LIF and/or fibroblast layer. The enzymatically active embryonic GAD44 is almost undetectable in mES cells shortly after plating, but is greatly upregulated at initial stages of differentiation and higher passages. Prolonged incubation with GABAAR or GABABR antagonists/agonists results in characteristic changes in the level of expression and spatial distribution of the GAD forms and GABA in mES clones, which could potentially be used as sensitive indicators for a differentiation shift.
Both vesicular and cellular membrane GABA transporters (VGAT and GAT, respectively) were highly expressed in undifferentiated mES cells suggesting that GABA can be released and uptaken by mechanisms similar to these operating in the GABAergic neurons. Evidence for vesicular trafficking is clearly demonstrated on electron micrographs of exponentially growing mES cells.
The GABAergic signalling components are expressed also at the morula stage and in the inner cell mass (ICM) of the blastocyst, where, similar to mES cells GABA may act selectively on GABAAR and GABABR to regulate proliferation and/or differentiation acting through [Ca2+]I changes. In conclusion, our data clearly show that undifferentiated mES cells express a fully functional GABA signalling, which is dynamically regulated with the transition to a more differentiated state.

Poster ID – 121

Teratogen Screening Using Transcriptome Profiling of Differentiating Human Embryonic Stem Cells

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Teratogens are substances that may cause defects in normal embryonic development while not necessarily being toxic in adults. Identification of possible teratogenic compounds has been historically beset by the species-specific nature of the teratogen response. To examine teratogenic effects on early human development we performed non-biased expression profiling of differentiating human embryonic and induced-pluripotent stem cells treated with several drugs; ethanol, lithium, retinoic acid, caffeine and thalidomide, which is known to be highly species specific. Our results point to the potency of specific teratogens and their affected tissues and pathways. Specifically, we could show that ethanol caused dramatic increase in endodermal differentiation, retinoic acid caused misregulation of neural development, and thalidomide affected both these processes. We thus propose this method as a valuable addition to currently available animal screening approaches.

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Poster ID – 122

Nitric oxide promotes cell survival and delays differentiation progress in embryonic stem cells 26

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Nitric oxide (NO) is a molecule with established functions as intracellular messenger in several cell systems, but its contribution to embryonic stem cell (ESC) biology has not been fully characterized. Exposure of ESC to low concentrations (2-20μM) of the NO donor DETA-NO confers protection from apoptotic DNA fragmentation elicited by culture in the absence of LIF. This action is linked to blockade of caspase 3 activation, PARP degradation, down regulation of pro-apoptotic genes Casp7,Casp9, Bax and Bak1 and upregulation of anti-apoptotic genes Bcl-2111, Bcl-2 and Birc6. These actions are also apparent in cells overexpressing high levels of eNOS. Exposure of LIF-deprived ESC cells to low NO also prevents against the loss of self-renewal genes (Oct4, Nanog and Sox2) and the loss of the SSEA surface markers. In addition, low NO blocked the differentiation process promoted by the absence of LIF and bFGF in mESCs and human ESCs (hESCs) respectively. Treatment with NO decreased the expression of early differentiation markers such as Brachyury, Gata6 and Gata4. Constitutive overexpression of eNOS in cells exposed to LIF deprivation maintained the expression levels of self-renewal markers, while differentiation genes are repressed.These actions were reversed in cells treated with the NOS inhibitor L-NMMA. Finally, cells grown in the presence of NO and in the absence of LIF for ten passages generated teratomas in SCID mice. Altogether, the data suggest that NO plays a role in the regulation of ESCs differentiation, delaying the entry into differentiation process, arresting the loss of self-renewal markers and promoting cell survival by inhibition of apoptosis

Poster ID – 123

The Impact of Human Induced Pluripotent Stem Cells (IPSC) on European and German Stem Cell and Regenerative Medicine Law

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Contrary to public opinion there are many opportunities for stem cell research in the European Union as well as in Germany. However, many European lawmakers prohibit the use of early totipotent embryonic cells and developmental stages to gain pluripotent embryonic stem cells and/or prohibit or restrict the use of pluripotent embryonic stem cells for research purposes. Considering the great hopes that stem cell research holds, e. g. the German lawmaker amended the national provisions in this area in 2008.

However, the above-mentioned amendment of the German stem cell law coincided with unexpected results of stem cell research in the field of the creation of ethically unloaded human stem cells by techniques of reprogramming. These techniques lead to so-called induced pluripotent stem cells (iPSC). But, the German Stem Cell Act contains a subsidiary provision which states that the import of human embryonic stem cells to Germany is not permitted if there is a scientific alternative for the use of human embryonic stem cells. Therefore, if the further import of embryonic stem cells is legally cut off there is the probability that further stem cell research in Germany flags. Due to legal restrictions for Germany-based researchers, the import of human embryonic stem cells is the only possibility of getting access to these cells. The scientific and medical success of reprogramming research could therefore legally inhibit the further import of and research with human embryonic stem cells in Germany, if iPSC were an alternative for the use of human embryonic stem cells. But, this subsidiary provision is only applicable if the "scientific alternative" could fully replace the scientific use of human embryonic stem cells. For this reason, the legal (and scientific) status of human iPSC was clarified for the first time in by this research project.

This research project also clarifies the regulatory and legal scope stem cell researchers have and focuses on the possibilities of purchasing embryonic stem cells in the European Union and especially in Germany. The project will also pay attention to the frequent questions as to what kind of international collaborations in Germany-based stem cell research are possible. Furthermore, the research project determines the legal conditions for the market approval of stem cell based therapies in the European Union, especially for iPSC-based therapies for the first time.

Poster ID – 124

Promoting Innovation to Benefit Society: Eye on Europe

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Morality as a concept is a complex issue for the intellectual property in general, but its application to biotechnological, including hESC inventions is clearly specifically problematic. The European Patent Office (EPO) has so far struggled in its approach and interpretations of morality concepts grounded in patent laws, its history and case law. Failure to articulate and elaborate on the notion of morality clause as such has led to precarious and disconcerting results both within the stem cell research and patent circles. Many are increasingly wary that patenting hESCs is on a slippery slope to a commodification of the human life, diminishing of human dignity and failure to provide shared ownership of key social goods.
Rather than building on the morality clause that the EU Biotechnology Directive and EU patent laws established, the patent system, has, at best, shown a very modest progress. Yet, meanwhile, patent examiners and the EPO face complex issues of patent morality and no tools provided in dealing with morally controversial innovation. Despite a remarkable opportunity to settle the morality issue with the WARF appeal, the EPO's WARF ruling in late 2008 failed to clarify the matter and further added to the general disappointment. Unsurprisingly, 'high' expectations are now pinned on the Brustle patent questions forwarded to the European Court of Justice. Nevertheless, when it comes to hESC patents, the EPO runs the risk of suffering worse than perpetual stagnation. European economy and its patent system could easily slip backwards and find itself unable to keep up, or catch up, to its fellow trans-Atlantic neighbour.
The Poster aims to outline present moral dilemma encircling hESC inventions, patent system as a moral censor and the legal fiasco at the EPO. Further, the Poster presents a summary of major challenges and criticism directed at the morality clause and depicts key reasons for the EPO's reluctance in interpreting the morality clause. The Poster then follows with a consideration of whether patent law can be subject to a sort of imm(p)unity from exercising ethics and sets forth a set of criteria required in order to adequately determine ethical and moral issues of hESC inventions. Finally, relying on the solution criteria, the Poster outlines a workable framework that can successfully be used as a basis in dealing with hESC inventions.

Parts of this work have been supported by funding for the ESTOOLS consortium under the Sixth Research Framework Programme of the European Union contract LSHG-CT-2006-018739

Poster ID – 125

Scientific Advances and the Ethics of Human Embryo

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Stem Cells, which are acknowledged to have differentiation capacity in any kind of tissue, hold the promise of becoming one of the most powerful and useful weapons of modern biological medicine. Certain therapies for genetic diseases and neurological disorders may be able to be developed thanks to the ability of these cells to repair damaged tissue. Their potential for research is beyond question, and there is a high likelihood of obtaining significant therapeutic results in this area.
Many researchers, neglecting the success that has been obtained in the research into Adult Stem Cells, argue that the greater potential for differentiation of the Embryonic Stem Cells is justification to pursue research projects using cellular lines derived from the internal mass of human blastocists (human embryos), collected from the surplus embryos that come from assisted procreation. In point of fact, this kind of research leads to the destruction of the embryos, and as such much unease has been expressed in relation to this destructive procedure of human entities that do not yet have a defined ontological or legal status, but which are certainly worthy of respect and dignity.
Given the controversy surrounding the research into Embryonic Stem Cells, I shall seek to argue, by aesthetic resources and ethical arguments, in favour of the exhaustive exploration of the conclusions drawn from the research into Adult Stem Cells, namely with regard to the iPS Cells, where analysis of the results obtained up to now have led to great expectations. Which one is the "tropo vero": human embryo as a focus of hope or human embryo as a new technological slave?

Poster ID – 126

An Italian journey

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Human assisted reproduction and research on embryonic stem cells have been one of the most divisive themes in Italian politics over the last decade.

In this poster we look at salient moments of this confrontation, from the initial attempts of the Dulbecco Commission to regulate the emerging technology of cloning to the referendum of 2005 to the recent debates on the public funding of stem cell science.

We aim at bringing a new perspective on this recent history, by highlighting the main resources, scientific, technological, legal and political, which were deployed to construct the public discourse on stem cell science. A parallel focus on the various actors who have shaped this debate (scientists, patient advocates, lawyers, political and religious institutions) allows us to draw a map of how particular strands of bioethical thinking aligned alongside new and at times contested understandings of life at the forefront of developmental biology.

THEATRE *Staminalia – a Dream and a Trial*

created by Valeria Patera
based on the book by Armando Massarenti

Thursday 27 May, Gulbenkian Foundation, 19:30
(venue and time to be confirmed)

Staminalia goes behind the science of stem cells to face and represent in aesthetic form the scientist of emotion and of human feeling. Staminalia is the story of a woman scientist who leads a stem cell research group but faces ethical and political limits to her freedom of scientific action that impact her public and private life, developing into conflict with her theo-conservative teenage daughter.

Valeria Patera, writer and theatre director, is president of TIMOS Teatro.eventi - a group that researches the connections between different theatre languages and the hybridisation of separate fields of knowledge. TIMOS Teatro.eventi promotes creative dialogue between Art & Science, and is creating a new artistic model of representation to capture voices and issues of contemporary life across art and science. Since 2005 Patera has produced and staged in Italy three key figures in science: Alan Turing, Max Perutz, and Charles Darwin (see Darwin video in Italian at www.valeriapatera.it/darwin/index.html).

Staminalia is the latest step in her series of the "science-in-theatre", premiering in Italy in late 2010 as a full-length production. This special 30 min production is created uniquely for the ESTOOLS symposium in Lisbon.

The research outputs of the stem cell field outline a new boundary where science, philosophy, politics and aspects of every day social life inextricably link. Data and philosophical references originate in the book "Staminalia. Le cellule etiche e i nemici della ricerca" by Armando Massarenti, eminent Italian intellectual who has analysed the last 15 years of discussions, conflicts, views and scientific achievement in the stem cell field within a philosophical and historical perspective. [Reviewed in Nature by Elena Cattaneo - *Science, dogmas and the state*, 456, 444-445, 2008].

TIMOS TEATRO

NeuroStemcell
European Consortium for Stem Cell Therapy in Neurodegenerative Diseases

CALOUSTE GULBENKIAN FOUNDATION

estools
Advances with Human Embryonic Stem Cells

uniStem
Università degli Studi di Milano Centro di Ricerca sulle Cellule Staminali

FONDAZIONE SIGMA-TAU

The University Of Sheffield.

UNIVERSITÀ DEGLI STUDI DI MILANO

Conference dinner, Thursday 27 May

Restaurante Estufa Real
Cç. do Galvão
Jardim Botânico da Ajuda
1400 LISBOA
Portugal

Buses will leave promptly at the end of the theatre presentation, at 20:15

Meeting point: Outside the front main entrance of the Gulbenkian Foundation

Return buses will stop at the top of *Rua Tomás Ribeiro*

Lisbon

There is much to be see and do in Lisbon. Below are a few highlights:

Tram 28
It starts at Praça Luis de Camões in the Chiado district up near the high Bairro Alto, then descends into downtown, climbs up through **Alfama** past the Sé Cathedral, Miradouro de Santa Luzia, Monastery of São Vicente de Fora and the National Pantheon, the old church at Graça, and finishes in Martim Moniz in the north.

Alfama is the oldest district of Lisbon and it is one that maintains the city's oldest traditions. This district is located on the slope between the Castle of Lisbon and the Tejo River.

Café Pastéis de Belém – Rua de Belém
If everybody in Brussels must see the Manneken Pis or the mermaid in Copenhagen, then Café Pastéis de Belem is the place to go in Lisbon. Very popular and busy, the place is big, so go and explore the rooms at the back, it might not be full. Open everyday from 8:00am to midnight.
In the area is the **Mosteiro de Jeronimos**, a 15th Century monastery classed as a 'World Heritage Site' of UNESCO.

Chapitô - Costa do Castelo
Sharing the premises of a circus school, this restaurant and bar has great views over Lisbon from its terrace. Join an audience of artists and bohemian students for live music or a theater show.

Café a Brasileira - Rua Garrett
Opened in 1905, Café a Brasileira has been the haunt of poets, writers and artists across the decades. Enjoy a bica (portuguese short&strong coffee) inside this Art Deco style bar and cafe.

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Richard **Losick**

KEYNOTE LECTURES

Cliff **Tabin**
Elizabeth **Blackburn**
Austin **Smith**
Michel **Haïssaguerre**

PLENARY LECTURES

Evolution of animal forms
Alejandro **Sánchez Alvarado**
Detlev **Arendt**
Marie-Anne **Félix**
Shigeru **Kuratani**

Systems biology & functional genomics
Marc **Vidal**
Edward (Eddy) **Rubin**
Ron **Weiss**
Olivier **Voinnet**

Signalling in development
Irma **Thesleff**
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WORKSHOPS

Dynamics of chromosome organization
Susan **Gasser** | Wendy **Bickmore**

Cellular tomography
Ohad **Medalia** | Achilleas **Frangakis**

Stem cells & regeneration
J C **Izpisúa Belmonte** | Brigitte **Galliot**

Gene network
Anne-Claude **Gavin** | Bart **Deplancke**

Modelling biological patterns
Edda **Klipp** | Olivier **Pourquie**

Metabolomics
Johan **Auwerx** | Juleen **Zierath**

Dynamics of molecule ensembles in cells
Philippe **Bastiaens** | Carsten **Schultz**

Cancer genomics & stem cells
María **Blasco** | Mariano **Barbacid**

Imprinting
Edith **Heard** | Azim **Surani**

DNA repair, cancer & aging
Jean-Marc **Egly** | Judith **Campisi**

Next generation sequencing
Thomas **Lemberger**

Transdifferentiation
Thomas **Graf** | Nadia **Rosenthal**

Cell contact & adhesion
Kathleen J **Green** | Marek **Mlodzick**

New insights in prokaryotes
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Long range gene regulation
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