GermanStemCellNetwork

1st Annual Conference 2013

of the German Stem Cell Network (GSCN)
in Berlin

www.gscn.org

November 11-13, 2013
Max Delbrück Center (MDC), Berlin

Program & Abstracts

Bundesministerium
für Bildung
und Forschung

MDC MAX DELBRÜCK CENTER FOR MOLECULAR MEDICINE BERLIN-BUCH
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Heidelberg, Germany

Nanostring Technologies Europe LTD
London, Great Britian

Fluidigm Europe B.V.
Amsterdam, The Netherlands

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GermanStemCellNetwork

1st Annual Conference
German Stem Cell Network (GSCN)

Max Delbrück Center (MDC)

Berlin

November 11 – 13, 2013

Organization

Michaela Langer / Timkehet Teffera / Lien-Georgina Dettmann
Max Delbrück Center for Molecular Medicine (MDC) Berlin Buch
Robert-Rössle-Str. 10
13125 Berlin

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Welcome Address

Dear Participants,

It is a great pleasure to welcome you to the

1st Annual Conference of the German Stem Cell Network e.V. (GSCN)

at the Max Delbrück Center for Molecular Medicine (MDC) in Berlin-Buch.

This conference will be the starting point of a series of annual conferences rotating among German cities. As the German capital, Berlin with its vibrant life science community at the Max Delbrück Center for Molecular Medicine (MDC) and other local scientific and clinical institutions provides an excellent venue for this conference.

The meeting will cover the latest topics in stem cell biology, including genetic and epigenetic mechanisms of reprogramming, maintenance of pluripotency, somatic stem cells in vertebrates and invertebrates, stem cells in disease and regenerative medicine, cancer stem cells and many more stem cell-related topics.

The key aim of this conference is to bring together researchers working on different aspects of stem cell biology and to promote networking and collaboration across regions and scientific disciplines. In addition to covering a large range of stem cell-related scientific areas, the meeting will feature sessions on highly relevant strategic topics such as emerging technologies, career development, funding opportunities and public outreach. Contributions on clinical applications in regenerative medicine and industry presentations are expected to reflect the strong translational impact of this fast-growing field.

Embedded between keynote lectures from international leaders in the field, the majority of the oral presentations will be given by junior scientists selected from the submitted abstracts. This special format reflects the major goal of this conference series to offer young researchers a high level forum for presenting and discussing their results and for initiating new interactions ranging from first scientific collaborations to the initiation of transregional research consortia. Two extensive poster sessions and a series of panel and group discussions will provide further opportunities for data sharing and intellectual exchange.

I hope you will enjoy this conference and our newly founded network as a stimulating platform for Germany's stem cell research community and wish you an interesting and enjoyable stay in Berlin!

Best wishes

Oliver Brüstle (GSCN Acting President)

For the program committee

Daniel Besser (Berlin) • Oliver Brüstle (Bonn) • Hans Schöler (Münster) • Elly Tanaka (Dresden) • Mathias Treier (Berlin) • Andreas Trumpp (Heidelberg)

Conference Information

Venue:
Max Delbrück Communications Center (MDC.C)
Robert-Rössle-Straße 10
13125 Berlin-Buch, Germany

Date:
Monday, November 11 to Wednesday, November 13, 2013

Registration:

Regular fee:	550 €
Regular member fee:	375 €
Student fee:	300 €
Student member fee:	200 €
Day ticket (onsite):	160 €

The registration fee includes attendance at all scientific sessions, November 11 – 13, conference documents, name badge, final program and abstract book, conference bag, conference dinner (downtown), coffee breaks (a.m./p.m.), lunch, dinner and free internet access.

Posters:
Posters will be displayed during the meeting in 2 sessions in the exhibition area (house 84), adjacent to the Communications Center and the conference hall.

The size of a single poster should not exceed 1m x 1,20m (width/height).
You will find the number of your poster in this abstract volume. According to this number, you may mount your poster in the exhibition area. Materials to fix your poster will be provided on site. The authors are asked to be present at their poster during the poster session.

Posters scheduled for Poster Session I (Poster numbers P1 - P94) can be viewed on Monday, November 11, 2013.
The posters in this session should be **set-up** on Monday, 12:00 h - 16:30 h and **removed** latest on Tuesday by 12:30 h.

Posters scheduled for Poster Session II (Poster numbers P95 – P193) can be viewed on Tuesday, November 12, 2013.
The posters in this session should be **set-up** on Tuesday, 12:30 h - 16:15 h and **removed** on Wednesday by 14:00 h.

Internet:
Internet access via Wireless LAN is free of charge in the MDC.C at the SSID "mdcguest". The current login and password is provided at the registration desk.

Social Events

Networking evening with dinner buffet downtown on Monday, November 11th, 20:00 h.
The bus shuttle for all participants to the event location will leave the MDC at 19:00 h.

The location is:

EVENTLOCATION ALTE PUMPE
Pumpe Gastronomie GmbH
Lützowstraße 42
10785 Berlin-Mitte

City maps are available at the registration desk.

Informal get-together at the MDC.C Foyer (exhibition area) on Tuesday, November 12th, 21:00 – 22:30 h.

PROGRAM OVERVIEW

Monday, November 11th, 2013

	Axon 1+2	Dentrit 2/3
12:00	Registration	
13:00	**Opening**	
13:30	**Keynote lecture I**	
14:00	*Michele de Luca*	
14:30	Coffee break / Industry exhibition	
15:00	**Keynote lecture II**	
15:30	*Vania Broccoli*	
16:00	Introduction of the GSCN strategic working groups	
16:30	**Poster session I** in the poster area	• Pluripotency and embryonic stem cells • Programing and reprograming • Stem cells in development • Stem cells in tissues of neural and non-neural ectoderm • Stem cells in tissues of mesodermal and endodermal origin
17:00		
17:30		
18:00		
18:30		
19:00	Bus Shuttle to Networking evening	
19:30		
20:00	**Networking evening with dinner buffet**	
00:30	at the "Alte Pumpe" (Lützowstr. 42, 10785 Berlin)	

Tuesday, November 12th, 2013

	Axon 1	Axon 2	Dentrit 2/3
	Concurrent scientific working group session I		
09:30	Pluripotency and embryonic stem cells	Cancer stem cells in solid tumors	Stem cells in tissues of meso- and endodermal origin
10:00			
10:30			
11:00	Coffee break / Industry exhibition		
	Concurrent strategic working group session A		
11:30	Carreer Development	Funding programs/policies	Outreach activities and public perception of SC therapies
12:00			
12:30	Lunch buffet / Industry exhibition		
13:00			
	Concurrent strategic working group session B		
13:30	Stem cell technologies	Clinical trials and regulatory affairs	
14:00			
14:30	Short break		
	Concurrent scientific working group session II		
14:45	Stem cells in regenerative therapies	Programing and reprograming	Stem cells in tissues of neural and non-neural ectoderm
15:15			
15:45			
16:15	**Poster session II** in the poster area	• HSCs and stem cells in hematopoietic malignancies • Cancer stem cells in solid tumors • Stem cells in regenerative therapies • Stem cells in disease modeling and drug development	
17:00			
17:30			
18:00			
18:45	**Dinner buffet** in the MDC.C Foyer		
19:00			
19:30	**Convention of GSCN members**		
20:00			
20:30			
21:00	**Informal GSCN Get-together** in the MDC.C Foyer		
21:30			

Wednesday, November 13th, 2013

	Axon 1	Axon 2	Dentrit 2/3
Concurrent scientific working group session III			
09:00 09:30 10:00	Stem cells in disease modeling and drug development	Stem cells in development	HSCs and stem cells in hematopoietic malignancies
10:30	Coffee break / Industry exhibition		
Industry session: "Technologies from GSCN industry partners"			
11:00 11:30 12:00	Peprotech GmbH Life Technologies GmbH Nanostrig Tech. LTD	BD Biosciences Eppendorf AG Apceth GmbH & Co. KG	Fluidigm, Europe B.V. Essen Bioscience AMS Biotechnology (Europe)
12:35 13:00 13:30	Lunch buffet / Industry exhibition		
14:00 14:30	**Keynote lecture III** *Christine Mummery*		
15:00	Coffee break / Industry exhibition		
15:30 16:00	**EMBO Lecture** *Austin Smith*		
16:30	**Poster award ceremony**		
16:45	**Outlook and closing remarks**		
17:00	End of conference		

Legend:

Keynote Lectures & EMBO Lecture
Concurrent scientific working group sessions
Concurrent strategic working group sessions
Poster Sessions and Poster award ceremony
Industry session
GSCN Evening events/Opening/Introduction/Outlook
Convention of GSCN members

PROGRAM

MONDAY, November 11th

12:00 – 13:00	Registration

Opening

13:00 – 13:10	Welcome to the Max Delbrück Center: *Walter Rosenthal* (Scientific Director, MDC)
13:10 – 13:30	Opening remarks *A Stem Cell Network for Germany – why the time is just right* *Oliver Brüstle* (Acting President, GSCN)
13:30 – 14:30	**Keynote lecture I** K1 - Regenerative medicine by somatic stem cells: The paradigm of epithelial cells *Michele de Luca*; Center for Regenerative Medicine "Stefano Ferrari", Modena, Italy (Chair: *Frank Emmrich*)
14:30 – 15:00	Coffee break / Industry exhibition
15:00 – 16:00	**Keynote lecture II** K2 - In vivo phenotypic and functional stability of iDA neurons and chemo-genetic enhancement of their therapeutic potential *Vania Broccoli*; San Raffaele Scientific Institute, Milan, Italy (Chair: *Mathias Treier*)
16:00 – 16:30	**Introduction of the GSCN strategic working groups**
16:30 – 19:00	**Poster session I:** P001 – P094 P001 – P019: Pluripotency and embryonic stem cells P020 – P052: Programing and reprograming P053 – P072: Stem cells in development P073 – P081: Stem cells in tissues of neural and non-neural ectoderm P082 – P094: Stem cells in tissues of meso- and endodermal origin
19:00	Bus shuttle to event location
20:00 – 0:30	Networking evening with dinner buffet at eventlocation "Alte Pumpe" Lützowstr. 42, 10785 Berlin (www.altepumpe.de) (Opening: *Albrecht Müller*) (included in the registration fee)

TUESDAY, November 12th

Concurrent scientific working group session I

Axon 1[1]:	**Pluripotency and embryonic stem cells** (chair: *Mathias Treier/James Adjaye*)
09:30 – 09:40	Introduction
09:40 – 10:00	T01 - Loss of Myc activity induces cellular dormancy in ES cells cultured in 2i mimicking the status of diapause embryos *Roberta Scognamiglio*; German Cancer Research Center, Heidelberg
10:00 – 10:20	T02 - Epigenetic regulation of lineage commitment *Gunnar Schotta*; Adolf Butenandt Institute, LMU Munich
10:20 – 10:40	T03 - Mll2 is the H3K4 trimethyltransferase for bivalent promoters in mouse embryonic stem cells *A. Francis Stewart*; Technical University Dresden
10:40 – 11:00	Group discussion
Axon 2:	**Cancer stem cells in solid tumors** (chair: *Andreas Trumpp/Thomas Brabletz*)
09:30 – 09:40	Introduction
09:40 – 10:00	T04 - The KC/IL8-mTOR axis activates stem cell maintenance and chemotherapy resistance of hepatic tumor-initiating cells *Uta Kossatz-Böhlert;* University Tübingen
10:00 – 10:20	T05 - The role of EVI1 in breast cancer cells *Hui Wang;* University Hospital Basel, Switzerland
10:20 – 10:40	T06 - Patient derived models for pancreatic cancer reveal four molecular subtypes associated with differential drug response and clinical outcome *Elisa Noll*; HI-STEM, Heidelberg
10:40 – 11:00	Group discussion
Dendrit 2/3:	**Stem cells in tissues of meso- and endodermal origin** (chair: *Thomas Braun/Heiko Lickert*)
09:30 – 09:40	Introduction
09:40 – 10:00	T07 - A lentiviral RNAi screen against the muscle stem cell proteome identifies novel regulators governing muscle regeneration *Johnny Kim*; MPI f. Heart and Lung Research, Bad Nauheim
10:00 – 10:20	T08 - Direct lineage conversion of liver to pancreas *Francesca M. Spagnoli*; Max Delbrück Center, Berlin
10:20 – 10:40	T09 - Early Growth Response transcription factors Egr1 and Egr2 regulate lineage commitment of bone marrow mesenchymal stromal progenitors *Konstantinos Anastassiadis*; BIOTEC, Technical University Dresden
10:40 – 11:00	Group discussion
11:00 – 11:30	Coffee break / Industry exhibition

[1] Axon 1 (240 seats), 2 (140 seats) and Dendrit 2/3 (80 seats) are the rooms in the MDC conference center

Concurrent strategic working group session A

Axon 1:	**Career development** (chairs: *Insa Schröder/NN*)
11:30 – 11:50	I1 – Alternative career pathways for life scientists *Thorsten Abs*; academics GmbH, Hamburg
11:50 – 12:30	Group/panel discussion
Axon 2:	**Funding programs/policies** (chairs: *Albrecht Müller/Ulrich Martin*)
11:30 – 11:50	I2 - Horizon 2020 - News on EU research funding *Jürgen Sautter*; European Commission, Brussels, Belgium
11:50 – 12:30	Group/panel discussion
Dendrit 2/3:	**Outreach activities and public perception of stem cell therapies** (chair: *Tobias Cantz/Ira Herrmann*)
11:30 – 11:40	Introduction (*Tobias Cantz*)
11:40 – 11:55	I3: EuroStemCell Toolkit for Public Engagement: Information, Education, Conversation *Emma Kemp,* EuroStemCell, Edinburgh, United Kingdom
11:55 – 12:10	I4: Ethical aspects of innovative therapies *Gisela Badura-Lotter,* University Ulm
12:10 – 12:30	Group/panel discussion
12:30 – 13:30	Lunch buffet / Industry exhibition

Concurrent strategic working group session B

Axon 1:	**Stem cell technologies** (chair: *Frank Emmrich/Andreas Bosio*)
13:30 – 13:50	I5: Stem cell technologies *Andreas Bosio;* Miltenyi, Berg. Gladbach, and *Frank Emmrich;* University Leipzig
13:50 – 14:30	Group/panel discussions
Axon 2:	**Clinical trials and regulatory affairs** (chair: *Torsten Tonn/Andreas Kurtz*)
13:30 – 13:50	I6: Implementation of cell- and gene therapy for clinical application: challenges and regulatory requirements *Christine Günther*; Apceth GmbH & Co. KG, Ottobrunn
13:50 – 14:30	Group/panel discussions
14:30 – 14:45	Short break

Concurrent scientific working group session II

Axon 1:	**Stem cells in regenerative therapies** (chair: *Michael Cross/Ulrich Martin*)
14:45 – 14:55	Introduction
14:55 – 15:15	T10 - Transplantation of genetically corrected human iPSC-derived neural stem cells in a mouse model of metachromatic leukodystrophy *Jonas Doerr*; Institute of Reconstructive Neurobiology, Bonn
15:15 – 15:35	T11 - MicroRNA-199a-5p: A novel regulator of differentiation of hepatocytes from embryonic stem cells *Selina Möbus*; REBIRTH, MH Hannover
15:35 – 15:55	T12 - Successful treatment of therapy-refractory acute Graft-versus-Host disease with mesenchymal stem cell-derived exosomes *Bernd Giebel*; Institute for Transfusion Medicine, Essen
15:55 – 16:15	Group discussion
Axon 2:	**Programing and reprograming** (chair: *Micha Drukker/Frank Edenhofer*)
14:45 – 14:55	Introduction
14:55 – 15:15	T13 - Analysis of signaling framework governing cardiac specification of human pluripotent stem cells *Boris Greber*; MPI f. molecular Biomedicine, Münster
15:15 – 15:35	T14 - Generation of mature megakaryocytes from human pluripotent stem cells by a transcription factor mediated forward programming approach *Thomas Moreau*; University of Cambridge, United Kingdom
15:35 – 15:55	T15 - A DNA methylation motifs discovery algorithm reveals the crosstalk among DNA methylation, transcription factors and histone marks in pluripotent cells *Marcos J. Arauzo-Bravo;* MPI f. Molecular Biomedicine, Münster
15:55 – 16:15	Group discussion
Dendrit 2/3:	**Stem cells in tissues of neural and non-neural ectoderm** (chair: *Walter Birchmeier/Ana Martin-Villalba*)
14:45 – 14:55	Introduction
14:55 – 15:15	T16 - Clonal lineage tracing of adult neural stem cells reveals features of adult neurogenesis *Filippo Calzolari*; Helmholtz Center Munich
15:15 – 15:35	T17 - The inflammatory gene AKNA, an at-hook containing transcription factor, regulates differentiation and survival of neural stem cells *Pia Johansson;* Helmholtz Center Munich
15:35 – 15:55	T18 - Oligodendrogenic and neurogenic adult subependymal zone neural stem cells constitute distinct lineages and exhibit differential responsiveness to Wnt *Benedikt Berninger*; University Mainz
15:55 – 16:15	Group discussion

16:15 - 18:45	**Poster session II:** P095 – P193 P095 – P116: HSCs and stem cells in hematopoietic malignancies P117 – P128: Cancer stem cells in solid tumors P129 – P166: Stem cells in regenerative therapies P167 – P193: Stem cells in disease modeling and drug development
18:45 – 19:30	Dinner buffet / Industry exhibition
19:30 – 21:00	**Convention of GSCN members**
21:00 – 22:00	Informal GSCN get-together (MDC.C Foyer)

WEDNESDAY, November 13th

Concurrent scientific working group session III

Axon 1:	**Stem cells in disease modeling and drug development** (chair: *Karl-Ludwig Laugwitz/Oliver Brüstle*)
09:00 – 09:10	Introduction
09:10 – 09:30	T19 - Induction of nuclear ataxin-3 inclusions in Machado-Joseph disease-specific neurons *Johannes Jungverdorben*; Institute of Reconstructive Neurobiology, Bonn
09:30 – 09:50	T20 - Restoration of GM-CSF depended functionalities in macrophages utilizing patient specific iPSC from a congenital pulmonary alveolar proteinosis patient *Nico Lachmann*; MH Hannover
09:50 – 10:10	T21 - Raman spectroscopy as novel marker for sensitive stem cell identification *Karin Schütze*; CellTool GmbH, Bernried
10:10 – 10:30	Group discussion
Axon 2:	**Stem cells in development** (chair: *Elly Tanaka/Jan Lohmann*)
09:00 – 09:10	Introduction
09:10 – 09:30	T22 - Tet-dependent processing of 5-methylcytosine, epigenetic stability and the maintenance of active higher order structures during differentiation *Achim Breiling*; German Cancer Research Center, Heidelberg
09:30 – 09:50	T23 - Aldh1b1 regulates the timing in pancreas lineage segregation and beta cell maturation *Elpinki Ninou;* Paul Langerhans Institute, Dresden
09:50 – 10:10	T24 - A transitional extracellular matrix instructs regenerative cell behavior *Hans-Georg Simon*; Northwestern University, Chicago, USA
10:10 – 10:30	Group discussion
Dendrit 2/3:	**HSCs and stem cells in hematopoietic malignancies** (chair: *Anthony Ho/Timm Schröder*)
09:00 – 09:10	Introduction
09:10 – 09:30	T25 - The genome wide molecular landscape of hematopoietic stem cells and their immediate progeny *Nina Cabezas-Wallscheid*; German Cancer Research Center, Heidelberg
09:30 – 09:50	T26 - Crosstalk between WNT/β-catenin and IRF8 in hematopoiesis and leukemia *Marina Scheller;* UKE, Hamburg
09:50 – 10:10	T27 - A stem cell intrinsic switch from canonical to non-canonical Wnt signaling regulates aging of hematopoietic stem cells *Maria Carolina Florian*; University Ulm
10:10 – 10:30	Group discussion

10:30 – 11:00 Coffee break / Industry exhibition

Industry session: "Technologies from GSCN industry partners"

Axon 1:	**Main supporters**
11:00 – 11:05	Introduction *(Mathias Treier)*
11:05 – 11:35	C1 - Development of a new serum-free media for the cultivation of pluripotent stem cells *Bärbel Icheln*; Peprotech GmbH
11:35 – 12:05	C2 - Novel reagents for pluripotent stem cell research and differentiation to neural lineages *Mohan C. Vemuri*; Life Technologies GmbH
12:05 – 12:35	C3 - Gene expression analysis down to the single cell level by digital quantification utilizing the nCounter system *Maik Prüß*; Nanostring Tech. LTD
Axon 2:	**Supporters**
11:00 – 11:05	Introduction *(Ulrich Martin)*
11:05 – 11:35	C4 – Flow cytometry applications for isolating and analyzing complex heterogeneous stem cell cultures *Yannick Marari*; BD Biosciences
11:35 – 12:05	C5 - Suspension culture and cardiomyogenic differentiation of human pluripotent stem cells in stirred bioreactor systems *Robert Zweigerdt*; MH Hannover, representing Eppendorf AG
12:05 – 12:35	C6 - Pharmaceutical stem cell therapy for advanced diseases entering clinical application *Veronika Reiter*; Apceth GmbH & Co. KG
Dendrit2/3:	**Supporters**
11:00 – 11:05	Introduction *(Michael Cross)*
11:05 – 11:35	C7 – Discover a New Approach to Single-Cell Genomics with the C1™ Single-Cell Auto Prep System Fluidigm, Europe B.V.
11:35 – 12:05	C8 - The IncuCyte system - new applications for iPSC production *Peter K. Djali*; Essen BioScience Ltd.
12:05 – 12:35	C9 - Development of extracellular matrices for stem cell culture *Maja Petkovic*; AMS Biotechnology (Europe) Limited

12:35 – 14:00 Lunch buffet / Industry exhibition

14:00 – 15:00 **Keynote lecture III**
K3 - Cardiomyocytes from human pluripotent stem cells: the new patient in safety pharmacology, drug discovery and disease
Christine Mummery; Leiden University Medical Center, Netherlands
(Chair: *Andreas Trumpp*)

15:00 – 15:30 Coffee break / Industry exhibition

15:30 – 16:30 **EMBO Lecture**
K4 - Design principles of pluripotency
Austin Smith; Wellcome Trust-MRC Stem Cell Institute, University of Cambridge, United Kingdom
(Chair: *Oliver Brüstle*)

Closing ceremony

16:30 – 16:45 **Poster award ceremony** (*Daniel Besser,* GSCN)

16:45 – 17:00 **Outlook and closing remarks**
Andreas Trumpp (Incoming president, GSCN)

End of conference

GSCN Travel Awards

The following participants have been selected for the **GSCN travel awards**:

- Bianca Buechner (Cells Hannover)
- André Görgens (University Hospital Essen)
- Leonid Schneider (Technical University of Darmstadt)
- Edda Schulz (Institut Curie, Paris, France)

Poster awards

Four poster awards are sponsored by PeproTech GmbH.
There will be two poster awards for each poster session:

Poster Session I: Monday, November 11, 2013, 16:30 – 19:00 h (P001-P094)

P001 – P019: Pluripotency and embryonic stem cells
P020 – P052: Programing and reprograming
P053 – P072: Stem cells in development
P073 – P081: Stem cells in tissues of neural and non-neural ectoderm
P082 – P094: Stem cells in tissues of meso- and endodermal origin

Poster Session II: Tuesday, November 12, 2013, 16:15 – 18:45 h (P095-P193)

P095 – P116: HSCs and stem cells in hematopoietic malignancies
P117 – P128: Cancer stem cells in solid tumors
P129 – P166: Stem cells in regenerative therapies
P167 – P193: Stem cells in disease modeling and drug development

Poster award for "Applied stem cell research"

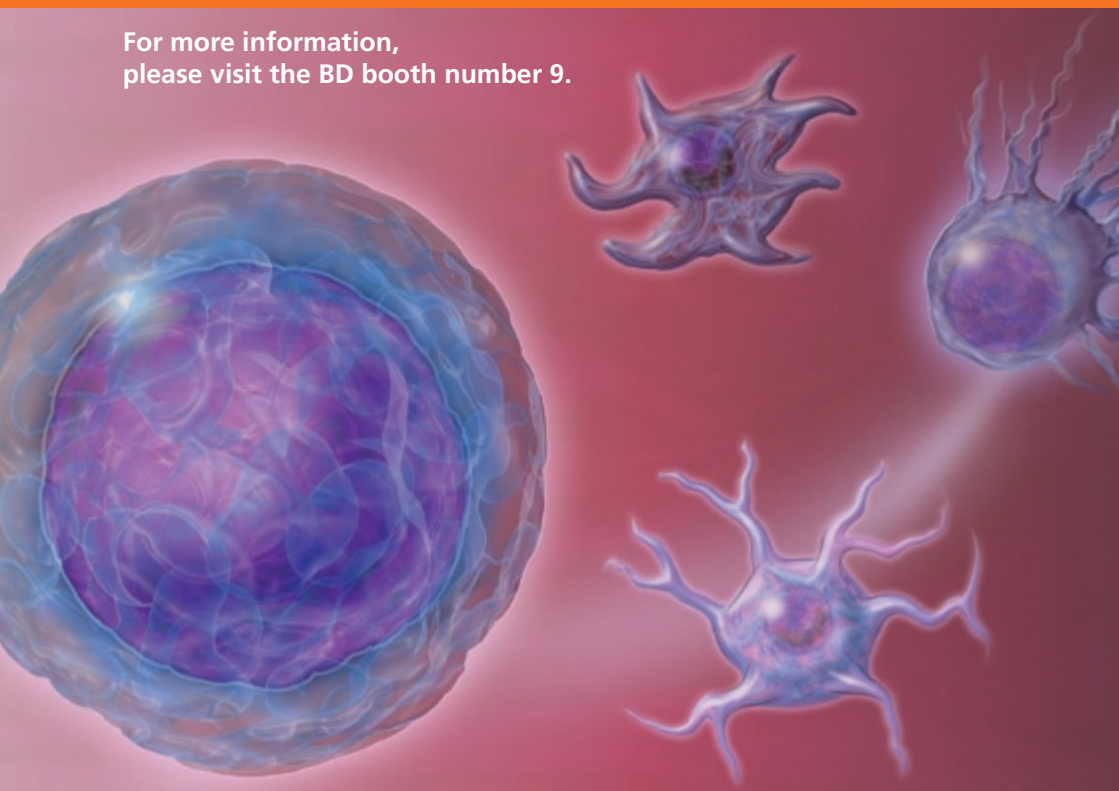
There will be one additional Poster award for applied stem cell research sponsored by Fraunhofer Institute for Cell Therapy and Immunology (IZI) and the Translational Centre for Regenerative Medicine (TRM Leipzig).

The poster award ceremony will take place on Wednesday, November 13, 2013 at 16:30 h.

Note: The awards will only be given, if the presenters of the posters are present at the ceremony.

BD Stem Cell Research Source

**For more information,
please visit the BD booth number 9.**



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Speaker Abstracts

1st Annual Conference
German Stem Cell Network

November 11-13, 2013

Keynote Lectures: K1 – K4

K1 Regenerative medicine by somatic stem cells: The paradigm of epithelial stem cells
Michele De Luca

K2 In vivo phenotypic and functional stability of iDA neurons and chemo-genetic enhancement of their therapeutic potential
Vania Broccoli

K3 Cardiomyocytes from human pluripotent stem cells: the new patient in safety pharmacology, drug discovery and disease
Christine Mummery

K4 Design principles of pluripotency (EMBO Lecture)
Austin Smith

Abstract No. K1

REGENERATIVE MEDICINE BY SOMATIC STEM CELLS: THE PARADIGM OF EPITHELIAL STEM CELLS

Michele De Luca [1,*]

[1]Centre for Regenerative Medicine "Stefano Ferrari", University of Modena and Reggio Emilia, Modena, Italy
*Presenting author

Adult stem cells are cells with a high capacity for self-renewal that can produce terminally differentiated progeny. Stem cells generate an intermediate population of committed progenitors, often referred to as transit amplifying (TA) cells, that terminally differentiate after a limited number of cell divisions. Human keratinocyte stem cells are clonogenic and are known as holoclones. Human corneal stem cells are segregated in the limbus while limbal-derived TA cells form the corneal epithelium. Self-renewal, proliferation and differentiation of limbal stem cells are regulated by the ΔNp63 (α, β and γ), C/EBPδ and Bmi1 transcription factors. Cultivated limbal stem cells generate sheets of corneal epithelium suitable for clinical application. We report long-term clinical results obtained in an homogeneous group of 154 patients presenting with corneal opacification and visual loss due to chemical and thermal burn-dependent limbal stem cell deficiency. The corneal epithelium and the visual acuity of these patients have been restored by grafts of autologous cultured limbal keratinocytes. In post hoc analyses, success was associated with the percentage of p63-bright holoclone-forming stem cells in culture. Graft failure was also associated with the type of initial ocular damage and postoperative complications. Mutations in genes encoding the basement membrane component laminin 5 (LAM5) cause junctional epidermolysis bullosa (JEB), a devastating and often fatal skin adhesion disorder. Epidermal stem cells transduced with a retroviral vector expressing the β3 cDNA can generate genetically corrected cultured epidermal grafts able to permanently restore the skin of patients affected by LAM5-β3-deficient JEB. The implication of these results for the gene therapy of different genetic skin diseases will be discussed.

Abstract No. K2

In vivo phenotypic and functional stability of iDA neurons and chemo-genetic enhancement of their therapeutic potential

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Novel genetic methods of direct lineage reprogramming enable the conversion of somatic differentiated cells into functional neurons. Moreover, these genetic procedures can be tailored to generate distinct neuronal subtypes. In this regard, several efforts are in place to produce induced dopaminergic (iDA) neurons suitable for Parkinson's disease (PD) cell therapy and modeling. However, little is known about the phenotypic and functional properties acquired by the reprogrammed neurons after brain transplantation. Here we show that iDA neurons retain a transgene-independent stable phenotype in vitro and in vivo and are able to functionally integrate in the host neuronal tissue exhibiting electrically excitable membrane, synaptic currents and dopamine release. Engrafted iDA neurons substantially alleviate motor symptoms in a PD animal model. Moreover, their beneficial effect can be enhanced remotely through a chemo-genetic approach. These data suggest that iDA neurons should be considered a future resource for PD cell therapeutics and highlight the potential of chemogenetics for enhancing cellular signaling in reprogrammed cell based approaches.

Abstract No. K3

Cardiomyocytes from Human Pluripotent Stem Cells: the new patient in Safety Pharmacology, Drug Discovery and Disease

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Derivation of heart cells from human pluripotent stem cells (hPSC) is an area of growing interest as a way of modelling disease phenotypes and as a platform for drug discovery and toxicity. Applying the underlying developmental mechanisms that control cardiac differentiation to hPSCs through the use of defined culture conditions in vitro is rapidly moving the field forward: cardiomyocyte differentiation is now a fairly efficient and reproducible process. Genetically marked hESCs have been produced in which expression of the green fluorescent protein marker is driven by specific lineage markers like Nkx2.5. We are now using these tagged lines in which GFP to select the progenitors of cardiomyocytes, endothelial cells and smooth muscle cells. Applications of hESC- and hiPSC-derived cardiomyocytes in drug discovery, disease and safety pharmacology will be shown. The field potential of hPSC-CM can be measured using commercially available multi electrode arrays. Systematic generation of dose response curves for cardiac and non-cardiac drugs show that hPSC-CM accurately predict reported drug effects on the human heart. These include blocking the hERG ion channel, resulting in QT-prolongation; this is associated with life-threatening arrhythmias. Dose responses of a wide range of compounds have been shown to predict clinical effects.
Induced pluripotent stem cells (hiPSC) from patients with genetic disease are source of somatic cells that can carry disease mutations and show disease phenotypes. Data generated using isogenic pairs of hPSC bearing the same cardiac disease mutation as well as a comparison of (mouse) iPSC and ESC-derived cardiomyocytes with adult heart cells will be shown.

Abstract No. K4 – EMBO Lecture
Design principles of pluripotency

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Pluripotency is the capacity of an individual cell to produce all lineages of the mature organism including the germline in response to extrinsic cues. In mice and rats this naïve state at the foundation of mammalian development can be captured in culture in the form of self-renewing embryonic stem (ES) cells. Our research is aimed at defining the transcription factor network that sustains the ground state and how this interfaces with extrinsic cues. Relatively homogenous propagation of ES cells is achieved using two selective kinase inhibitors that target the mitogen-activated protein kinase (Erk) cascade and glycogen synthase kinase-3 (Gsk3) respectively. ES cells maintained using the two inhibitors (2i) are anchored in a self-renewing ground state. The cytokine leukaemia inhibitory factor (LIF) potently enhances ES cell self-renewal and also promotes both somatic cell reprogramming to naïve pluripotency and conversion of primordial germ cells into pluripotent EG cells. We have recently identified a critical effector induced by LIF. We are now exploring how the pluripotency transcription factor hub is dissolved in order for ES cells to exit the ground state and embark upon multi-lineage differentiation.

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Abstract No. T01

Loss of Myc activity induces cellular dormancy in ES cells cultured in 2i mimicking the status of diapause embryos

Roberta Scognamiglio [1,*], *Nina Cabezas-Wallscheid* [1], *Sandro Altamura* [2], *Alejandro Reyes* [2], *Larissa Carnevalli* [1], *Wolfgang Huber* [2], and *Andreas Trumpp* [1]

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Mouse ESCs cultured in medium containing fetal calf serum (FCS) plus leukemia inhibitory factor (LIF) are metastable. Substitution of FCS by two inhibitors (2i) of the MAPK and GSK3b pathways reveals ESCs that more closely resemble the naïve state of pluripotency of the inner cell mass of the blastocyst. To genetically address the role of c-Myc and N-Myc in naïve ESCs, both genes were deleted using a Cre/loxP approach. Myc-dKO cells exit the cycle, arrest in a G0-G1 phase without signs of apoptosis and form smaller colonies, remaining undifferentiated with unchanged expression of Oct4, Nanog and Sox2. Whole transcriptome analysis (RNA-seq) confirmed the expression of the core pluripotency network but revealed down-regulation of all metabolic and biosynthetic aspects of cellular physiology in dKO ESCs (cell cycle activity, DNA replication, ribosomal biogenesis and DNA/protein synthesis). The cellular and molecular phenotype of Myc-dKO ESCs is consistent with a status of “metabolic dormancy”. Strikingly, the signature of dKO ESCs is remarkably similar to an expression signature previously reported for diapause arrested pre-implantation embryos (Hamatani et al., PNAS, 2004). In mice, diapause of early embryos is i.e. observed in mothers still feeding a litter and is critical for the isolation of ES cells. Very low expression of networks such as DNA synthesis, cell division, metabolic activity but high activity of the IGF pathway are observed in both diapause embryos and Myc-dKO ES cells. In summary, our data identify c/N-Myc activity as a key element controlling the entire metabolic and proliferative machinery of naïve ESCs without affecting the pluripotency network, thus separating the biological process of self-renewal into two distinctly regulated networks. Moreover, our data raise the possibility that Myc controls the reversible arrest of diapause embryos suggesting that Myc regulates the spectrum of overall cellular activity ranging form dormancy to unleashed metastatic growth.

Abstract No. T02
Epigenetic regulation of lineage commitment

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Mouse embryonic stem (ES) cells are pluripotent and have the potential to generate all cell lineages of an embryo. ES cells are isolate from the inner cell mass (ICM) of the blastocyst. The ICM gives rise to the pluripotent epiblast or differentiates into the extra-embryonic endoderm lineage. Loss of pluripotency of the epiblast during gastrulation coincides with the differentiation of the three germ-layers: endoderm, ectoderm and mesoderm. When cells have made a lineage commitment, transcription programs stably change by epigenetic mechanisms. This means that in homeostasis and under normal physiological conditions most differentiated cell types are unable to dedifferentiate and to change fate to other cell lineages. Formally, reprogramming is possible by nuclear cloning or by forced expression of pluripotency-associated transcription factors, however, these techniques are low-efficient, supporting the hypothesis that epigenetic mechanisms have the potential to stabilize transcription programs. It is currently unclear at what stage of lineage commitment into the embryonic and extra-embryonic endoderm lineages, undifferentiated cells lose their pluripotency. Also the epigenetic mechanisms that mediate this transition are largely unknown.

In order to determine generic and specific epigenetic mechanisms that mediate the exit from pluripotency we performed a comparative analysis of embryonic stem cells that leave pluripotency towards embryonic endoderm. We have investigated the genome-wide transcriptional and epigenomic changes in functionally defined cell populations. Our results indicate that activation of Foxa2, the master transcription factor for mesendodermal lineages coincides with exit from the pluripotent state although core components of the pluripotency transcription factor network are still functional in these cells. We will furthermore discuss the connections between epigenomic and transcriptional changes during endoderm commitment.

Abstract No. T03

Mll2 is the H3K4 trimethyltransferase for bivalent promoters in mouse embryonic stem cells

Sergei Denissov [1], *Helmut Hofemeister* [2], *Andrea Kranz* [2], *Giovanni Ciotta* [2], *Hendrik Marks* [1], *Sukhdeep Singh* [2], *Ashish Gupta* [2], *Davi Coe Torres* [2], *Konstantinos Anastassiadis* [2], *Henk Stunnenberg* [1], and *A. Francis Stewart* [2,*]

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Trimethylation of histone 3 lysine 4 (H3K4me3) at promoters of actively transcribed genes is a universal epigenetic mark and a key product of Trithorax-Group action (1). We report that Mll2, one of the six Set1/Trithorax-type H3K4 methyltransferases in mammals, is required for trimethylation of bivalent promoters in mouse embryonic stem cells. Mll2 is bound to bivalent promoters but also to most active promoters, which do not require Mll2 for H3K4me3 or mRNA expression. In contrast, the Set1C subunit Cxxc1 is primarily bound to active but not bivalent promoters. This indicates that bivalent promoters lose H3K4me3 because they only have bound Mll2 and not Set1C. Removal of Mll1, sister to Mll2, had almost no effect on any promoter unless Mll2 was also removed indicating functional back-up between these enzymes. Except for a subset, loss of H3K4me3 on bivalent promoters did not prevent responsiveness to retinoic acid thereby arguing against a priming model for bivalency. Because Mll2 is essential and the major H3K4 trimethyltransferase during oogenesis and the early cleavages after fertilisation (2), we propose that Mll2 is the pioneer trimethyltransferase for promoter definition in the naïve epigenome and Polycomb-Group action on bivalent promoters blocks premature establishment of active, Set1C bound, promoters.

Roguev A, Schaft D, Shevchenko A, Pijnappel WWM, Wilm M, Aasland R and Stewart AF. (2001) The S. cerevisiae Set1 complex includes an Ash2-like protein and methylates histone 3 lysine 4. EMBO J. 20, 7137-7148.

Andreu-Vieyra CV, Chen R, Agno J, Glaser S, Anastassiadis K, Stewart AF and Matzuk MM. (2010) Mll2 is required in oocytes for bulk histone 3 lysine 4 trimethylation and global transcriptional silencing. PLoS Biology, 17, 8(8).

Abstract No. T04

The KC/IL8-mTOR axis activates stem cell maintenance and chemotherapy resistance of hepatic tumor-initiating cells

Benita Wolf [1], *Christine Falk* [2], *Christina Gilot* [3], *Hakim Djaballah* [4], *Matthias Heikenwalder* [5], *Marc Ringelhahn* [5], *Hidegard Keppeler* [6], *Nisar P. Malek* [6], and *Uta Kossatz-Böhlert* [7,*]

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*Presenting author

We identified a mechanism by which a hepatic stem cell is turned into a tumor stem cell. These tumor stem cells are characterized by an aggressive, invasive growth and display a high chemotherapy resistance. To identify mechanisms, which regulate the resistance in these cells we asked if extrinsic factors may contribute. Interestingly we found that the Keratinocyte Chemoattractant Factor, the mouse homologue of human IL8, is secreted in significant amounts by liver cancer stem cells. To analyze the function of KC/IL8 on mouse hepatic progenitor cells we developed an immunofluorescence based assay, which allowed us to follow the fate of hepatic progenitor cells over time. We found that hepatic progenitors accumulate under KC treatment due to the inhibition of differentiation a process, which is depending on the activity the mTOR kinase. We show that in vitro inhibition of mTOR significantly reduces the number of Doxorubicin negative cells.
Because high serum IL8 levels correlate with a poor prognosis of HCC patients, we were interested if the amount of circulating tumor stem cells is related to serum IL-8 concentrations. We used CD45, CD90, CD44 to identify cancer cells in peripheral blood samples of HCC patients. We could not detect a correlation between tumor size and serum IL-8 levels, but a moderate and significant correlation between the amount of CD44/CD90+ cells in the blood and increased IL-8 serum levels. We next investigated the importance of our findings for the development of new treatment strategies. We used Xenotransplants in which we monitored the growth of tumors generated by HepG2 and HUH7 cells. Animals treated with combination of both, Doxorubicin and the mTOR inhibitor, showed a significant reduced tumor growth compared to control animals.
Our results closely link stemness to chemotherapy resistance and argue for a druggable chemoresistance pathway in liver cancer stem cells.

Abstract No. T05

The role of EVI1 in breast cancer cells

Hui Wang [1,*], *Selina Reich* [2], *Lothar Kanz* [2], and *Claudia Lengerke* [1]

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The transcription factor EVI1 has been mainly studied as a gene expressed in long-term repopulating HSC and an oncogene in myeloid leukaemia where high expression was found to predict poor outcome. EVI1 expression has also been reported and associated with high-risk disease in a variety of other tumour types, including breast cancer (Patel 2011). Here, we explore the functional role of EVI1 in breast cancer and its relationship to breast cancer stem cells (CSCs).
qRT-PCR and western blot analysis show robust expression of EVI1 and its isoforms (ΔEVI1, MDS/EVI1) in breast cancer cell lines and patient samples. EVI1 expression was modulated in three breast cancer cell lines by transduction with inhibitory siRNAs or lentivirus mediated shRNAs. Knockdown of EVI1 in these cells affects cell proliferation by inducing a G0/G1 arrest. Consistently, EVI-1 knockdown cells showed reduced tumor growth compared to control cells after in vivo xenotransplantation in NSG mice. Furthermore, EVI-1 knockdown cells displayed enhanced apoptosis sensitivity in response to both intrinsic and extrinsic stimuli, as previously shown in ALL (Konantz 2013). Surprisingly, modulation of EVI1 expression did not significantly alter expression of tumor stem cell antigens (CD24, CD44), in vitro tumor spheres formation capacity or gene expression of previously reported CSC markers (SOX2, OCT3/4 or ALDH1). Microarray gene expression profiling, however, revealed a significant over-representation of genes involved in cell communication in control versus EVI1 knockdown cells: Sema4D, for example, which regulates angiogenesis and progression of breast cancer tumor cells by modulating their interaction with the microenvironment or FBLN1, which was shown to act as an inhibitor of in vitro cell adhesion and motility. Taken together, our findings indicate that, in breast carcinoma, EVI1 acts as an oncogene influencing tumor cell proliferation, apoptosis and cell communication with the environment, but does not specifically regulate the cancer stem cell compartment.

Patel et al, Control of EVI-1 oncogene expression in metastatic breast cancer cells through microRNA miR-22, Oncogene, 2011
Konantz et al, EVI-1 modulates leukemogenic potential and apoptosis sensitivity in human acute lymphoblastic leukemia, Leukemia, 2013

Abstract No. T06

Patient derived models for pancreatic cancer reveal four molecular subtypes associated with differential drug response and clinical outcome

Christian Eisen [1], *Elisa Noll* [1,*], *Elisa Espinet* [1], *Albrecht Stenzinger* [2], *Franziska Zickgraf* [1], *Jan Engelhardt* [1], *Teresa Rigo-Watermeier* [1], *Thomas Hoefner* [1], *Vanessa Vogel* [1], *Corinna Klein* [1], *Peter Neuhaus* [3], *Marcus Bahra* [3], *Markus Buechler* [4], *Jens Werner* [4], *Wilko Weichert* [2], *Andreas Trumpp* [1], and *Martin Sprick* [1]

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Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive disease with dismal prognosis. Despite the discovery of several promising drug candidates, recent trials with targeted therapies have shown limited or no benefit. Subclasses have been recognized for a range of cancers. Although three molecularly distinct subtypes have been described for PDAC, it is therapeutically still treated as a single disease. Hence, one of the key challenges for effective treatments for PDAC is to faithfully recapitulate the human disease in order to facilitate the development of personalized treatment strategies. To approach this, we established a patient-derived PDAC model system that allows stratified analysis of the three human PDAC subtypes: classical, exocrine-like and quasi-mesenchymal (QM); both in vitro and in vivo. Especially noteworthy is the first functional characterization of the exocrine-like subtype for which no tumour cell line has been reported so far. Additionally, using a combined in silico and in vitro approach a two-marker-set was established to perform patient stratification by routine immuno-histopathology. These three subtypes differ in survival and drug sensitivity towards frontline therapies, with the exocrine-like subtype demonstrating resistance towards all standard treatments. Interestingly, we found a significant variability in the drug response within the QM subtype. Based on the differential drug sensitivities, we were able to further refine the QM subtype into a responsive (QM-R) and a non-responsive (QM-NR) subtype. Molecular analyses of these novel subtypes revealed differential expression of multiple interferon-responsive genes and significant enrichment of several immune- and interferon-related gene signatures. Using a gene signature of multiple immune-regulatory genes the existence of both QM-NR and QM-R subtypes was validated in larger patient cohorts. Collectively, our patient-derived model system preserves all known PDAC subtypes, providing the first opportunity to characterize the full spectrum of this disease, unravelling novel drug targets to improve personalized patient care.

Collisson, E. A. et al. Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy. Nature medicine 17, 500-503, (2011)
Eisen et al., to be submitted

Abstract No. T07

A lentiviral RNAi screen against the muscle stem cell proteome identifies novel regulators governing muscle regeneration.

Johnny Kim [1,*], *Ting Zhang* [1], *Stefan Günther* [1], *Krishna-Moorthy Sreenivasan* [1], *Janine Beetz* [1], *Mario Looso* [1], *Marcus Krüger* [1], *Marc Bickle* [2], and *Thomas Braun* [1]

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Skeletal muscle contains a resident population of muscle-specific stem cells known as satellite cells that enable muscle regeneration throughout most of adult life. Hence, satellite cells can serve as a paradigm to uncover regulators that govern the self-renewal and regenerative potential of adult stem cells.

To contribute to a systems level understanding of muscle stem cell mediated regeneration, we determined the protein expression profile of undifferentiated, FACS-purified satellite cells via high-resolution mass spectrometry. By differential display of proteomes of fractionated muscle tissue we identified novel biomarkers of muscle stem cells. Subsequently, we performed a high-content RNAi screen against these genes with an arrayed and redundant lentiviral shrna library of ~2,000 short hairpin RNAs. This approach led to the systematic identification of genes required for muscle stem cell self-renewal or differentiation in vitro. Intriguingly, conditional ablation of hits specifically in adult satellite cells completely abolished muscle regeneration in vivo.

Our findings validate the RNA interference screen and highlight a systematic approach that has unveiled new molecules that govern muscle stem cell self-renewal and tissue-regenerative potential.

Abstract No. T08
Direct lineage conversion of liver to pancreas

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Reprogramming of somatic cells into pancreatic beta-cells represents a promising strategy for cell-based therapy of diabetes. The liver is an ideal tissue source for generating new beta-cells, due to its close developmental origin with the pancreas and regenerative ability. However, the molecular basis of hepatic and pancreatic cellular plasticity is still poorly understood.
Here, we identify the TALE Homeodomain factor TG-interacting factor 2 (TGIF2) as a potential regulator of pancreas versus liver fate decision and capable to promote conversion of liver to pancreas. Upon stable lentiviral expression of TGIF2 in murine embryonic and adult liver cells, we observe 1) repression of the hepatic transcriptional program and 2) induction of pancreatic genes. Importantly, the fate switch between the two lineages is rapid and accompanied by transient cell cycle arrest. Taken together, our results suggest that TGIF2 is sufficient to convert hepatocytes to a pancreatic progenitor state, promoting a binary lineage switch decision rather than induction of hybrid phenotypes. Current efforts focus on studying further differentiation and beta-cell functional properties acquisition of the reprogrammed TGIF2+ pancreatic progenitors in vivo upon transplantation into the mouse. Finally, direct lineage conversion provides a new arena to study the activity of transcription factors and “pioneer” factors. Our study uncovers the reprogramming activity of TGIF2 and investigation into the mechanistic aspects of the liver-to-pancreas lineage conversion will be discussed.

Abstract No. T09

Early Growth Response transcription factors Egr1 and Egr2 regulate lineage commitment of bone marrow mesenchymal stromal progenitors

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Bone marrow stroma contains a complex of mesenchymal progenitors generating cells of skeletal tissues, such as osteocytes, chondrocytes and adipocytes. These progenitors are difficult to expand ex vivo and in order to characterize them on the molecular level we generated transgenic mice that carry a conditional immortalization system based on the expression of SV40 Large T-Antigen regulated by Dexamethasone and Doxycycline. We established single-cell derived bone marrow mesenchymal stromal cells (BM-MSC) subpopulations by limiting dilutions, and screened them for the potential to generate osteo- ("O"), adipo- ("A") and chondrocytes ("C"). We identified seven types of progenitors: tripotential ("OAC"), bipotential ("OA", "OC", "AC") and monopotential ("O", "A", "C") and characterized their transcriptome using Illumina RNAseq. We identified distinct transcriptional signatures for the osteogenic and adipogenic lineages (all clones that contain "O" or "A" property, respectively), and found that progenitors with both potentials ("OAC" and "OA") combine those signatures, and do not express unique genes. Based on the RNA profiling analysis, we selected differentially expressed members of signaling pathways and examined their role in the commitment of "O" and "A" clones. From the chosen candidates only IFNg and Wnt3a had a dual effect on the osteogenic and adipogenic differentiations by promoting the "A" clones to generate osteoblasts and inhibiting their adipogenic potential. RNAseq analysis revealed that "O" progenitors express lower levels of early growth response genes Egr1 and Egr2 than "A", and that treatment with IFNg or Wnt3a resulted in downregulation of Egr1 and Egr2 in "A" cells. Knockdown of Egr1 or Egr2 using shRNA abolished the adipogenic potential and promoted osteogenesis in "A" progenitors. These results lead us to propose that the two pathways (canonical Wnt and INFg) regulate cell fate choice between osteogenic and adipogenic lineages in BM-MSCs, acting at least in part, via attenuation of Egr1 and Egr2 levels.

Abstract No. T10

Transplantation of Genetically Corrected Human iPSC-Derived Neural Stem Cells in a Mouse Model of Metachromatic Leukodystrophy

Jonas Doerr [1,*], *Annika Böckenhoff* [2], *Benjamin Ewald* [1], *Jérôme Mertens* [3], *Julia Ladewig* [1], *Ulrich Matzner* [2], *Matthias Eckhardt* [2], *Volkmar Gieselmann* [2], *Oliver Brüstle* [1], and *Philipp Koch* [1]

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Metachromatic leukodystrophy (MLD) is a lysosomal storage disorder resulting from a deficiency of arylsulfatase A (ArsA), an enzyme which catalyzes the first step in the degradation of 3-O-sulfoglactosylceramide (sulfatide). This membrane lipid is found in various cell types, including certain types of neurons and, in particularly high concentrations, in myelin. For this reason, sulfatide storage in MLD mainly affects oligodendrocytes and Schwann cells, causing a progressive demyelination. Several therapeutic approaches have been explored, including bone marrow transplantation, autologous hematopoietic stem cell-based gene therapy, enzyme replacement and substrate reduction. Although positive effects were reported for some somatic tissues and the peripheral nervous system, long-term treatment of the blood-brain-barrier-protected central nervous system remains a challenge. In previous work we could demonstrate that mouse embryonic stem cell (ESC) derived glial precursors overexpressing human ArsA (hArsA) lead to a reduction of sulfatide deposits in the vicinity of engrafted cells (Klein et al., Gene Therapy 2006). We set out to translate this approach to iPS cell-derived neural stem cells (lt-NES cells; Koch et al., PNAS 2009) generated from MLD patients. MLD lt-NES cells equipped with a lentivirus-based hArsA overexpression system showed high enzymatic ArsA activity. Immunodeficient ArsA-deficient MLD mice were transplanted with MLD lt-NES cells expressing either GFP or GFP and hArsA, or with GFP expressing control lt-NES cells. While MLD lt-NES cells without the enzyme failed to improve the sulfatide accumulation phenotype, wt control cells sufficed to reduce size and amount of immunoreactive sulfatide deposits. The most prominent effect was obtained after transplantation of hArsA overexpressing MLD lt-NES, where sulfatide deposits were decreased in direct vicinity of the graft up to 400 fold with the effect declining with increasing distance to the graft. Thus, lt-NES cells generated via reprogramming from MLD patients can be engineered to ameliorate sulfatide accumulation in the ArsA-deficient brain.

Klein et al., Gene Therapy 13:1686-95, 2006
Koch et al., PNAS 106:3225-30, 2009

Abstract No. T11

MicroRNA-199a-5p: A novel regulator of differentiation of hepatocytes from embryonic stem cells

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The complex process of hepatocyte formation from embryonic stem cells (ESCs) is controlled at the transcriptional and posttranscriptional level. MicroRNAs (miRNAs) are posttranscriptional regulators, which have been reported to regulate hepatocyte cell fate during liver development. We aimed to identify and analyze hepatogenic miRNAs for their potential to improve hepatocyte differentiation from ESCs. We profiled miRNAs from ESCs, hepatocyte-like cells (HLCs) differentiated from ESCs, fetal liver cells and murine adult primary hepatocytes to identify candidate hepatogenic miRNAs. We found that inhibition of miR-199a-5p in HLCs, where it is highly expressed, facilitates efficient hepatocyte differentiation from mouse as well as human ESCs. Furthermore, miR-199a-5p inhibition in human ESCs-derived HLCs enhanced the engraftment and repopulation capacity in the liver of immunodeficient fumarylacetoacetate knockout mice. Our findings demonstrate that miR-199a-5p inhibition in mouse as well as in human ES-derived HLCs leads to improved hepatocyte differentiation. Upon transplantation, ESCs-derived HLCs are able to engraft and repopulate the liver of immunodeficient fumarylacetoacetate knockout mice. We provide evidence that miRNA modulation serves as a promising approach to generate hepatocytes from stem cell sources for the treatment of liver diseases.

Abstract No. T12

Successful treatment of therapy-refractory acute Graft-versus-Host Disease with mesenchymal stem cell-derived exosomes

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Graft-versus-Host Disease (GvHD) is a major cause of morbidity and mortality after allogeneic stem cell transplantation. Several studies reported a beneficial effect of systemically applied mesenchymal stem cells (MSCs) for preventing or treating acute GvHD. Apparently MSCs secrete immune-modulatory factors, which impair inflammation and thus help to suppress GvHD. Even though the nature of these factors remains elusive, small MSC-derived membrane vesicles, exosomes, might exert these functions. These small membrane vesicles are released by a huge variety of different cell species, including MSCs.
Aiming to treat a 22-years female patient suffering from severe therapy-refractory cutaneous and intestinal GvHD grade IV with MSC-derived exosomes, exosomes from bone marrow derived MSCs of four different unrelated stem cell donors were enriched. The immune modulatory properties of the exosome preparation with the highest content of anti-inflammatory cytokines were confirmed in vitro, before it was administered in escalating doses to the patient. Following application of third-party MSC-derived exosomes the patient revealed significant clinical improvements of the GvHD symptoms. Apart of showing that exosome-administration did not cause any unwanted side-effects, our data propose MSC-derived exosomes as a promising new tool to treat in GvHD and maybe other immune-reactive diseases.

Abstract No. T13

Analysis of Signaling Framework Governing Cardiac Specification of Human Pluripotent Stem Cells

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Human pluripotent stem cells (hPSCs) are capable of differentiating into diverse somatic cell types, which is driven by extrinsic cues. It is, however, not well understood how specific signaling molecules orchestrate these complex processes.
We sought to investigate how a set of signaling molecules may specifically drive the conversion into mesoderm and, subsequently, into cardiomyocytes. To this end, a simple 2-step protocol was devised to drive cardiac fate from hPSCs at efficiencies up to ~90%, both in 2D and 3D culture settings, under chemically defined conditions devoid of any complex media additives, and with only minimal perturbations to the signaling environment. Overall cardiac induction in this experimental platform was critically dependent on the initial stimulation of the BMP and WNT signaling pathways. Interestingly, these cascades appeared to closely cooperate with one another in that reduced BMP input could be compensated for by increased WNT activity and vice versa (cardiac corridor model). Specific BMP and WNT activity combinations outside this cardiac-permissive range instead gave rise to endodermal and neuroectodermal lineages, suggesting that graded BMP and WNT signals play a key role in determining cell fate of all three germ layers in hPSCs. Subtractive whole-genome expression analysis was then used to identify key downstream target genes of the signaling cues mediating cardiac induction. This revealed that during mesoderm induction, BMP and WNT cooperate in specifically suppressing the pluripotency controlling transcription factor SOX2, whereas inducible SOX2 overexpression in turn abolished cardiac mesoderm formation. In the second stage of the protocol, WNT inhibition was required for subsequent cardiac specification. Unbiased analysis of downstream gene expression revealed that this leads to suppression of the transcription factor MSX1, whereas inducible MSX1 overexpression in turn abolished cardiac specification. We propose that a dual repressive mechanism underlies cardiomyocyte differentiation from hPSCs.

Abstract No. T14

GENERATION OF MATURE MEGAKARYOCYTES FROM HUMAN PLURIPOTENT STEM CELLS BY A TRANSCRIPTION FACTOR MEDIATED FORWARD PROGRAMMING APPROACH

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Human pluripotent stem cells and in particular induced pluripotent stem cells (iPSCs) derived from adult tissue have recently opened novel and promising avenues for cell therapy. Among them, in vitro production from genetically defined iPSC lines of high demand transfusion products such as red blood cells and platelets might ultimately revolutionize the field of transfusion medicine. Yet, major hurdles need to be overcome to achieve this potential, including the development of reliable and efficient GMP compliant differentiation protocols and dedicated cell culture devices compatible with large scale cell production.

We have developed a novel approach to efficiently generate mature megakaryocytes from human induced pluripotent stem cells (hiPSCs) in chemically defined conditions using a forward programming strategy relying on forced expression of selected transcription factors (TFs). Using a transcriptome and protein interactome guided process we generated a rational candidate gene list and experimentally identified a minimal combination of three TFs – GATA1, FLI1 and TAL1 – driving differentiation of a variety of hiPSC lines towards megakaryocytes. Further optimisation of the culture conditions achieved a pure population of highly mature megakaryocytes without sorting procedures (>95% CD41a+ and >50% CD42a+) with up to a 50 times fold increase from the hiPSC input in 20 days using a standardised, small footprint and reduced cytokine setting. Generated hiPSC-megakaryocytes shared key features with their cord blood derived counterpart at the genetic, epigenetic and ultrastructural levels, and are able to release functional platelet like particles in vitro. These results surpass existing protocols offering wider perspectives for biological and clinical applications of human iPSCs derived MKs.

Concurrent work on both improvement of hiPSC MK terminal differentiation and development of physiologically relevant 3D scaffolds for bioreactors should ultimately help us to go a step closer towards in vitro platelet production for transfusion.

Abstract No. T15 / P-T15

A DNA methylation motifs discovery algorithm reveals the crosstalk among DNA methylation, transcription factors and histone marks in pluripotent cells

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Gene expression regulation is gated by promoter methylation states modulating transcription factor binding. The known DNA methylation/unmethylation mechanisms are sequence unspecific, but different cells with the same genome have different methylomes. Thus, additional processes bringing specificity to the methylation/unmethylation mechanisms are required. Searching for such processes, we demonstrated that CpGs methylation states are influenced by the sequence context surrounding the CpGs (Luu et al, 2013). We used such property to develop a CpG methylation motif discovery algorithm. The newly discovered motifs reveal "methylation/unmethylation factors" that could recruit the "methylation/unmethylation machinery" to the loci specified by the motifs. Our methylation motif discovery algorithm provides a synergistic approach to the differently methylated region (DMR) algorithms. Since our algorithm searches for commonly methylated regions inside the same sample, it requires only a single sample to operate. The motifs found discriminate between hypomethylated and hypermethylated regions. The hypomethylation-associated motifs have high CG content, their targets appear in conserved regions near transcription start sites, tend to co-occur within transcription factor binding sites, are involved in breaking the H3K4me3/H3K27me3 bivalent balance, and transit the enhancers from repressive H3K27me3 to active H3K27ac during ESCs differentiation. The new methylation motifs characterize the pluripotent state shared between ESCs and iPSCs. Pluripotent methylomics profiles show higher variability than pluripotent transcriptomics profiles and are more sensible to detected variations between reprogrammed cells than transcriptomics profiles. Thus, methylomics are a more suitable than transcriptomics methods to understand the cellular reprogramming mechanism and disclose the somatic memory. In fact, we found a collection of motifs associated with the somatic memory inherited by the iPSCs from the initial fibroblast cells, thus revealing the existence of epigenetic somatic memory on a fine methylation scale.

Luu PL, Schöler HR, Araúzo-Bravo MJ. Disclosing the crosstalk among DNA methylation, transcription factors and histone marks in human pluripotent cells through discovery of DNA methylation motifs. Genome Research. In Press 2013

Abstract No. T16

Clonal lineage tracing of adult neural stem cells reveals features of adult neurogenesis

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In mammals, adult neural stem cells (NSCs) reside in restricted regions in the forebrain, one being the subependymal zone (SEZ). This NSC population is responsible for life-long generation of a heterogeneous population of neurons destined to the olfactory bulb. While individual NSCs are seen as long-term self-renewing and infrequently dividing, only indirect or population-level data support this view, and it is unclear how dynamics of proliferation and differentiation at the clonal level determine the diversity and magnitude of the output from this neurogenic population. We addressed this issue by tracing the progeny of single adult NSCs of the SEZ under physiological conditions, by performing genetic fate mapping based on the "confetti" multicolor reporter and an inducible Cre expressed in adult NSCs (GLAST::CreERT2).

Our data show that some NSCs repeatedly undergo division, producing multiple waves of progeny, which can remarkably expand at the transient-amplifying progenitor stage. Interestingly, the degree of progeny amplification doesn't carry an obvious lineage-specific signature, suggesting stochastic factors influence this process.

Our observations also indicate that differential abundance of subtype-restricted NSCs contributes to explain the known biases in the composition of the adult-born neuronal population, as we show that individual NSCs are restricted in the subtype of granule neurons (GNs) they produce, generating either deep or superficial GNs, with a preponderance of deep GN-restricted NSCs.

Finally, our data show that some NSCs divide surprisingly often, yet rarely or never in a symmetric self-renewing manner and that, on the other hand, NSC exhaustion may be a common occurrence, at least among the highly active population labeled using our approach.

Overall, our clonal observations provide support for existing concepts regarding lineage progression, help explaining known features of the general adult-born neuronal population, and suggest revisions to long-held ideas about the behavior of NSCs.

Abstract No. T17

THE INFLAMMATORY GENE AKNA, AN AT-HOOK CONTAINING TRANSCRIPTION FACTOR, REGULATES DIFFERENTIATION AND SURVIVAL OF NEURAL STEM CELLS.

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Changes in brain development and neurogenesis have previously been shown to be affected upon activation of inflammatory signaling pathways as a result of maternal inflammation. However, little is known of the role, if any, of these signaling pathways and their down-stream regulators in the absence of apparent inflammation. In the present study we found that acute down-regulation of the inflammatory regulator Akna (an AT-hook transcription factor) altered both survival and differentiation of neural stem cells in the developing mouse forebrain.

We used in utero electroporation of small hairpin-containing plasmids (directed against Akna mRNA) in the cerebral cortex at embryonic day (E) 13 to assess the effect of acute down-regulation of Akna. This approached revealed a dramatic increase in cell death (TUNEL-assay) already two days after electroporation. At this time point as well as 5 days after electroporation the surviving cells were defective in the process of proceeding through the neuronal differentiation cascade (as revealed by an increase in stem and progenitor cells 2 days after electroporation and a decrease in the proportion of the surviving cells being positive for the neuronal marker NeuN after 5 days). This suggested that Akna is necessary for neuronal stem cells to differentiate into neurons in the developing forebrain.

In agreement with the down-regulation results, overexpression of Akna, showed that an increased proportion of the electroporated cells had left the left the stem and progenitor cell stage and become post-mitotic already 24 hours after electroporation. This suggests that Akna is sufficient to induce neuronal differentiation in the embryonic forebrain.

The results from the present study indicate that genes and molecules normally associated with the immune response might play an alternative role during organogenesis and in the regulation of stem cell behavior.

Abstract No. T18

Oligodendrogenic and neurogenic adult subependymal zone neural stem cells constitute distinct lineages and exhibit differential responsiveness to Wnt

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

Abstract No. T19

Induction of nuclear ataxin-3 inclusions in Machado-Joseph disease-specific neurons

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Machado-Joseph Disease (MJD) or spinocerebellar ataxia type 3 is the most frequent form of spinocerebellar ataxia in Europe, Japan and the United States. It is associated with an expansion of the polyQ repeat in the C-terminus of ataxin-3, resulting in ataxin-3 positive neuronal nuclear and cytoplasmic inclusions. Since MJD is a late onset disorder, a key challenge is to model pathological features in a short-lived induced pluripotent stem (iPS) cell-based in vitro system. Previous data showed that glutamate-driven excitation activates calpains in iPSC-derived long-term self-renewing neuroepithelial stem (lt-NES) cell-derived neurons and that proteolytic activity selectively generates SDS-insoluble ataxin-3 microaggregates in MJD neurons (Koch et al., 2011). A drawback of this approach is that induction of the aggregation phenotype relies on mature human neurons expressing glutamate receptors, which requires extended in vitro differentiation of lt-NES cells across at least 4-6 weeks. Here we explore direct modulation of intracellular calcium homeostasis as an alternative route to induce aggregate formation in lt-NES-derived neuronal cells. Using this approach we were able to induce SDS-insoluble aggregates in MJD neurons already at 6 days of neuronal differentiation. Stimulated MJD cultures showed prominent neuronal intranuclear inclusions (NIIs) in up to 80% of the neurons, whereas cultures generated from unaffected donors were free of NIIs. The aggregates were strongly ataxin-3-positive and, similar to ataxin-3 aggregates found in the brain of MJD patients, co-stained with ubiquitin. Our data suggest that dysregulation of intracellular calcium homeostasis offers a rapid avenue to model pathological protein aggregation already at an early stage of neuronal differentiation.

Koch P, Breuer P*, Peitz M*, Jungverdorben J* et al., Nature 2011 "Excitation-induced ataxin-3 aggregation in neurons from patients with Machado–Joseph disease"*

Abstract No. T20

Restoration of GM-CSF depended functionalities in macrophages utilizing patient specific iPSC from a congenital Pulmonary Alveolar Proteinosis patient

Nico Lachmann [1,*], *Adele Mucci* [1], *Christine Happle* [1], *Doreen Lüttge* [1], *Mania Ackermann* [1], *Nicolaus Schwerk*[1], *Martin Wetzke*[1], *Sylvia Merkert*[1], *Axel Schambach*[1], *Gesine Hansen*[1], and *Thomas Moritz*[1]

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Introduction: Pulmonary Alveolar Proteinosis (PAP) due to a deficient GM-CSF/IL-3/IL-5 receptor on monocytes/macrophages (M/M) constitutes a severe lung disease caused by the functional insufficiency of alveolar macrophages, which require GM-CSF signalling for terminal maturation and intracellular processing of phospholipids. While treatment options in PAP are rare, intra-tracheal (i.t.) transplantation of healthy M/M may represent an effective treatment. Thus, we have evaluated the suitability of iPSC-derived M/M for functional disease modelling and (after gene correction) as a donor source for i.t. transplants. Methods and Results: PAP-iPSC were generated from CD34+ bone marrow cells of an GM-CSF α-chain (CSF2RA) deficient PAP patient by OCT4/SOX2/KLF4/c-Myc-based reprogramming and three clones were obtained demonstrating SSEA4/Tra1-60 expression, reactivation of endogenous OCT4, SOX2, and NANOG, OCT4-promoter demethylation, differentiation into cells of all three germlayers, as well as lack of chromosomal abnormalities by fluorescence-R banding and array-CGH. Hematopoietic differentiation yielded M/Ms of typical morphology and phenotype (CD14, CD11b, CD45) for all clones. Upon functional analysis GM-CSF independent characteristics of M/Ms (cytokine secretion, basal phagocytosis) were maintained, whereas GM-CSF dependant functions (CD11b activation, GM-CSF uptake, and downstream signalling by STAT5) were profoundly impaired, thus establishing M/M differentiation of PAP-iPSC as a functionally relevant disease model. When a PAP-iPSC clone was transduced with a 3rd gen. SIN-lentiviral vector expressing a codon-optimized CSF2RA-cDNA from a combined ubiquitous chromatin opening element (UCOE)/ EFS1a-promoter sequence, moderate but stable CSF2RA-expression was observed with no detectable effects on iPSC growth, pluripotency, or differentiation capacity. Furthermore, upon differentiation to M/Ms CSF2RA-expression was maintained and complete reconstitution of GM-CSF dependent functions (see above) was achieved. Discussion: In summary, we have established iPSC-lines from a CSF2RA-deficient PAP-patient. M/Ms differentiated from these cells represent a functional disease model in which complete reconstitution of GM-CSF dependent functions is observed upon CSF2RA gene transfer.

Abstract No. T21

Raman spectroscopy as novel marker for sensitive stem cell identification

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Introduction

Raman spectroscopy (RS) is a highly sensitive analytical method for marker-free and non-invasive identification and characterization of cells.

We demonstrate the feasibility of RS to detect invading glioblastoma cells within engineered neural tissue (ENT), and to discover neuronal differentiation of rat neural precursor cells (NPCs) primed with Transforming Growth Factor (TGF)-β1.

Material & Methods

ENTs were established from human pluripotent stem cells and grown on a semi-permeable membrane. Patient-derived glioblastoma cells were co-cultured on top of the ENTs to allow invasion [1]. After 2 weeks, tissues were fixed and cut into 30um slices. The embedding medium was removed and slices were rehydrated for Raman measurements. Straight glioblastoma spheres and healthy ENTs were tested as internal controls.

NPCs isolated from spinal cord of 3-month old female Fischer 344 rats (Charles River, Sulzfeld, Germany) were grown under proliferation conditions as previously described and treated with 10 ng/ml TGF-β1 on day 1, 4 and 7. Afterwards, samples were fixed with 4% paraformaldehyde for analysis with Raman spectroscopy.

Raman measurements were carried out with the BioRam® system (CellTool GmbH, Bernried). Data processing was performed with customized BioRam®-software followed by statistical Principal Component Analysis (PCA).

Results

Neuronal cells could not be discriminated by bright field microscopy. Raman spectroscopy shows significant differences between neuronal and glioblastoma cells and detects the cell ratio within an unknown mixture. TGF-ß treatment leads to differentiation into electrophysiologically functional neuronal cells [2]. Preliminary Raman measurements indicate spectral differences between treated and untreated cells.

Outlook

RS provides information about the entire metabolome of a single cell that is as characteristic as a “fingerprint”. RS is a novel marker for gentle yet highly specific detection of sensitive neuronal cells and engineered tissue.

Acknowledgement

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S. Koch ; H. Walles ; K. H. Krause ; M. Baquié ; M. L. Hansmann; K. Schütze. Novel cell identification: markerfree and suitable for living cells, Proc. SPIE 8798, Clinical and Biomedical Spectroscopy and Imaging III, 87980J (2013); doi:10.1117/12.2033542

S. Kraus, B. Lehner, N. Reichhart, S. Couillard-Despres, K. Wagner, U. Bogdahn, L. Aigner, O. Strauß. Transforming growth factor-beta1 primes proliferating adult neural progenitor cells to electrophysiological functionality. Glia 61,11: 1767-1783, (2013)

Abstract No. T22

Tet-dependent processing of 5-methylcytosine, epigenetic stability and the maintenance of active higher order structures during differentiation

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Lineage specific differentiation in the developing embryo is accompanied by extensive reprogramming of the epigenome, resulting in the repression of stemness specific factors and the transcriptional maintenance of activated lineage-specific genes. Using the Hoxa cluster as a model system to follow changes in DNA methylation during induced differentiation, we show that the activated part of the cluster becomes increasingly enriched in 5-hydroxymethylcytosine (5hmC), which is paralleled by the reduction of 5-methylcytosine (5mC). Interestingly, this 5mC-5hmC conversion follows closely the colinear activation pattern of the cluster. 5mC can be converted to 5hmC by proteins of the Ten-Eleven-Translocation (TET) family, which either maintains activated transcription or acts as intermediate for demethylation pathways. By analysing tissue samples from Tet2-/- mice, we found the maintenance of activated Hoxa transcription significantly disturbed. In addition, we found that Tet2 deficient mouse embryonic fibroblasts (MEFs) show a greatly reduced differentiation potential. These data suggest that gene specific 5mC-5hmC conversion by Tet2 is crucial for the maintenance of active chromatin states at lineage-specific loci. Currently we analyse if long term maintenance of active higher order structures depends on Tet2-mediated conversion of 5mC to 5hmC.
Mice mutant for either Tet1 or Tet2 are viable, raising the question of whether these enzymes have redundant roles in development. We have therefore generated Tet1 and Tet2 double-knockout (DKO) embryonic stem cells (ESCs) and mice. DKO ESCs were depleted of 5hmC, but retain pluripotency. While a fraction of doublemutant embryos exhibited various developmental defects and perinatal lethality, also viable and overtly normal Tet1/Tet2-deficient mice were born. DKO mice showed reduced 5hmC and increased 5mC levels throughout the genome, in particular abnormal methylation at various imprinted loci. Our data suggests that loss of both enzymes is compatible with development but promotes hypermethylation, compromises imprinting and leads to epigenetic instability.

M.T. Bocker, F. Tuorto, G. Raddatz, T. Musch, F.C. Yang, M. Xu, F. Lyko & A. Breiling (2012). Hydroxylation of 5-methylcytosine by TET2 maintains the active state of the mammalian HOXA cluster. Nat Commun. 3: 818.
M.M. Dawlaty, A. Breiling, T. Le, G. Raddatz et al. (2013). Combined Deficiency of Tet1 and Tet2 Causes Epigenetic Abnormalities but Is Compatible with Postnatal Development. Developmental Cell 24(3): 310-323.

Abstract No. T23

Aldh1b1 regulates the timing in pancreas lineage segregation and beta cell maturation

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Aldehyde dehydrogenase activity is increasingly associated with stem and progenitor cells but its functional significance remains uncertain. We identified Aldh1b1 as a marker for pancreas progenitor cells. Aldh1b1 is exclusively expressed in the emerging pancreatic buds in a Pdx1 dependent manner. Subsequently, Aldh1b1 is expressed in both the tips and trunks of the developing pancreatic epithelium and its expression in the latter is Ngn3 dependent. During late fetal development Aldh1b1 expression persists only in acinar progenitors where it is extinguished by birth. Expression in the adult persists in very rare centroacinar-like cells but the number of Aldh1b1+ cells expands greatly during the regenerative response that follows streptozotocin or caerulein treatment. Thus Aldh1b1 expression is associated with pancreas stem and progenitor cells during development and in the adult.

To address the role of Aldh1b1 in pancreas development we generated a lacz knock-in mouse strain. Aldh1b1 null mice are viable and fertile but defects in pancreas morphogenesis are evident already during embryo development. The timing of the emergence of endocrine progenitors and differentiated acinar and ductal cells is altered in the null embryos. In newborn null animals, islet morphology and insulin expression are disrupted and this is not corrected during postnatal development. Beta cell mass is not significantly affected in the adult null mice but there is a shift towards larger islets and this is coupled with both increased mitotic activity and cell death in the islets. Accordingly, there is a disruption of islet architecture, lower ratio of mature to immature secretory granules in the beta cells as well as associated glucose intolerance and age dependent onset of hyperglycaemia.

Therefore, Aldh1b1 regulates the timing of pancreas lineage specification and this regulation is important for subsequent functional maturation of beta cells. Incomplete maturation manifests later in life with hyperglycaemia and glucose intolerance.

Abstract No. T24

A Transitional Extracellular Matrix Instructs Regenerative Cell Behavior

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The ability to functionally repair tissues damaged by disease or injury remains a significant challenge for regenerative medicine. Using the newt, a salamander that naturally regenerates lost structures and injured tissues, my laboratory is investigating the underlying mechanisms that control the differentiated state of the cell and regulate regenerative processes. Using a multi-tissue approach, employing regenerating fore- and hindlimbs, tail, spinal cord, brain, and heart, we identified concerted gene activities indicative of a molecular signature of regeneration. A striking feature shared by the different regenerating tissues in the newt and regenerating cardiac muscle in the zebrafish was an extensive and dynamic remodeling of the extracellular matrix (ECM) at the wound site. In particular, we find that the collagen and laminin-rich extracellular environment of differentiated tissues and cells is rapidly replaced by a transitional matrix rich in hyaluronic acid, tenascin-C, and fibronectin. Using skeletal and cardiac muscle as examples, we present evidence that these regeneration-specific ECM components directly influence the generation and behavior of progenitor cells, including proliferation and migration. Collectively, these results provide a novel understanding of tissue regeneration, suggesting that an evolutionarily conserved, regeneration-specific matrix instructs distinct cell activities that direct in a spatial and temporal sequence the re-building of lost or damaged tissues. Thus, the engineering of nature-tested ECMs may provide new strategic opportunities for the enhancement of regenerative responses in mammals.

Abstract No. T25

The Genome Wide Molecular Landscape of Hematopoietic Stem Cells and their Immediate Progeny

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Dormant hematopoietic stem cells (HSCs) harbor the highest self-renewal activity and generate active HSCs and subsequently multipotent progenitors (MPP) that differentiate into lineage-committed progenitors and finally mature cells. To explore essential HSC features such as multipotency, self-renewal and quiescence, we performed an extensive global analysis combining quantitative proteome and whole transcriptome (RNA-seq) analyses on ex vivo-isolated and FACS-sorted mouse HSC, MPP1, MPP2, MPP3 and MPP4 populations, as previously described by us (Wilson et al., Cell, 2008). By employing stable isotope dimethyl labeling and high-resolution tandem-mass spectrometry, more than 6,000 proteins were identified. Expression profiling highlights energy metabolism, immune response, cell cycle and DNA repair to be modulated during lineage progression. To our knowledge, these data represent the first global protein signature of HSCs defined at this level. Furthermore, using in-depth RNA-seq we achieved more than 11-fold coverage of the genome enabling the robust quantification of 27,000 expressed genes. Bioinformatic analysis reveals specific expression clusters of cell adhesion molecules and TFs and uncovers a novel landscape of 70 differentially expressed lncRNAs in HSC-MPPs. To address differentiation potential of MPP2-MPP3-MPP4 we complement our OMICS data with functional reconstitution experiments. Finally, to identify first markers and molecular pathways distinctly employed by dormant HSCs (dHSCs), dormant/label-retaining versus active/non-label-retaining HSCs were also analyzed by RNA-seq. This revealed striking differences at the transcriptome level between both HSCs. The most significantly enriched clusters in dHSCs include: metabolism, immune response, signaling (TGFb, Kit, senescence/autophagy) and cell surface-receptors. Strikingly, we uncovered 200 receptors expressed in dHSCs representing potential novel surface markers to prospectively identify dHSCs. In summary, the transcriptome and proteome signatures for stemness and multipotency defined in this study represent a novel genome wide resource for the scientific community and will significantly extend the current understanding of HSC-progenitor biology at the global level.

Abstract No. T26

Crosstalk between WNT/β-catenin and IRF8 in hematopoiesis and leukemia

Marina Scheller [1,*] , and *Achim Leutz* [2]

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[2]Max-Delbrueck-Center for Molecular Medicine
*Presenting author

Chronic myelogenous leukaemia (CML) originates from a pluripotent hematopoietic stem cell that carries the oncogenic BCR-ABL kinase translocation. Disease etiology involves progression from chronic phase to fatal blast phase that resists Imatinib treatment. The molecular events involved in disease progression are poorly understood but frequently correlate with activation of β-catenin/WNT signaling and down-regulation of the interferon regulatory factor 8 (IRF8). Targeted mouse genetics and a BCR-ABL-model of CML now revealed crosstalk between both pathways. In normal hematopoiesis, activation of β-catenin up-regulates IRF8, which in turn limits β-catenin functions. In IRF8-deficient animals that develop a myelo-proliferative CML-like disease, hematopoiesis becomes dependent on β-catenin. Combining IRF8 deletion with constitutively activated β-catenin in the murine BCR-ABL leukemia model caused disease progression into blast crisis, enhanced leukemic stem cell function and resistance to the BCR-ABL inhibitor Imatinib. A pre-existing Irf8-deficient gene signature was enhanced by β-catenin, identifying WNT-signaling as an amplifier of gene regulation during shift from chronic phase to blast crisis. Our results thus reverberate down-regulation of IRF8 and activation of β-catenin/Wnt signaling as major events in the induction of the blast phase. Collectively, the results uncover IRF8 as a roadblock for β-catenin-driven leukemia and imply both factors as targets in combinatorial therapy.

Scheller, M., Schönheit, J., Zimmermann, K., Leser, U., Rosenbauer, F., Leutz, A., Crosstalk between Wnt/β-catenin and Irf8 in leukemia progression and drug resistance. J. Exp. Med., (2013), in press
Scheller, M.; Huelsken, J.; Rosenbauer, F.; Taketo, M.M.; Birchmeier, W.; Tenen, G.D. and Leutz, A. Hematopoietic stem cell and multi lineage defects by β-catenin activation. Nature Immunology, (2006), 7:1037-1047

Abstract No. T27

A STEM CELL INTRINSIC SWITCH FROM CANONICAL TO NON-CANONICAL WNT SIGNALLING REGULATES AGING OF HEMATOPOIETIC STEM CELLS

Maria Carolina Florian [1], *Kalpana Nattamai* [2], *Karin Dörr* [1], *Gina Marka* [1], *Yi Zheng* [2], and *Hartmut Geiger* [3,*]

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*Presenting author

Many organs with a high cell turnover (e.g., skin, intestine and blood) are composed of short-lived cells that require continuous replenishment by somatic stem cells1, 2. Ageing results in the inability of these tissues to maintain homeostasis and it is believed that somatic stem cell ageing is one underlying cause of tissue attrition with age or age-related diseases like leukemia. Ageing of haematopoietic stem cells (HSCs) is associated with impaired haematopoiesis in the elderly3-6. Despite a large amount of data describing the decline of HSC function upon ageing, the molecular mechanisms of this process remain still largely unknown, which precludes rational approaches to attenuate stem cell ageing of target aging-associated leukemia.
Here we report an unexpected shift from canonical to non-canonical Wnt signalling due to elevated expression of Wnt5a in aged HSCs that causes stem cell ageing. Wnt5a treatment of young HSCs induces ageing associated stem cell apolarity, reduction of regenerative capacity and an ageing-like myeloid-lymphoid differentiation skewing via activating the small RhoGTPase Cdc42. Conversely, Wnt5a haploinsufficiency attenuates HSC ageing, while stem cell intrinsic reduction of Wnt5a expression results in functionally rejuvenated aged HSCs. In summary our data demonstrate a critical role for stem cell intrinsic non-canonical Wnt5a signalling in HSC ageing.

Impulse Talks: I1 – I6

I1 Alternative career pathways for life scientists
Thorsten Abs

I2 Horizon 2020 – News on EU research funding
Jürgen Sautter

I3 EuroStemCell toolkit for public engagement: Information, education, conversation
Emma Kemp

I4 Ethical aspects of innovative therapies
Gisela Badura-Lotter

I5 Stem cell technologies
Frank Emmrich/Andreas Bosio

I6 Implementation of cell- and gene therapy for clinical application: challenges and regulatory requirements
Christine Günther

Abstract No. I1
Alternative career pathways for life scientists

Thorsten Abs [1,*]

[1]academics GmbH, *www.academics.de*
*Presenting author

Abstract not available.

Abstract No. I2
Horizon 2020 – News on EU research funding

Jürgen Sautter [1,*]

[1]European Commission
*Presenting author

In June 2010, Europe's leaders presented the Europe 2020 Strategy, a roadmap to put research and innovation at the forefront of Europe's efforts to transform the economy and provide growth and jobs. More funding will be provided through Horizon 2020 (H2020), the new EU programme for research and innovation that will start at the beginning of next year.

One of H2020's biggest innovations is to bring together, for the first time, all EU research and innovation funding in one place. Another important change from the previous Framework Programmes is that H2020 covers the whole innovation chain from basic research to products. Financed through the Multiannual Financial Framework 2014-2020, H2020 should have a budget of € 79 billion*.

Health and Life Sciences will be tackled in H2020 as a priority in the societal challenge "Health, Demographic Change and Wellbeing". This part of the programme will be funded with a total of € 7.4 billion*. Research under this challenge will have to consider an ageing population, pursue the path to more personalised medicine, enhance translational research, encourage private sector capability, coordinate national efforts, reduce costs, and expand global cooperation.

The diversity of challenges in the health and life sciences sectors may require flexible instruments to tackle them on all levels and at different scales. Funding will be provided through focused research on individual basis (e.g. ERC) or collaborative efforts on different level (e.g. collaborative research projects, public-private partnerships, ERA-NET).

Collaborative research projects will be based on non-prescriptive topics without fixed budget indication, easy two-stage application procedure, simplified set of rules and simplifications for reimbursement of costs. Horizon 2020 will provide opportunities for research to tackle biomedical challenges including in the area of regenerative medicine and stem cell research, which will be presented.

*Pending final adoption by European Parliament and Commission

Abstract No. I3

EuroStemCell Toolkit for Public Engagement: Information, Education, Conversation

Emma Kemp [1,*]

[1]EuroStemCell, www.eurostemcell.org
*Presenting author

EuroStemCell is an EU-funded partnership of scientists, clinicians, ethicists, social scientists and science communicators from across Europe. Our aim is to help Europe's citizens make sense of stem cells – providing information, supporting education and encouraging conversation about all aspects of stem cell biology and its impact on society. Our multilingual website eurostemcell.org receives more than 10,000 visitors per week and includes an ever-increasing range of materials in German, delivering:

- reliable information for non-specialists about the latest research;
- road-tested educational and engagement tools – for schools, open days and public discussion events.

We are keen to explore opportunities to collaborate with other relevant organisations such as the German Stem Cell Network, to maximize the impact we can all achieve together. In this impulse talk, you will learn more about our educational Toolkit and how it can support public events and activities.

Abstract No. I4
Ethical aspects of innovative therapies

Gisela Badura-Lotter [1,*]

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*Presenting author

Abstract not available.

Abstract No. I5
Stem cell technologies

Frank Emmrich [1,*], and *Andreas Bosio* [2]

[1]University Leipzig - Translationszentrum für Regenerative Medizin (TRM)
[2]Miltenyi Biotec GmbH
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Abstract not available

Abstract No. I6

Implementation of cell- and gene therapy for clinical application: challenges and regulatory requirements

Christine Günther [1,*]

[1]Apceth GmbH & Co. KG, Ottobrunn; *www.apceth.com*
*Presenting author

Abstract not available.

Industry Talks: C1 – C9

C1 Development of a new serum-free media for the cultivation of pluripotent stem cells.
Bärbel Icheln

C2 Novel reagents for pluripotent stem cell research and differentiation to neural lineages
Mohan C Vemuri

C3 Gene expression analysis down to the single cell level by digital quantification utilizing the nCounter system
Maik Prüß

C4 Flow cytometry applications for isolating and analyzing complex heterogeneous stem cell cultures
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C5 Suspension culture and cardiomyogenic differentiation of human pluripotent stem cells in stirred bioreactor systems
Ruth Olmer

C6 Pharmaceutical stem cell therapy for advanced diseases entering clincial application
Veronika Reiter

C7 Discover a new approach to single-cell genomics with the C1™ single-cell auto prep system
Fluidigm Europe B.V

C8 The IncuCyte system - new applications for iPSC production
Peter K. Djali

C9 Development of extracellular matrices for stem cell culture
Maja Petkovic

Abstract No. C1

Development of a new serum-free media for the cultivation of pluripotent stem cells.

Bärbel Icheln [1,*], and *Rick I. Cohen* [2]

[1]PeproTech GmbH, Hamburg, Germany
[2]Rutgers University, N. J., USA
*Presenting author

INTRODUCTION:
Pluripotent stem cells have the potential to play a key role in the treatment of many diseases, and are therefore the main target of many ongoing research projects. In order to work with these cells media are needed that sustain these cells in their pluripotent state.
To induce the differentiation of stem cells into more specialized cells that are able to cure the targeted defect or disease, high quality cytokines and growth factors are needed.
OBJECTIVE:
In cooperation with the Rutgers stem cell course (University of New Jersey, USA), PeproTech has developed a novel serum-free and insulin-free media for the cultivation of pluripotent stem cells. This media has been tested extensively regarding f. e. the competence to propagate initial IPSC colonies, the growth of cells on Matrigel™ and Peprotech's xeno-free Vitronectin. immunostaining for pluripotency markers (Oct4, Nanog, Tra-1-60), flow cytometry for pluripotency markers (SSEA4/Oct4) and testing on other PPSC lines (hESCs).
In addition PeproTech will offer a range of ancillary proteins that are Animal-derived Component Free and manufactured in compliance with GMP, which simplifies the use of these cytokines in ex vivo manufacturing processes, as described in USP Chapters 92 and1043.
SUMMARY:
PeproTech's novel stem cell media was found to be suitable for the culturing of hESCs and iPSCs in the undifferentiated, pluripotent state (SSEA4+/Oct4+), and demonstrates less than 15% spontaneous differentiation as indicated by flow cytometry.
PeproTech's range of ancillary proteins for cellular therapy complements this stem cell media.

Abstract No. C2

Novel Reagents for Pluripotent Stem Cell Research and Differentiation to Neural lineages

Mohan C Vemuri [1,*]

[1]Life Technologies
*Presenting author

Mohan C Vemuri, Ph.D. MBA
Director, Research & Development
Cell Biology & Stem Cell Sciences
Life Technologies
Frederick, MD 21702, USA

Pluripotent stem cells (PSC) are excellent candidates for cell replacement therapy and tissue engineering. In order to efficiently expand and differentiate pluripotent stem cells, reliable reagents that are defined, qualified, and preferably prepared from animal origin–free raw materials are desirable. At Life technologies we have developed; a) Novel reagents for the derivation and culture of human PSC b) Tools for pluripotent stem cell characterization, and c) Efficient Differentiation methods for PSC to neural lineages.

The combination of these reagents enables researchers a novel cell culture platform with serum-free and feeder-free environment that can be adapted for large scale manufacture. Together with the ability to transfer hiPSCs directly into this GMP reagents for large scale expansion, this set of tools helps move the research field one step closer to a GMP'able process to manufacture pluripotent stem cells in large scale and use them in a wide variety of regenerative applications.

Abstract No. C3

Gene Expression Analysis Down to the Single Cell Level by Digital Quantification Utilizing the nCounter System

Maik Prüß [1,*]

[1]Field Applications Support
*Presenting author

The nCounter Analysis System utilizes a digital color-coded barcode technology that is based on direct multiplexed measurement of gene expression and offers high levels of precision and sensitivity (>1 copy per cell). The technology uses molecular barcodes and single molecule imaging to detect and count hundreds of unique transcripts in a single reaction.

Each color-coded barcode is attached to a single target-specific probe corresponding to a gene of interest. Mixed together with controls, they form a multiplexed CodeSet.

- Multiplex hundreds of gene targets in a single reaction
- High sensitivity (< 1 copy per cell)
- Fully-automated system
- Exceptionally easy-to-use
- No enzymes required to perform assay
- Multiplex 800 regions

With protocols starting from 100ng or less of total RNA, raw cell or blood lysate, and Formalin-Fixed Paraffin-Embedded (FFPE) extracts, the nCounter Gene Expression CodeSets offer the ultimate in sample input flexibility, all with excellent performance.

The introduction of the nCounter® Single Cell Gene Expression Assay greatly expands the types of studies that can be analyzed on the nCounter Analysis System, allowing researchers to address biological questions that have previously been impossible to answer. Entire biological pathways and custom gene signatures can now be studied for single cells without the necessity to match the gene number to the format of a microfluidic PCR consumable.

Geiss GK et al.: Direct multiplexed measurement of gene expression with color-coded probe pairs. Nat Biotechnol. 2008 Mar;26(3):317-25
http://www.nanostring.com/applications/single_cell

Abstract No. C4

Flow cytometry applications for isolating and analyzing complex heterogeneous stem cell cultures

Yannick Marari [1,*]

[1]BD Biosciences
*Presenting author

The differentiation of stem cells often yields inconsistent and heterogeneous cell populations that are problematic for transplantation and quantitative or comparative analyses.

One way to address this challenge is to identify unique combinations of markers to facilitate the isolation of near-pure cell populations from heterogeneous cell cultures. In addition, there is a need for robust and standardized flow cytometry tools and methods for analyzing heterogeneous stem cell cultures.

Applications discussed in this seminar will include:

- Cell surface marker screening for the isolation of hESC-derived neurons by FACS
- Tips and tricks for efficient cell sorting of hESC, hIPSC, NSC and neurons
- Quantification of differentiation status by flow cytometry
- Assessment of MSC phenotype by flow cytometry

Abstract No. C5

Suspension culture and cardiomyogenic differentiation of human pluripotent stem cells in stirred bioreactor systems

Ruth Olmer [1], *Sebastian Selzer* [2], *Christina Kropp* [1], *Henning Kempf* [1], *Ulrich Martin* [1], and *Robert Zweigerdt* [1,*]

[1]Hannover Medical School
[2]DASGIP / Eppendorf
*Presenting author

Therapeutic and industrial applications of human pluripotent stem cells (hPSCs) and their derivatives require large cell quantities generated in defined conditions. Utilizing the medium mTeSRTM1 (STEMCELL Technologies) we have recently established single cell inoculated suspension cultures of hPSCs (Zweigerdt et al., Nature Prot. 2011), forming aggregates in stirred tank bioreactors (Olmer et al., Tissue Eng. 2012). Since stirred reactors allow straightforward up scaling and comprehensive monitoring of process parameters these systems are widely used for the mass culture of mammalian cell lines aiming at protein production. Application of stirred reactors to hPSC culture, however, is in its infancy. Aiming at low medium consumption but integration of all probes relevant for process monitoring (pO2, pH, biomass) “mini bioreactors” consisting of 4 individually controlled vessels (DASGIP / Eppendorf) were utilized. After establishing stirring-controlled aggregate formation up to 2x108 hiPSCs were generated per run in 100 ml scale applying batch-feeding. Yet, only linear growth rates were achieved resulting a relative low density of ~2x106 hiPSC / ml, suggesting suboptimal conditions. Here we present data on how perfusion feeding can be established in mini-bioreactors resulting in substantially improved process characteristics and cell yields. Cardiomyogenic differentiation in stirred tank reactors was achieved as well. Technical modifications of the system will be highlighted including: impeller design, online biomass sensor integration, establishing a cell retention system and utilization of disposable bioreactor vessel (BioBLU® 0.3) combined with the DASbox® culture control system.
Funding sources: REBIRTH Cluster of Excellence (DFG EXC62/3), BMBF (VDI grant no. 13N12606), BIOSCENT (FP7/2007-2013, grant no. 214539) and StemBANCC (Support from the Innovative Medicines Initiative joint undertaking under grant agreement n° 115439-2, resources of which are composed of financial contribution from the European Union (FP7/2007-2013) and EFPIA companies’ in kind contribution). STEMCELL Technologies (Vancouver, Canada), DASGIP / Eppendorf (Germany)

Scalable expansion of human pluripotent stem cells in suspension culture. Zweigerdt R, Olmer R, Singh H, Haverich A, Martin U. Nat Protoc. 2011 May;6(5):689-700.
Suspension culture of human pluripotent stem cells in controlled, stirred bioreactors. Olmer R, Lange A, Selzer S, Kasper C, Haverich A, Martin U, Zweigerdt R. Tissue Eng Part C Methods. 2012 Oct;18(10):772-84

Abstract No. C6

Pharmaceutical stem cell therapy for advanced diseases entering clincial application

Veronika Reiter [1,*]

[1]apceth GmbH & Co. KG
*Presenting author

Cell-based therapies are expected to revolutionize clinical medicine in the same way as monoclonal antibodies did. Although the idea to use cells as pharmaceutical compounds is not new, the clinical application of the Somatic Cell Therapeutics represent a new challenge for a pharmaceutical applicant with regard to the current regulatory requirements for manufacturing, quality control and clinical trial approval in many parts of the world. The guidelines and standards are currently harmonized within the EU / FDA area of responsibility.
Within the North American / European regulatory area, somatic Cell Therapeutics must comply with the regulations for advanced therapies (ATMP). The GMP-compliant manufacturing and quality control of cell-based products follow the pharmaceutical requirements for medicinal products starting from cell culturing and cell biology, rigorous GMP/GLP standards to regulatory affairs. The procurement, the handling of cells/ tissues and the ex vivo expansion require highly-qualified and continuously trained personnel. The quality control of the cells and cell products comprises the extensive characterization of somatic cells with regard to identity, purity, potency, genetic stability and sterility. The non-cellular components such as media or growth factors have to be vigorously tested. All measures contribute to the significant cost of goods (COGS) for a somatic cell product.
Most somatic cell therapeutics are presently mainly based on adult cells, including mesenchymal (stromal) stem cells (MSC), hematopoietic (progenitor) stem cells (HSC) and even iPS / hES cells as well as tissue specific progenitor cells. Numerous studies with somatic cells have been started with particular attention to the function of (stem) cells in reconstitution and functional integrity of diseased organs, their tissue regeneration capacity (e.g. wound healing, vascularization), immunomodulatory properties (e.g. treatment of autoimmune diseases), induction of immune tolerance (e.g. organ transplantation) and their potential for usage as carriers of therapeutic genes (e.g. cancer gene therapy).

Abstract No. C7

Discover a New Approach to Single-Cell Genomics with the C1™ Single-Cell Auto Prep System

speaker tba [1,*]

[1]Fluidigm Europe B.V., *www.fluidigm.com*
*Presenting author

Fluidigm develops, manufactures, and markets life science systems based on integrated fluidic circuits (IFCs). This technology furthers research by minimizing costs and enhancing sensitivity for applications such as single-cell gene expression, high-throughput SNP genotyping, and next-generation sequencing. Single-cell gene expression profiling has recently emerged as a powerful method to uncover heterogeneity in cell populations. In response to this, Fluidigm has developed a streamlined and automated workflow for capturing and analyzing single cells. The new C1™ Single-Cell Auto Prep System isolates single cells starting with low cell number input.

Abstract No. C8

The IncuCyte system - new applications for iPSC production

Peter K. Djali [1,*], and *Meagan Roddy* [2]

[1]Essen BioScience Ltd.
[2]Essen Bioscience Inc
*Presenting author

With over 200 publications worldwide the IncuCyte is widely known for remote monitoring of cells from within a standard tissue culture incubator. Here we present new work in our lab using the IncuCyte to monitor iPSC reprogramming. The IncuCyte is used to monitor whole wells in multiple vessels at the same time and allow observation of stem cell colony formation from your own desk or another remote location. The ability to mark colonies within the software and place a physical mark on the tissue culture vessel significantly reduces the time cells spend outside the incubator during maintenance and observation. Technician time in observing cells manually is also significantly reduced, representing significant labour savings and technician discomfort working for long periods in a TC hood.

Dr Djali has a background in computing, engineering and biology and is a fellow of the Royal Microscopical Society. He has been an invited speaker on many microscopy, imaging, degree and postgraduate courses. His role, now with Essen BioSience, is to promote new business relationships and partnerships across Europe.

Abstract No. C9
Development of extracellular matrices for stem cell culture

Maja Petkovic [1,*]

[1]AMS Biotechnology (Europe) Limited; *www.amsbio.com*

*Presenting author

Abstract not available.

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Contract GMP manufacturing services and support

Poster Abstracts

Poster session I: P001 – P094

P001 – P019	Pluripotency and embryonic stem cells
P020 – P052	Programing and reprograming
P053 – P072	Stem cells in development
P073 – P081	Stem cells in tissues of neural and non-neural ectoderm
P082 – P094	Stem cells in tissues of meso- and endodermal origin

Poster session II: P095 – P193

P095 – P116	HSCs and stem cells in hematopoietic malignancies
P117 – P128	Cancer stem cells in solid tumors
P129 – P166	Stem cells in regenerative therapies
P167 – P193	Stem cells in disease modeling and drug development

Pluripotency and embryonic stem cells: P001 – P019

P001 Glucose influences embryonic stem cell osteogenic differentiation by affecting nuclear beta-catenin activity
Anke Dienelt

P002 Regulation of Human Embryonic Stem Cell Pluripotency and Self-renewal by a Novel RNA Binding Protein L1TD1
Maheswara Reddy Emani

P003 Towards a mathematical model of pattern formation in mESC cultures
Maria Herberg

P004 Wnt/ ß–Catenin Signaling Regulates Telomerase in Stem Cells and Cancer Cells
Katrin Hoffmeyer

P005 Excision of viral reprogramming cassettes by Cre protein transduction enables rapid, robust and efficient derivation of transgene-free human iPS cells
Asifiqbal Kadari

P006 Cardiomyogenic differentiation of human pluripotent stem cells (hPSC) in defined suspension culture by the application of small molecules
Henning Kempf

P007 E-cadherin and beta-catenin are cleaved by calpain in suspension cultures of human pluripotent stem cells
Sarah A. Konze

P008 The European human Pluripotent Stem Cell Registry and CellFinder: From registry to research tool
Andreas Kurtz

P009 Single-cell analysis of protein dynamics in mouse embryonic stem cells
Carsten Marr

P010 The Histone 3 Lysine 4 Methyltransferase Mll2 Primes Bivalent Promoters for Neural Differentiation
Katrin Neumann

P011 Zfp819, a novel KRAB-zinc finger protein, interacts with KAP1 and functions in genomic integrity maintenance of mouse embryonic stem cells
D.V. Krishna Pantakani

P012 Mad2l2-deficient mouse embryonic stem cells are unstable and deviate into primitive endoderm
Mehdi Pirouz

P013 Two active X chromosomes block exit from the pluripotent state by inhibiting the Fgf-MAPK signaling pathway
Edda G. Schulz

P014 Single-cell quantification of cellular fluorescence and molecular properties in time-lapse microscopy
Michael Schwarzfischer

P015 Evaluation of dynamic aggregate-based suspension cultures for human pluripotent stem cells in a fully automated bioreactor system
Annika Sommer

P016 LINE-1 mediated retrotransposition in human pluripotent stem cells: Consequences for genomic stability of hES and hiPS cells and their derivatives
Anett Witthuhn

P017 Primate iPS cells as tools for evolutionary analyses
Stephanie Wunderlich

P018 Polycomb group protein Pcgf6 represses mesodermal- and spermatogenesis-specific genes in ES cells and replaces SOX2 in iPS reprogramming
Daniela Zdzieblo

P019 Male stem cell lines derived from WKY/Ztm rats
Nils-Holger Zschemisch

Abstract No. P001

Glucose influences embryonic stem cell osteogenic differentiation by affecting nuclear beta-catenin activity

Anke Dienelt [1,*], *Kevin Keller* [2], and *Nicole zur Nieden* [2]

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[2]University of California Riverside
*Presenting author

Along with the aging population, the increasing occurrence of diabetes and musculoskeletal degeneration highlights the need to understand the impact of hyperglycemia on the formation and maintenance of musculoskeletal tissue.
Pathological attributes of diabetes includes low bone turnover, increased fracture risk, and malformations of the developing embryonic skeletal system if persistent during pregnancies. Mirroring these embryonic consequences, we have previously demonstrated that in the presence of high glucose concentrations both murine and primate embryonic stem cells (ESCs) are negatively affected in terms of pluripotency maintenance and differentiation along the osteogenic lineage.

Central to the Wnt signaling pathway, beta-catenin is a key regulator of osteogenic differentiation of ESCs and embryonic bone formation. In addition to its normal functions, perturbations to the Wnt signaling pathway have been connected to many pathologies including type 2 diabetes.

Thus, we hypothesized that the osteogenic impairment of embryos of diabetic mothers is directly related to an altered beta-catenin activity during development. Using our established ESC osteogenic differentiation model, we set forth to examine the link between beta-catenin signaling and glucose-dependent changes to osteogenic differentiation.
By comparing murine ESCs differentiating in either high (diabetic) or low (physiological) levels of D-glucose, we were able to ascertain differences in early and late bone cell differentiation. We could show that osteogenesis is diminished in hyperglycemic conditions, which was evident from day 7 of differentiation onward. The reduced formation of osteogenic precursors was further assessed in connection to the involvement of the Wnt signaling pathway. Hence, we examined the nuclear activity of beta-catenin and its interacting transcription factors LEF/TCF and FOXO during the early stages of differentiation. We proved that the beta-Catenin activity is altered during early differentiation events in the two conditions analyzed. Furthermore, we have implicated Akt kinase signaling as a potential mediator of the altered beta-catenin activity.

Abstract No. P002

Regulation of Human Embryonic Stem Cell Pluripotency and Self-renewal by a Novel RNA Binding Protein L1TD1

Maheswara Reddy Emani [1,*], *Elisa Narva* [1], *Nelly Rahkonen* [1], *Kari Nousiainen* [2], *Miro Viitala* [1], *Harri Lähdesmäki* [2], *Riikka Lund* [1], and *Riitta Lahesmaa* [1]

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[2]Department of Information and Computer Science, Aalto University, Konemiehentie 2, FI-02150 Espoo, Finland
*Presenting author

Human pluripotent stem cells (hPCSs), including embryonic and induced pluripotent stem cells (hESCs and hiPSCs), offer a wide range of opportunities for cell therapy and disease modeling. The detailed understanding of the molecular mechanisms regulating the stem cell status is still elusive. We have identified a novel RNA binding protein, L1TD1, with previously unknown function that is highly expressed in hESCs and iPSC, but not in normal somatic cells. Here we demonstrate that L1TD1 regulates self-renewal and is a novel marker for hESCs. L1TD1 is highly expressed in hESCs and rapidly down-regulated during differentiation. Moreover, our results show that silencing of L1TD1 induces downregulation of known regulators of pluripotency OCT4, NANOG and SOX2 leading to differentiation of the cells. L1TD1 is also highly expressed in testicular and colorectal cancers. We have further demonstrated that L1TD1 interacts indirectly (via RNA) with LIN28 and directly with RNA helicase A (RHA), and is required for hESC self-renewal and cancer cell proliferation. We hypothesize that L1TD1, in complex with RHA-LIN28, regulates OCT4 translation in hESCs and plays an important role in maintaining hESCs pluripotency and self-renewal

Närvä E, Rahkonen N, Emani Maheswara Reddy et al.,, RNA-binding protein L1TD1 interacts with LIN28 via RNA and is required for human embryonic stem cell self-renewal and cancer cell proliferation. Stem Cells. 2012 Mar;30(3):452-60.

Abstract No. P003

Towards a mathematical model of pattern formation in mESC cultures

Maria Herberg [1,*], *Ingmar Glauche* [1], *Tuzer Kalkan* [2], and *Ingo Roeder* [1]

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Mouse embryonic stem cells (mESCs) typically form clusters of cells establishing a microenvironment vital for the maintenance of pluripotency and potentially involved in the regulation of cell fate decisions. It appears that differences in the state of an individual cell are not only detectable on a molecular level, but are also reflected in their morphology and the spatial arrangement of multiple mESCs in their colony structures. While mESCs cultured in 2i media form spherical, dens clusters of cells, which express pluripotency factors at constantly high levels, the same cells spread out under LIF/serum forming rather flat and spatially extended colonies, which are characterized by heterogeneous expression patterns.

Our aim is to study the spatial organization of mESCs cultures and to identify potential (cor-)relations between a cell's intrinsic state and its local environment. Therefore, we first developed an intracellular network model, which consistently describes transcription factor dynamics under different culture conditions. We explicitly integrated FGF4/Erk signalling into a network of pluripotency factors (namely Oct4, Sox2, Nanog and Rex1) deriving predictions on the alteration of molecular heterogeneity and state transitions under LIF/serum conditions. Second, to arrive at a more realistic population model incorporating both intra- and intercellular aspects of pluripotency regulation, we are currently analysing live-cell image data on the evolution of mESCs colonies. In particular, we establish an automated colony-tracking framework to objectively quantify changes in structural properties, like shape and internal motion and design measurements describing the spatial heterogeneity in and between cell colonies. Third, we use these analyses to extend our intracellular model by a spatial dimension, incorporate different modes of cellular interaction, and compare it to the experimental results.

The resulting agent-based model eventually allows to test hypothesis about culture-dependent interactions between mESCs and to study the impact of cell properties on pattern formation in mESC cultures.

Abstract No. P004

Wnt/ ß–Catenin Signaling Regulates Telomerase in Stem Cells and Cancer Cells

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ß-catenin is the central player in the canonical Wnt signalling pathway, where it regulates gene expression by binding to transcription factors (TFs). Wnt/ß-catenin signalling has been demonstrated to maintain pluripotency in mouse and primate stem cells. Mouse embryonic stem (mES) cells have characteristically long telomeres which are regulated by the activity of telomerase (tert). Telomerase expression is one of the key features of ES cells and is a prerequisite to undergo unlimited divisions.

Comparing the expression profile of wild-type mES with ß-catenin-/- (ß-cat-/-) mES cells, we found that tert mRNA was significantly reduced in ß-cat-/- cells. Interestingly, a mES cell line harbouring the stabilized form of ß-catenin (ß-catΔEx3/+) expressed an even higher level of tert mRNA as compared to wt mES cells. Importantly, the changes in tert expression resulted in different length of the telomeres. These effects are most likely due to direct transcriptional regulation of tert expression by ß-catenin, as shown by presence of ß-catenin on the tert promoter in chromatin immunoprecipitation assays.

We identified Klf4 as the TF which recruits ß-catenin to activate the tert promoter. Remarkably, in ß-cat-/- cells the tert promoter is silenced as monitored by loss of active chromatin marks. Furthermore, ß-catΔEx3/+ mES cells show a strong increase in active histone marks at the tert promoter compared to wt ES cells. We identified Setd1a, a histone lysine methyltransferase of the trithorax group of proteins, as new direct interaction partner of ß-catenin, providing insight into the mechanism by which ß-catenin promotes tert promoter activation in mouse ES cells.

Finally, the recruitment of ß-catenin to the tert promoter can also be detected in adult stem cells and in human carcinoma cell lines, pointing towards more general role of ß-catenin as the regulator of tert expression.

Abstract No. P005

Excision of viral reprogramming cassettes by Cre protein transduction enables rapid, robust and efficient derivation of transgene-free human iPS cells

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Emergence of induced pluripotent stem cells (iPSC) technology has paved novel routes for regenerative medicine. iPSCs offer the possibilities of disease modeling, drug toxicity studies as well as cell replacement therapies by autologous transplantation. Classical protocols of iPSC generation harness integrative retro- or lenti-viruses. However, the presence of viral transgenes in iPSC is undesirable as it holds the risk of insertional mutagenesis leading to malignant transformation and has also been shown to affect the differentiation potential. More recently, alternative protocols have been explored to derive transgene-free iPSC, such as use of transposons, mRNA transfection, episomal plasmid transfection, and use of non-integrating viruses such as sendai virus. However, the utility of such protocols remains limited due to low efficiency and narrow range of cell specificity. Hence viral transduction still appears the most practical approach regarding reliability and efficacy. In this study we aim at combining the robustness of lentiviral reprogramming with the high efficacy of Cre recombinase protein transduction to readily delete reprogramming transgenes from iPSCs. We demonstrate rapid generation of transgene-free human iPSCs by excising the loxP-flanked reprogramming cassette employing direct delivery of biologically active Cre protein. By genome-wide analysis and targeted differentiation towards the cardiomyocyte lineage, we show that transgene-free iPSCs do resemble more to human ESCs and has better differentiation potential than iPSCs before Cre transduction. Our study provides a simple, rapid and robust protocol for the generation of superior transgene-free iPSCs suitable for disease modeling, differentiation and drug toxicity analysis.

Abstract No. P006

Cardiomyogenic differentiation of human pluripotent stem cells (hPSC) in defined suspension culture by the application of small molecules

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Estimations suggest that more than one billion cardiomyocytes per patient will be required for regenerative therapies aiming at the replacement of cardiac tissue loss after myocardial infarction. Human pluripotent stem cells (hPSC) present an attractive cell source to generate large quantities of functional human cell types in vitro, which will not only enable cell therapies but drug discovery and drug safety assays as well.

For this to occur, efficient hPSC expansion and differentiation processes are mandatory, preferentially under defined and scalable conditions. We have recently developed scalable suspension culture of undifferentiated hPSCs including transition into stirred, controlled bioreactors (1, 2). However, efficient cardiomyogenic differentiation in suspension culture was not described yet. Here we have tested chemical compounds including p38 MAPK- and Wnt- pathway modulators in a multi-well assay and monitored cardiac differentiation by an Nkx2.5-eGFP reporter cell line as well as flow cytometry and qRT-PCR analysis for numerous early and late cardiomyocyte specific markers. Promising compounds which resulted in >60% cardiomyocyte induction in lab-scale suspension culture were applied to hPSC mass suspension cultures in Erlenmeyer Flasks and stirred bioreactors in up to 100 ml culture scale. Up to 90% cardiomyocyte purity was achieved in this process without additional enrichment strategies. The generated cardiomyocytes were characterized by immunofluorescent staining, quantitative gene expression, electrophysiological assessment and were successfully applied for cardiac tissue engineering.

Taken together, we have achieved an efficient hPSC differentiation process based on small molecules enabling the derivation of large amounts of functional cardiomyocytes in defined and scalable suspension culture in stirred bioreactors.

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Abstract No. P007

E-cadherin and beta-catenin are cleaved by calpain in suspension cultures of human pluripotent stem cells

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The desired clinical and industrial application of human pluripotent stem cells has driven the development of suspension culture protocols that enable mass production of cells. Understanding molecular changes that accompany the transfer from adherent to suspension culture is of utmost importance because this information may directly impact on the development of optimized culture conditions. In this study we have assessed gene expression of human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) grown in surface-adherent culture (2D) versus free-floating suspension culture spheroids (3D). We combined a quantitative proteomic approach based on stable isotope labeling by amino acids in cell culture (SILAC) with Deep-Sequencing-based transcriptomics. In accordance with morphological changes, cells in 3D culture showed a significantly reduced expression of proteins forming structural components of cell-cell and cell-extracellular-matrix junctions. However, we also identified a significant upregulation of secreted inhibitors of the canonical Wnt signaling pathway and a reduction in the level of active beta-catenin and in the expression of Wnt target genes. The protease calpain was shown to cleave E-cadherin and beta-catenin under 3D culture conditions. Our data allowed the development of a model in which calpain cleavage of E-cadherin induces the disruption of focal cell-cell contacts and generates a 100 kDa E-cadherin fragment required for the formation of three-dimensional cell-cell contacts in spheroids. The parallel release of beta-catenin and its potential activation by calpain cleavage is counter-balanced by the overexpression of soluble Wnt pathway inhibitors. According to this model calpain holds a key function at the interplay between E-cadherin and beta-catenin-mediated intercellular adhesion and the canonical Wnt signaling pathway. Supporting this model we show that pharmacological modulation of calpain activity prevents spheroid formation and causes disassembly of pre-existing spheroids, thereby providing novel strategies for improving suspension culture conditions for human pluripotent stem cells in the future.

Abstract No. P008

The European human Pluripotent Stem Cell Registry and CellFinder: From registry to research tool

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Human embryonic stem cells and induced pluripotent stem cells have developed into a formidable research tool on the threshold to enter clinical and pharmaco-toxicological application. An overview over the available pluripotent stem cells is required on ethical provenance, source data and scientific quality to advance the knowledgebase and reduce risks pervading the field. The information should be useable for characterization, comparison, standardization and quality control of the cell lines, but also provide information on their application and usage. We present the efforts to establish and develop pluripotent stem cell registries using a European example and relate it to international efforts of banking and registration. We provide an outlook on the development of the registry into a research platform for cells and their derivatives, CellFinder. CellFinder provides an open database and platform for integration of all available data relating to a specific cell type. This allows for their comparison, analysis and classification based on a comprehensive background of curated and validated information.

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Abstract No. P009

Single-cell analysis of protein dynamics in mouse embryonic stem cells

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The role of the key transcription factors Nanog and Oct4 for the maintenance of pluripotency has been scrutinized and modeled in recent years. However, up to now, it was not possible to study the dynamics of protein levels over generations in single cells.
Here, we present the dynamic analysis of two VENUS fusion lines that faithfully report Nanog and Oct levels, respectively. Both transcription factors exhibit considerable heterogeneity in protein expression in steady state. Quantifying protein expression time courses over up to 8 generations allows to study the repopulation dynamics from in silico sortings. For both Nanog and Oct, repopulating the steady state distribution takes many generations. On a population level, we find a good qualitative agreement between a Markov model-based repopulation and the repopulation of protein levels. Thus, all the memory of the protein kinetics is encoded in the present protein state. Similarly, dynamics of compartments of protein expression are well fitted with an exponential decay. We further investigate the onset of Nanog protein expression from negative sorted cells. We find a pronounced heterogeneity in the dynamics of Nanog onsets, refuting the notion of simple dynamic patterns arising from simple regulatory network motifs.

Abstract No. P010

The Histone 3 Lysine 4 Methyltransferase Mll2 Primes Bivalent Promoters for Neural Differentiation

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In embryonic stem cells (ESC) promoters of lineage-specific genes are characterized by transcriptionally activating histone 3 lysine 4 methylation (H3K4me) and repressing H3K27me. These so-called bivalent domains are thought to prime promoters for fast activation or final silencing upon differentiation.

We and others identified Mixed Lineage Leukemia 2 (Mll2) as the H3K4 methyltransferase responsible for bivalent promoters. The knockout of Mll2 in mouse ESC leads to reduction of H3K4me on many bivalent promoters, most of them being neural lineage-specific. Hence, these knockout cells fail to differentiate to neural stem cells (NSC) in vitro due to enhanced apoptosis. Rescuing the cells from apoptosis by over-expression of B cell leukemia/lymphoma 2 (Bcl2) restored proliferation but did not improve differentiation capacity of Mll2 knockout ESC.

Surprisingly, only a minority of Mll2 target genes fail to get up-regulated during neural differentiation despite the reduced H3K4me levels. One of them, nuclear transport factor 2-like export factor 2 (Nxt2) could partially rescue the differentiation defect when over-expressed together with Bcl2.

Mll2 binding to the Nxt2 promoter decreases during the differentiation from ESC over Epiblast stem cells to NSC. Knockout of Mll2 in ESC leads to a reduction of H3K4me that is inherited during following cell divisions and finally prevents up-regulation of Nxt2 transcription in neural precursors. However, when Mll2 is inactivated in NSC where Nxt2 is already highly transcriptionally active, the following reduction of H3K4me at its promoter does not influence mRNA levels.

Thus, Mll2 and its bivalent target gene Nxt2 represent an example where H3K4me indeed functions as priming mechanism that is required for subsequent transcriptional activation of a promoter rather than for fine-tuning or maintenance of transcription levels.

Abstract No. P011

Zfp819, a novel KRAB-zinc finger protein, interacts with KAP1 and functions in genomic integrity maintenance of mouse embryonic stem cells

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Pluripotency is maintained by both known and unknown transcriptional regulatory networks. In the present study, we have identified Zfp819, a KRAB-zinc finger protein, as a novel pluripotency-related factor and characterized its role in pluripotent stem cells. We show that Zfp819 is expressed highly in various types of pluripotent stem cells but not in their differentiated counterparts. We identified the presence of non-canonical nuclear localization signals in particular zinc finger motifs and identified them as responsible for the nuclear localization of Zfp819. Analysis of the Zfp819 promoter region revealed the presence of a transcriptionally active chromatin signature. Moreover, we confirmed the binding of pluripotency-related factors, Oct4, Sox2, and Nanog to the distal promoter region of Zfp819, indicating that the expression of this gene is regulated by a pluripotency transcription factor network. We found that the expression of endogenous retroviral elements (ERVs) such as Intracisternal A Particle (IAP) retrotransposons, Long Interspersed Nuclear Elements (LINE1), and Short Interspersed Nuclear Elements (SINE B1) is significantly upregulated in Zfp819-knockdown (Zfp819_KD) cells. In line with the activation of ERVs, we observed the occurrence of spontaneous DNA damage in Zfp819_KD cells. Furthermore, we tested whether Zfp819 can interact with KAP1, a KRAB-associated protein with a transcriptional repression function, and found the interaction between these two proteins in both in vitro and in vivo experiments. The challenging of Zfp819_KD cells with DNA damaging agent revealed that these cells are inefficient in repairing the damaged DNA, as cells showed presence of γH2A.X foci for a prolonged time. Collectively, our study identified Zfp819 as a novel pluripotency-related factor and unveiled its function in genomic integrity maintenance mechanisms of mouse embryonic stem cells.

Abstract No. P012

Mad2l2-deficient mouse embryonic stem cells are unstable and deviate into primitive endoderm

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Pluripotent mouse embryonic stem cells (ESCs) can undergo infinite self-renewal upon culture in the conventional culture condition (LIF/Serum). In this study, we identified an essential role of the Mad2l2 gene in maintaining pluripotency of ESCs. Mad2l2-deficient ESC lines cultured in LIF/Serum differentiated spontaneously into epithelial-like cells with the molecular characteristics of primitive endoderm. They divided with a differentiation-type cell cycle, failed to incorporate into chimeras, deviated to the extraembryonic lineage at the expense of pluripotency, and manifested an epigenetic configuration distinct from normal ESCs. Differentiation was hampered when the cultures were grown in chemically defined medium supplemented with inhibitors of the Erk pathway and GSK3β (LIF/2i). However, Mad2l2-deficient LIF/2i cells still had a significantly different gene expression profile. ESC-specific interacting partners of Mad2l2 were identified. A model describing the molecular function of Mad2l2 in the regulation of pluripotency will be discussed.

Abstract No. P013

Two active X chromosomes block exit from the pluripotent state by inhibiting the Fgf-MAPK signaling pathway

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During early development of female mouse embryos, both X chromosomes are transiently active. X gene dosage is then equalized between the sexes through the process of X chromosome inactivation (XCI). Whether the double dose of X-linked genes in females compared to males leads to sex-specific developmental differences has remained unclear. Using embryonic stem cells (ESC) with distinct sex chromosome compositions as a model system, we show that two X chromosomes stabilize the naive pluripotent state by inhibiting the Fgf-MAPK signaling pathway. We observe a global impact of X chromosome number on autosomal gene expression, which affects MAPK target genes as well as DNA-methylation sensitive genes due to MAPK-dependent regulation of de novo methyltransferases Dnmt3a/b. Since Fgf signaling is required to exit the pluripotent state, differentiation is therefore blocked in female cells as long as both X chromosomes are active. We show that this differentiation block is released once XX cells have undergone X inactivation, by experiments either triggering XCI precociously or by preventing it. We propose that two active X chromosomes in female cells block differentiation thus ensuring a tight coupling between X-chromosome dosage compensation and development.

Abstract No. P014

Single-cell quantification of cellular fluorescence and molecular properties in time-lapse microscopy

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Continuous, single-cell quantification of molecular properties is crucial when analyzing the dynamic behavior of heterogeneous cell populations and their molecular control. Non-invasive, live and long-term single-cell observation typically results in very large numbers of images for which existing software tools are largely inadequate. Indeed, lack of robust, high-throughput quantification methods poses a major hurdle to the more widespread application of long-term time-lapse microscopy in mammalian cell systems.

We therefore developed a tool for efficient computer-assisted single-cell segmentation and quantification of cellular fluorescence signals, which incorporates sophisticated, automatic background and illumination correction. Importantly, all software modules allow efficient manual data input or correction of computer generated data through graphical user interfaces.

We apply our pipeline to two strains of mouse embryonic stem cells containing either a NanogVENUS or Oct4VENUS fusion protein, respectively. We perform movies with cycloheximide to inhibit protein synthesis. The single-cell resolution allows observing individual protein half-life behaviors and reveals a large heterogeneity for both proteins. However, the overall half-life can be well compared to Western Blot analysis. Furthermore, we see that a subpopulation of cells exist that have a stable fraction of proteins which do not fully decay. Lastly, we apply our toolbox to rare cell populations and determine PU.1 and Gata1 half-lives in primary hematopoietic stem and progenitor cells.

Abstract No. P015

Evaluation of dynamic aggregate-based suspension cultures for human pluripotent stem cells in a fully automated bioreactor system

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The translation of discoveries in fundamental stem cell biology into viable cell technologies is still challenging and requires a broad set of expertise. Since the end of 2010, the multidisciplinary research project StemCellFactory develops an automated system which facilitates the production and differentiation of human induced pluripotent stem cells (hiPSC). An integral part of the StemCellFactory is the development of bioreactor technologies, which enables a standardised and large-scale production of hiPSC for further downstream biomedical applications.
We present the application of a fully controlled fermentation bioreactor (MicroSHAKE, BTS) for the aggregate-based expansion of hiPSC. This reactor represents the miniaturized version of the BaySHAKE bioreactors developed by BTS, which is in use for mass production with animal cells in scales from 2l to 1000l. To establish a scalable bioreactor-based protocol for the expansion of hiPSC, cultivation parameters including inoculation density, rotation speed, medium supply and culture periods were adapted to the MikroSHAKE system on the basis of a protocol developed for the aggregate-based cultivation of hiPSC in the BioLevitator (Poster Andreas Elanzew). Growth analysis revealed a more then 12-fold increase after 7 days of cultivation in the MikroSHAKE. FACS analysis demonstrated that more than 90% of the cells were positive for the pluripotency-associated marker Tra-1-60. Immunocytochemical analysis of replated aggregates confirmed homogenous expression of pluripotency-associated markers Oct4 and SSEA3, while expression of germ layer markers AFP, SMA and Pax6 could not be observed. For monitoring metabolic activity of cells during cultivation within the MicroSHAKE, glucose consumption and lactate production were analysed. Metabolic analysis indicated an initial lag phase (d1–d3), followed by an exponential growth phase (d4–d7), yielding cell numbers up to 1,4 x 10^5 cells/ml. We expect automated bioreactor-based expansion to facilitate the standardized and automated scale-up of hiPSCs and their derivates for further downstream biomedical applications.

Abstract No. P016

LINE-1 mediated retrotransposition in human pluripotent stem cells: Consequences for genomic stability of hES and hiPS cells and their derivatives

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Human pluripotent stem cells (hPSCs) are considered as favourite cell source for regenerative medicine. However, recent findings indicate that potential tumorigenic chromosomal abnormalities and mutations in hPSCs might either be perpetuated from parental cells or arise during their generation, expansion and differentiation. Such mutations could be induced by human non-LTR retrotransposons (LINE-1, Alu, SVA). It has been reported that the reprogramming process towards hiPSCs might enhance the activation of LINE-1 elements. Similar levels of active LINE-1s were found in hESCs and hiPSCs but not in parental cells. Hence, we aim to ascertain whether their mobilization affects the genomic integrity of hPSCs and their differentiated derivatives. This study aims to investigate LINE-1 retrotransposition rates and preferential integration sites in hPSCs and their derivatives. Using a novel retrotransposition reporter assay, we will assess whether LINE-1 activity may cause genetic aberrations. LINE-1 expression levels will be assessed using immunoblottings, immunofluorescence stainings and RT-PCR. Furthermore, we will analyse LINE-1 mediated genomic destabilization and preferential integration sites via Array-CGH and high-throughput sequencing. We adapted retrotransposition reporter vectors to the use in hPSCs and for stable integration into the AAVS1 safe harbour locus. Successful reporter gene expression and retrotransposition in hiPSCs were proven by expression of retrotranspositon dependent G418 resistance. Furthermore, we detected increased LINE-1 protein levels in immunoblottings and immunofluorescence stainings. We successfully proved that retrotransposition of engineered LINE-1s is supported in hiPSCs. Experiments are ongoing to assess retrotransposition rates and integration preferences in hPSCs during long-term culture as well as during differentiation. Furthermore, ongoing experiments will demonstrate whether LINE-1 retrotransposition may induced genetic aberrations and can be considered as one underlying reason for the reported genomic instabilities of hiPSCs and hESCs.

Abstract No. P017

Primate iPS cells as tools for evolutionary analyses

Stephanie Wunderlich [1], *Martin Kirchner* [2], *Beate Vieth* [2], *Alexandra Haase* [1], *Sylvia Merkert* [1], *Jennifer Beier* [1], *Gudrun Göhring* [1], *Silke Glage* [1], *Axel Schambach* [1], *Elisa C. Curnow* [3], *Svante Pääbo* [2], *Ulrich Martin* [1,*], and *Wolfgang Enard* [4]

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Induced pluripotent stem cells (iPSCs) are regarded as a central tool to understand human biology in health and disease. Similarly, iPSCs from closely related species should be a central tool to understand human evolution and to identify conserved and variable patterns of iPSC disease models. Here, we have generated human, gorilla, bonobo and cynomolgus monkey iPSCs. We show that these cells are well comparable in their differentiation potential and generally similar to human, cynomolgus and rhesus monkey embryonic stem cells (ESCs). RNA sequencing reveals that expression differences among clones, individuals and stem cell type are all of very similar magnitude within a species. In contrast, expression differences between closely related primate species are three times larger and most genes show significant expression differences among the analysed species. However, pseudogenes differ more than twice as much, suggesting that evolution of expression levels in primate stem cells is rapid, but constrained. These patterns in pluripotent stem cells are comparable to those found in other tissues except testis. Hence, primate iPSCs reveal insights into general primate gene expression evolution and should provide a rich source to identify conserved and species-specific gene expression patterns for cellular phenotypes.

Abstract No. P018

Polycomb group protein Pcgf6 represses mesodermal- and spermatogenesis-specific genes in ES cells and replaces SOX2 in iPS reprogramming

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Polycomb group (PcG) proteins comprise a large group of evolutionary conserved factors with essential functions for embryonic development and adult stem cell self renewal. PcG proteins constitute multiprotein polycomb repressive complexes (PRC). Two main PRC complexes have been characterized: PRC2 is involved in the initiation of gene silencing, whereas PRC1 participates in stable gene repression. The literature describes the existence of functionally distinct PRC1 complexes that are defined by Polycomb group RING finger protein (PCGF) paralogs. So far, six PCGF paralogs (PCGF 1-6) have been identified. By performing expression analyses of all six PCGF paralogs, we observed that Pcgf6 showed the highest expression level in undifferentiated mouse ES cells and testes. When ES cells differentiate, Pcgf6 expression strongly declined. To investigate the function of Pcgf6, we established dox-inducible shRNA knockdown (KD) ES cells. Following Pcgf6 KD the expression of pluripotency genes decreased, while mesodermal genes were de-repressed. In parallel, Pcgf6-KD ES cells showed increased hemangioblastic and hematopoietic potential consistent with the elevated expression of mesodermal markers. Microarray analyses further revealed de-repression of spermatogenesis-specific genes upon Pcgf6 KD suggesting a role for Pcgf6 during spermatogenesis. Finally, iPS reprogramming analyses showed that PCGF6 can replace SOX2 but not OCT4, KLF4 or c-MYC in the generation of germline-competent iPS cells. Together, these analyses show that Pcgf6 is non-redundantly involved in repressing mesodermal- and testis-specific differentiation programs in undifferentiated ES cells. Additionally, PCGF6 functions up/down stream or in parallel to SOX2 in iPS reprogramming.

Abstract No. P019
Male stem cell lines derived from WKY/Ztm rats

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Extracellular matrix components secreted by feeder cells and 2i medium containing the growth factor LIF and inhibitors blocking the MEK/ERK and GSK3β pathways are essential to maintain rat pluripotent stem cells in vitro. Despite of this substantial progress derivation of rat embryonic stem cells (ESC) from the inner cell mass of blastocysts background resulted in predominantly female ESC lines under these culture conditions independent of the rat genetic background. Therefore, we tested different cell lines as feeder cells to improve the establishment of male ESC lines using blastocytes from our favoured rat strain WKY/Ztm. Optimal clonal growth of undifferentiated WKY-ESCs was supported through the immortalized cell line TRF-O3 isolated from a Dnd1-deficient ovarian teratoma. Pluripotency of the established ESC lines was demonstrated by positive staining for alkaline phosphatase and immunofluorescent stainings for NANOG, OCT4, SSEA-1, SSEA-3 and DDX4/MVH. 80% of the ESCs were diploid and gave rise to tissues derived from all three germ layers in teratoma assays. Moreover, blastocyst injection of male ESCs resulted in germ-line transmissible chimeric rats. Gender determination using the Sry-PCR revealed that up to 20% of the ESC lines were male. Moreover, cultivation of pre-migratory and genital ridge-derived primordial germ cells resulted in 28% and 38% male pluripotent embryonic germ cell lines, respectively. Therefore, the spindle-shaped TRF-O3 were analysed in more detail. Expression of the fibroblast specific genes Col1a2, Vimentin, P4ha2 and smooth muscle α actin characterized TRF-O3 cells as tumor derived myofibroblasts. Moreover BMP4 but no FGF2, SCF and LIF transcription were detected performing by RT-PCR supporting the maintenance of undifferentiated ESCs and EGCs. These results suggested that the optimized culture conditions using TRF-O3 feeder cells and 2i-LIF medium facilitated the establishment of male stem cell lines from WKY/Ztm rats that may represent a superior, permissive genetic background.

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Abstract No. P020

Generation of Transgene-free iPSCs From Parkinson's Disease Patients

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Patient-derived induced pluripotent stem cells (iPSCs) offer exciting potential in both cell therapy and in vitro disease modeling. Efficient reprogramming of patient somatic cells to iPSCs plays a key role in realization of this potential. Many reprogramming methods have been optimized for use with cell lines or robust primary cells, but lead to technical challenges in converting adult or disease somatic cells to iPSCs consistently and with high efficiency. Traditional methods relying on integrating virus or plasmid to reprogram could potentially result in multiple insertions and risk of tumorigenicity. Reprogramming with episomal vectors, mRNAs and miRNAs typically leads to low reprogramming efficiency or requires multiple rounds of transfection. Sendai virus is a negative-strand RNA virus that replicates in the cytoplasm of infected cells and does not integrate into the host genome. Recent literature demonstrates that Sendai virus delivering the four Yamanaka factors is a highly efficient method to reprogram normal human foreskin fibroblasts, peripheral blood mononuclear cells and CD34+ cells to generate integration-free iPSCs. In this study, fibroblasts from skin biopsies of three Parkinson's disease (PD), one Multiple System Atrophy (MSA) and two age-matched control individuals were efficiently reprogrammed to iPSCs using the Sendai reprogramming method. These iPSCs are transgene-free and karyotypically normal, express known pluripotency markers and are able to differentiate into embryoid bodies that present three germ layer lineages. Gene expression profiles clearly distinguished these iPSCs from their parental fibroblasts and demonstrate a high level of consistency with expression patterns from control iPSCs and H9 ESC line. Given the efficiency, speed and ease with which we were able to reprogram adult disease fibroblasts, we anticipate the Sendai reprogramming method being applied to large scale reprogramming of multiple disease lines potentially in an automated fashion.

Abstract No. P021

Detecting gross chromosomal abnormalities from gene expression microarray data

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Gross chromosomal abnormalities can be found in cancer cells as well as in cell cultures undergoing adaptation to cell culture conditions. In the context of reprogramming somatic cells to induced pluripotent cells, the genomic integrity of the donor cells and their resulting iPS cells is challenged by harsh experimental protocols designed to force transcriptional and epigenetic changes in a relatively short time. Although gene expression microarrays were not explicitly developed to detect chromosomal abnormalities, it is possible to use the genome-wide detection of mRNA levels to discern gross duplications and deletions. We have developed a software package in R called CAFE (Chromosomal Aberration Finder in Expression data), which can detect gross chromosomal gains and losses from microarray expression data, with resolution at the chromosome, arm and cytoband level. The analysis basically recapitulates the workflow published by (Ben-David et al. 2013) and has the following basic steps: 1) The user provides CAFE with microarray data from controls and test cases; 2) relative expression for each sample is calculated with respect to the median expression of the entire dataset, and over/under-expressed probesets are flagged; 3) a Fisher test tests for enrichment of over or under-expressed probesets per chromosome, arm or cytoband; 4) relative expression values for test samples can be plotted for chromosomes of interest to visualize areas that were found to be enriched for over/under expression. We stress that CAFE is not intended to replace specific techniques that are used to detect chromosomal aberrations, such as karyotyping or array-based comparative genomic hybridization. Rather, mRNA microarray data could be used to provide some initial insight into the genomic status of the clones in the absence of karyotyping data.

Ben-David U, Mayshar Y, Benvenisty N. Virtual karyotyping of pluripotent stem cells on the basis of their global gene expression profiles. Nat Protoc. 2013 May;8(5):989-97.

Abstract No. P022

A General Strategy for Cellular Reprogramming: the Importance of Transcription Factor Cross-Repression

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Transcription factor cross-repression is an important concept in cellular differentiation. A bistable toggle switch constitutes a molecular mechanism that determines cellular commitment and provides stability to transcriptional programs of binary cell fate choices. Experiments support that perturbations of these toggle switches can interconvert these binary cell fate choices, suggesting potential reprogramming strategies. However, more complex types of cellular transitions could involve perturbations of combinations of different types of multistable motifs. Here we introduce a method that generalizes the concept of transcription factor cross-repression to systematically predict sets of genes, whose perturbations induce cellular transitions between any given pair of cell types. Furthermore, to our knowledge, this is the first method that systematically makes these predictions without prior knowledge of potential candidate genes and pathways involved, providing guidance on systems where little is known. Given the increasing interest of cellular reprogramming in medicine and basic research, our method represents a useful computational methodology to assist researchers in the field in designing experimental strategies.

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Abstract No. P023

Influence of epigenetic memory on neural induction of human induced pluripotent stem cells

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Epigenetic memory in induced pluripotent stem (iPS) cells in regard to their donor cell type of origin could lead to variations in their differentiation capacities. We have generated human iPS cells from fetal neural stem cells (fNSC-iPSCs), hematopoietic stem cells (CD34-iPSCs) as well as dermal fibroblasts (Fib-iPSCs) and evaluated their potential to differentiate into neural progenitor cells (NSCs).

We have performed whole genome expression analysis on enriched populations of nestin-positive and sox1-positive NSCs derived from fNSC-iPSCs, CD34-iPSCs as well as Fib-iPSCs and carried out an analysis of neural and positional marker gene expression in these cells. Here, we found that fNSC-iPSC-NSCs cluster and separate from CD34-iPSC-NSCs and Fib-iPSC-NSCs both in whole genome and in neural gene expression analysis. Our data show that fNSCs and fNSC-iPSC-NSCs express an exclusive set of genes. In addition, we performed whole genome methylation analysis of each donor cell, iPSC as well as NSC population and performed bisulfite sequencing and histone methylation analysis on memory genes to decipher the influence of epigenetic memory on the observed differences in gene expression profiles.

Our data indicate that the origin of cell for reprogramming does not influence the efficiency of NSC-derivation but has impact on the neural identity of iPSC-derived NSCs.

Abstract No. P024

Generation of neural precursor cells from human cord blood

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Reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) represents an attractive approach for generating donor cells for neuro-restorative approaches employing cell transplantation. However, the validation and differentiation of human iPSCs is time-consuming, and extended culture periods may favour the acquisition of mutations. More recently, it has become possible to directly convert somatic cells into induced neurons. While this strategy provides a shortcut by bypassing the lengthy transit through a pluripotent stage, it is hampered by the lack of a scalable intermediate. We set out to address these limitations and used neonatal human cord blood-derived CD34+ cells to explore direct conversion of this easily accessible cell type into induced neural precursor cells (iNPCs). Upon transduction of CD34+ cells with non-integrating Sendai viruses coding for SOX2 and c-MYC we were able to obtain primary iNPC colonies within 20 days. After serial passaging clonal iNPC lines were found to be free of viral replicons and to express typical neural progenitor markers such as PAX6, SOX2, NESTIN and PLZF. INPCs could be expanded for more than 30 passages and were able to differentiate into various neuronal and glial subtypes. Neurons derived from these iNSCs were able to fire action potentials upon current injection and exhibited spontaneous postsynaptic currents indicating formation of neuronal circuits. Conversion of cord blood-derived cells into transgene-free iNPCs may enable the establishment of a patient-specific cellular resource for neural cell replacement and other biomedical applications.

Abstract No. P025

Direct conversion of mouse fibroblasts into neurons without cell divisions

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The investigation of neuron activity and its relationship to disease has been limited by a lack of physiologically relevant cell models. Recently it was found that ectopic expression of the transcription factors Ascl1, Brn2 and Myt1l in fibroblasts generates a mixed population of neuronal cells. This transdifferentiation approach opens broad possibilities for neural tissue repair and cell-based replacement therapies. However, little is known about the nature and the sequence of epigenetic events accompanying this process.

Cell division is a key event in epigenetic reprogramming to pluripotency. To investigate the role of cellular divisions in the transdifferentiation process we studied direct conversion of fibroblasts to neural cells in the presence of the cell cycle inhibitors. We were able to generate neurons from fibroblasts in the presence of the cytostatic agent aphidicolin; however the yield of neurons was low (probably due to a toxic effect of aphidicolin). To improve cell viability and increase the number of transdifferentiated cells we reduced aphidicolin treatment to 2-4 days followed by inhibitor-free cultivation for further 6-8 days. Under these conditions most of the fibroblasts were converted into neurons without cell division, as confirmed by BrdU staining. Moreover, even in the absence of inhibitors some transdifferentiated cells were BrdU negative. We were also able to repeat these experiments using another cell cycle inhibitor, mimosine. We then studied how activation of cell cycle affects transdifferentiation efficiency. We found that forced expression of the cell cycle activator cMyc does not increase the yield of neurons in contrast to previous reports showing improved efficiency of reprogramming to the pluripotent state in the presence of cMyc.

Overall our results indicate that cell divisions are not essential for direct conversion of fibroblasts into neuronal phenotype. However, further studies are required to clarify the details of the cell transdifferentiation process.

Abstract No. P026

A Journey to Pluripotency and Back: Generation of Mesenchymal Stromal Cells from MSC-derived iPS Cells.

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Human mesenchymal stromal cells (MSC) frequently exhibit heterogeneity and lose differentiation potential during expansion in culture. Generation of MSCs from induced pluripotent stem cells (iPS cells) might therefore represent an appealing option to obtain standardized and homogenous MSC populations with potent multilineage differentiation potential. In our recent work, we generated iPS cells from bone marrow MSC (Shao et al., Mol. Therapy 2013). Notably, senescence-associated DNA methylation changes were almost entirely reversed during reprogramming, indicating that iPS cells are rejuvenated on the epigenetic level (Koch et al., Genome Res. 2013). Here, we describe that these iPS cells can be differentiated back into functional MSC-like cells using a simple one-step protocol. This protocol involves the same culture conditions as used for initial MSC culture with human platelet lysate (hPL) as serum supplement. Within two weeks, iPS cell-derived-MSC (iPS-MSC) lost expression of pluripotency markers and displayed a typical fibroblast-like morphology. The immunophenotype of iPS-MSC closely resembled that of primary MSC (CD14−, CD29+, CD31−, CD34−, CD45−, CD73+, CD90+, and CD105low). Furthermore, iPS-MSC were induced to differentiate towards adipogenic, osteogenic, and chondrogenic lineages. Gene expression profiles of iPS-MSC demonstrated that several marker genes were up-regulated similar to parental MSC - particularly genes of mesodermal lineage. However, there were also differences in gene expression profiles of MSC and iPS-MSC. Notably, also DNA methylation profiles of iPS-MSC changed towards those of MSC – and reacquired senescence-associated DNA methylation changes. Our results indicate that iPS-MSC provide an attractive, unlimited and more standardized source of MSC-like cells with new perspectives of use in regenerative medicine.

Abstract No. P027

Generating NSCs from rat fibroblasts: Induced pluripotent stem cell-derived NSCs are more similar to fetal NSCs than directly converted fibroblasts

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The transplantation of fibroblast-derived neural stem cells (NSCs) into animal models provides prototypes of cell therapies for several human neurological disorders. Such syngeneic NSCs would avoid the need for immunosuppression and ensure full compatibility of trophic factors and survival. For the rat, however, generation of induced pluripotent stem (iPS) cells and subsequent neural differentiation has been proven to be challenging. Thus, we evaluated two possible protocols to obtain NSCs from rat fibroblasts.

First, fibroblasts were infected with an inducible OSKM lentivirus and PL-SIN-EOS-S(4+)-EiP pluripotency reporter to generate iPSCs. Neural differentiation was then induced via embryoid body formation. Second, fibroblasts were infected with lentiviruses encoding Sox2, FoxG1-Brn2, and hTERT in a direct conversion approach. At each passage, qRT-PCR analyses and immunofluorescence staining were performed and tripotency was evaluated. Brain-derived fetal rat NSCs served as positive control.

Rat iPSCs were successfully generated and stably expanded. Their differentiation into NSCs improved dramatically by addition of 0.1 µM retinoic acid during the hanging drop period. Also in the direct approach, the infected fibroblasts showed profound morphological changes. Cells of both methods displayed morphological features of NSCs, were highly proliferative and spontaneously formed neurospheres. Immunostaining indicated that they expressed Nestin, Sox2 and Musashi1. However, mRNA expression of Sox2, Mash1, Musashi1 and Pax6 was considerably higher in iPS-NSCs and reached the levels observed in fetal NSCs. Both types of fibroblast-derived NSCs were capable to differentiate into neurons, astrocytes and oligodendrocytes, but neuronal differentiation was remarkably better for iPS-NSCs even leading to synaptophysin expression.

We developed new protocols to derive NSCs from rat fibroblasts and demonstrated that iPS-derived NSCs are superior to directly converted iNSCs. This suggests that more profound (epigenetic) changes occur during reprogramming and may be required to alter cell fate satisfactorily. Currently, additional factors and epigenetic modulations are tested to improve direct conversion.

Abstract No. P028

Direct conversion of adult human dermal fibroblast into hematopoietic cells.

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Abstract

The direct conversion of fibroblast cells to another without an intermediate pluripotent stage is also possible but, at present, requires the viral transfection of appropriate transcription factors, limiting its therapeutic potential.

DNA methylation is a biochemical modification that, in human cells, primarily affects cytosines when they are part of the symmetrical dinucleotide CpG. Methylation of promoter-associated CpG islands is indispensable for maintaining the genes' silenced state. Aza inhibits DNA methyltransferases, and as a result, triggers silenced (methylated) genes. We sought to determine whether Aza could convert easily accessible cells such as human dermal fibroblast cells (HDF) into hematopoietic cells and followed by differentiating culture conditions. Adult human skin fibroblasts were exposed for 18 hours to 1 μM 5-aza. Cells were allowed to recover in embryonic stem cell medium for 3 h, then followed by hemangioblast differentiation protocol that lasted 37 d. we observed the efficient formation of erythroid cells after 37 days. Our findings revealed after 8 days, cells displayed morphological changes. Whereas, most of cells became round on day 24 and about 50% of cells expressed hemoglobin clearly. Additionally, Morphological changes were accompanied by the up-regulation of hematopoietic genes (RUNX-1, CD34, FOG-1 and HbG). This experiment is ongoing in details.

Similarly, we examined potential of OCT-4 protein to induce hematopoietic lineages specially cells as well as OCT-4 gene without having to pass through a pluripotent state which has been already demonstrated. Using OCT4 protein together with specific cytokine treatment induced hematopoietic genes expression, including (RUNX-1, GATA-2, GATA-1, TAL-1 and FOG-1). Gene expression analysis indicated most of genes upregulated after 37 days, are related to early hematopoietic development including RUNX-1, GATA-2 and TAL-1.

In addition, transdifferentiated fibroblasts revealed low to undetectable levels of genes associated with pluripotency, such as NANOG and SOX2. This experiment is ongoing in details.

Abstract No. P029

Differentiation of human iPS cells into definitive endoderm cells using signaling molecules and IDE1, in 2 and 3-dimensional cell culture

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Human induced pluripotent stem cells (hiPSCs) are potentially considered to differentiate into all human cell lineages and hold promise as an unlimited source for cell replacement therapies in clinical applications. Formation of definitive endoderm (DE) is the first and crucial step in the development of visceral organs such as liver, lung, pancreas, etc. Therefore, efficient generation of DE cells ensures the efficient generation of eventual target cells used in cell therapy. In the present study, culturing and differentiation of hiPSCs into derivation of DE cells were investigated on Matrigel coated poly lactic acid/gelatin (PLA/gelatin) nanofibrous scaffolds. Analyses of DE-specific markers including Sox17, FoxA2 and Gooscoid (Gsc) genes in levels of mRNA and protein revealed higher levels of expression in the hiPSCs differentiated cells cultured on PLA/gelatin scaffolds compare to cells differentiated in two- dimensional (2D) system. Our results showed that using small molecules such as inducer of definitive endoderm 1 (IDE1) and other molecular signaling such as Activin A and Wnt3a could induce differentiation of hiPSCs in to DE cells. Also, three-dimensional (3D) culture conditions showed better results in differentiation process compared with 2D one. Activin A and Wnt3a demonstrated more promoting results in differentiation of hiPSCs in both 2D and 3D culture conditions. The results of this study may have impact in tissue engineering and cells replacment therapy of visceral organs-related diseases.

Abstract No. P030

Telomer length is preserved in reprogrammed cells

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Proliferation and differentiation properties of induced pluripotent stem cells (iPSCs) are generally not different from their embryonic counterparts (ESCs). The self-renewal potential is accompanied by increased activity of telomerase reverse transcriptase (TERT) which is responsible for the de novo synthesis of the telomers in highly proliferating cells. In this study we established and compared qPCR, qFISH, and Southern blot analysis in order to analyze the telomeric length of fibroblasts and pluripotent stem cells of mus musculus and rattus norvegicus. There was an increase in the telomer length in reprogrammed fibroblasts of both, mice and rats, compared to the starting cells. Of note, the distribution of the telomer lengths was substantially more spread in the iPSCs compared to fibroblasts (15-50 kbp vs 15-25 kbp in iPSC vs fibroblasts, respectively). Also of note, there was no critical decrease in telomer length of the iPSCs over 30 passages of cultivation. Such self-renewal potential similar to ESCs was further emphasized by similar cell doubling times of < 15 h in iPSCs and ESCs compared to > 30-50 h in fibroblasts. Finally, telomer length was significantly greater in rat embryonic fibroblasts (rEF) compared rat tip tail fibroblasts (rTTF), but only the former could successfully be reprogrammed to become iPSCs. This would suggest that the observed increase in TERT mRNA expression in P5 riPSCs is just too late to compensate for initially critically short telomers. Therefore, these studies help to better understand not only the properties of pluripotent stem cells, but also the programmability of potential somatic starting cells and thus to increase reprogramming efficiency, ultimately. However, if a somewhat “over-compensating” lengthening of the telomers is in fact necessary and if the cells with longer telomers within the inhomogeneous distribution are qualitatively superior remains to be investigated.

Abstract No. P031

Reprogramming of patient-specific B cells for the generation of disease-specific neural cells

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Responding to a growing interest on psychiatric diseases, research on the causal connection to neurologic dysfunction becomes very important. Psychiatric diseases are multifactorial disorders, which also rely on dysfunction of neural cells. Genome-wide association studies indicate that copy number variation (CNVs) and/or single nucleotide polymorphisms (SNPs) are related to appearance, progression, and treatment of psychiatric disorders. To broaden the knowledge on psychiatric diseases and to further analyze the impact of DNA variations and/or mutations, patient-specific iPS cells provide a potent tool for the analysis of psychiatric diseases. Accordingly, Epstein-Barr virus-immortalized B-lymphoblastoid cell lines (B-LCLs) provide a well-characterized pool of somatic donor cells.

B-LCLs obtained from healthy donors were studied after delivery of episomal reprogramming vectors carrying Oct4, Sox2, Nanog, Lin28, Klf4, and c-Myc. Efficiency of vector delivery was analyzed by flow cytometry. Transcript analysis by semi-quantitative PCR included about 30 pluripotency marker genes. Protein expression of crucial reprogramming factors was verified by IF analysis. Reprogramming conditions included feeder cells, different matrices, and hypoxic conditions.

Transcript analysis of about 30 pluriportency-associated marker genes in B-LCLs revealed that crucial reprogramming factors are absent. Episomal reprogramming vectors induced Oct4, Sox2, Nanog, Lin28, Klf4, and c-Myc. Clonal growth and ES cell-like morphology was maintained for more than 10 passages. Episomal vectors induced Oct4, Sox2, and SSEA4. High efficient vector delivery affected cell viability demonstrated by flow cytometry. Feeder cells as well as matrices were suitable for pre-iPS cell formation. Hypoxia reduced number of colonies. Transcript analysis of spontaneously differentiated cells revealed expression of primary germ layer marker genes suggesting that reprogrammed B lymphoblastoid cells are suitable for the generation of neurons and glia cells.

In conclusion, patient-specific B-LCL-derived iPS cells provide a powerful tool to study psychiatric disorders within disease-specific differentiation models.

Abstract No. P032

Better safe than sorry: LINE1 retrotransposon reactivation in human induced pluripotent stem cells and its impact on hiPSC genome stability

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The ability to reprogram human somatic cells to human induced pluripotent stem cells (hiPSCs) represents an invaluable tool for regenerative medicine depicting an autologous source for cell-based therapies. Unfortunately, recent surveys show that hiPSCs in vitro accumulate genomic abnormalities probably aggravating their usage for stem cell therapies. One cause of this genomic instability can be reactivation of human endogenous non-LTR retrotransposons (LINE-1 (L1), Alu, SVA). 35% of human genome sequence is generated by non-LTR retrotransposons. L1 activity can cause various forms of genetic instability including deletions, duplications, chromosomal translocations, and can affect gene expression. Currently, 90 cases of genetic disorders/tumorigenic diseases have been linked to L1 activity. Thus, it is important to understand if L1 activity impacts genomic integrity in hiPSCs.

To test if endogenous L1 activity affects genome stability of differently generated hiPSCs, we analysed CpG methylation of the L1 promoter region by bisulfite sequencing, and expression of functional L1 elements by qRT-PCR and immunoblot analyses. To map individual L1, Alu, and SVA de novo insertions, we used Retrotransposon Capture Sequencing (RC-Seq) applying custom sequence capture arrays targeting full-length L1, Alu, and SVA elements followed by Deep Sequencing. Detected de novo transposition events were validated by insertion site PCR and capillary sequencing.

We see reduced methylation of the L1 promoter in hiPSCs which correlates well with enhanced full-length L1 mRNA and protein expression compared to parental cells. We identified 66 putative de novo L1 and Alu insertions out of which 25 are novel non-reference genome insertions validated via PCR and capillary sequencing. In parallel, we characterize karyotypic abnormalities caused by L1 in hiPSCs applying mFISH, and array-CGH. Taken together, our data demonstrate that reprogramming of human somatic cells into hiPSCs irrespective of the reprogramming method used results in mobilization of endogenous L1 elements which may affect genome stability.

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Abstract No. P033

TaqMan® Human Pluripotent Stem Cell Scorecard

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The revolutionary technology of converting adult somatic cells to pluripotent stem cells has led to the rapid progress of efficient, footprint-free methods for generation of induced pluripotent stem cells (iPSC). The resulting iPSC derived from diverse patient sources using different methods and conditions has created a challenge for rapid and comprehensive characterization. Traditional characterization methods are based on biomarker expression utilizing a combination of in vitro and in vivo cellular analysis to confirm pluripotency and trilineage differentiation potential. Such methods are subjective and not amenable to high throughput confirmation.

TaqMan® hPSC Scorecard™ Panel is a comprehensive gene expression real-time PCR assay that can be utilized for rapid generation of quantitative transcriptome data. High density and medium density gene expression analyses were used to identify the optimal genes that define the pluripotent state and detect early stages of differentiation into cell types representative of the three germ layers. The resulting TaqMan® Human Pluripotent Stem Cell Scorecard consists of 93 assays comprising a combination of controls, pluripotent and lineage specific genes. Resulting expression data is analyzed using cloud-based analysis software that compares the expression pattern against a reference standard generated using multiple functionally validated ESC and iPSC lines. This system was successfully used to test several ESC and iPSC lines to confirm their pluripotency and determine their trilineage differentiation potential.

Block, C. etal (2011) Reference Maps of Human ES and iPS Cell Variation Enable High-Throughput Characterization of Pluripotent Cell Lines. Cell 144(3), 439-452

Abstract No. P034

Transcriptome analysis of the adult neural stem cell progeny

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Neural stem cells exist not only in the developing mammalian nervous system but also in the adult mammalian brain. They serve as a basis for adult neurogenesis due to their capacity to self-renew and generate new neurons in restricted brain areas, called neurogenic niches. Although the architecture of subependymal neurogenic niche (SEZ), stem cell identity and neurogenic lineage have been well defined, the genetic program for adult neurogenesis taking place in the subependymal zone is poorly understood. Therefore, transcriptome analysis of neurogenic cell populations is required to identify genes and biological processes involved in generation of new neurons. While our lab has previously determined the genome-wide expression pattern of neural stem cells (Beckervordersandforth et al., 2010), the transcriptome of their progeny, the neuroblasts (NBs) or oligodendrocyte progenitor cells (OPCs), has not yet been characterized. Therefore, we prospectively isolated the neuroblasts, OPCs and oligodendrocytes utilizing surface antigens and fluorescence activated cell sorting method (FACS). Using Affymetrix Gene 2.0 ST arrays we determined the transcriptional profile of these three populations. In further analysis we characterized the genes enriched specifically in different populations and revealed biological processes and pathways in which are these genes involved. Our analysis identified the neuronal differentiation and chromatin remodeling, as prominent biological processes enriched in progeny of neural stem cells. We aim to further characterize the role of chromatin remodeling in the adult neurogenesis, by performing expression analysis and functional analysis of selected candidates.

Beckervordersandforth R. et al. 2010. In vivo fate mapping and expression analysis reveals molecular hallmarks of prospectively isolated adult neural stem cells. Cell Stem Cell, 7, 6: 744-758

Abstract No. P035

Reprogramming to pluripotency through a somatic stem cell intermediate

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In recent years, direct reprogramming and transdifferentiation from one somatic cell lineage into another has become an important area of stem cell research. We recently reported the direct reprogramming of mouse embryonic fibroblasts (MEFs) into induced neural stem cells (iNSCs). In this study, we have reprogrammed iNSCs into induced pluripotent stem cells (iPSCs), which we termed iNSC-derived iPSCs (iNdiPSCs). iNdiPSCs are truly pluripotent, as evidenced by a pluripotent gene expression profile and the ability to differentiate into all three germ layers, both in vitro and in vivo. While iNSCs maintain a residual transcriptional profile of MEFs, we could show that iNdiPSCs do not retain epigenetic memory of either NSCs or the initial MEF population. Furthermore, upon directed or undirected differentiation, iNiPSCs do not seem to exhibit a bias toward any of the three cell lineages. In conclusion, our data provide evidence that iNSCs can give rise to bona fide iPSCs, which are indistinguishable from other pluripotent stem cells, and free of any epigenetic memory.

Han DW, Tapia N, et al. (2012) Direct Reprogramming of Fibroblasts into Neural Stem Cells by Defined Factors. Cell Stem Cell.

Abstract No. P036

Characterisation of endoderm progenitors from integration-free episomal plasmid based-iPSCs generated from human fetal foreskin fibroblasts

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Human embryonic stem cells (hESCs) have two fundamental characteristics. First is pluripotency, i.e. the ability to differentiate to all cell types of the three germ layers endoderm, ectoderm and mesoderm in vitro (formation of embryoid bodies) and in vivo (teratoma formation in immunodeficient mice) Second, hESCs have the capability to self-renew indefinitely. Embryonic stem cells express pluripotency associated markers such as OCT4, NANOG and SOX2 also the surface markers SSEA-4, TRA-l-60, TRA-1-81 and TRA-2-49 but not SSEA-1.

Induced pluripotent stem cells (iPSCs) are embryonic-like cells and can be generated from somatic cells derived from individuals with known genetic characteristics by the over-expression of OCT4 and SOX2 in combination with either KLF4 and c-MYC or NANOG and LIN28.

We have generated episomal-derived and integration-free E-iPSCs from human fetal foreskin fibroblast cells (HFF1) and compared the transcriptome to that of retro-viral derived HFF1-cells (V-iPSCs) generated in our laboratory. The transcriptome of E-iPSCs are closer to that of hESCs (R2 = 0.9363) in comparison to V-iPSC (R2 = 0.8176). This viral-free method has the advantage over viral-based protocols because of the lack of integrations which otherwise leads to chromosomal re-arrangements of the host genome. Using the E-iPSC line we have derived and characterized hepatocyte-like cells (HLCs) and endodermal progenitors (EPs).

Further studies are planned involving the use of the E-iPSCs derived EPs to generate hepatocyte and pancreatic cells. These studies will enable uncovering the genes and associated pathways that specify a bipotential EP to differentiate to either liver or pancreas. Additionally, these E-iPSCs and derived EPs provide unique resources for disease modeling, developmental studies, drug screening and toxicology studies.

Abstract No. P037

The effect of donor age on the induction of pluripotency in bone marrow derived Mesenchymal Stem Cells from young and aged individuals

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The in vitro expansion and application potential of human bone marrow derived mesenchymal stem cells (hBM-MSCs) isolated from aged donors is limited by their short life span in culture and restricted differentiation potential. Age-related features such as elevated levels of reactive oxygen species (ROS), genome instability and senescence and their role in reprogramming have been analysed in a limited number of studies in human fibroblasts. However, little is known about the role of age-associated molecular changes during reprogramming of hBM-MSCs and the effect on the derived induced pluripotent stem cells (iPSCs). To obtain new insights into the potential roles of age in reprogramming of hBM-MSCs we induced pluripotency in hBM-MSCs from fetal femur (55 days post conception) and hBM-MSCs from aged donors (60-70 years of age). Higher levels of ROS, phosphorylated γH2AX and decreased proliferation could be detected in hBM-MSCs from aged individuals. Karyotype abnormalities were not detected in BM-MSCs of both groups. Microarray-based comparative transcriptome analyses identified genes involved in p53 and hypoxia pathways, cell cycle regulation and glutathione metabolism as down-regulated in hBM-MSCs from aged donors in comparison to fetal femur hBM-MSCs. Whether the identified age related features in hBM-MSCs are altered during the reprogramming process is under investigation. Human BM-MSCs from fetal femur could be reprogrammed more efficiently and faster compared to BM-MSCs from aged donors. We derived a fully reprogrammed iPSC line from BM-MSCs from an aged donor (60 years of age) using retroviruses and three fully reprogrammed iPSC lines from fetal femur BM-MSCs using episomal plasmids. In conclusion, we have demonstrated, that the efficiency of inducing pluripotency in hBM-MSCs is potentially affected by the age of the donor. Age-associated features such as elevated ROS levels, altered transcriptomes and cell cycle regulation diminish the pace and efficiency of inducing pluripotency in BM-MSCs from aged donors.

Abstract No. P038

Epigenetic repressor function of RUNX1 contributes to hematopoietic differentiation

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During hematopoietic differentiation progenitor cells undergo a hierarchical progression, in which a specific lineage identity is adopted. In this process the transcription factors Tal1 and RUNX1 play an important role. The mechanism how Tal1 and RUNX1 contribute to lineage differentiation is not fully understood. We found that Tal1 interacts with RUNX1 and we detected the transcription factors concomitantly present at the promoters of common target genes. Our data show that RUNX1 provides repressor functions to RUNX1/Tal1 target genes. RUNX1 takes part in the recruitment of epigenetic corepressor proteins, such as PRMT6. This results in increase of the repressive histone modification H3R2me2a, which inhibits the transition of H3K4me2 to the active H3K4me3 mark in progenitor cells. This way, genes are kept in an epigenetically repressed but poised state in progenitor cells. Finally, we demonstrate that the repressive function of RUNX1 influences the balance between erythroid and megakaryocytic differentiation. We conclude that RUNX1 contributes to repression of differentiation genes in progenitor cells and to the suppression of the erythroid gene expression program during megakaryocytic differentiation.

Abstract No. P039

YOUNG VERSUS AGED CELL SOURCES - GENETIC ABERRATIONS IN iPS CELLS AND THE EFFECT OF PRIMARY CELL PROLIFERATION ON REPROGRAMMING EFFICIENCY

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OBJECTIVE:
The quality of iPSCs derived from somatic cells of aged individuals may be critical for the production of clinically useful cell products. Indeed, the proliferation of primary cells from aged individuals is typically lower than in juvenile cells. Additionally, genomic and mitochondrial mutations appear to accumulate over time and have been suggested to contribute to aging and cancer formation.
Therefore, our aim is to compare reprogramming efficiencies and frequency of mutations in iPSCs from old versus young sources. To possibly exclude cell-type-specific effects, we focused on endothelial cells (ECs) derived from different sources including cord blood and umbilical cord, as well as peripheral blood and saphena veins of elderly donors.
METHODS:
Population doublings (PDs) were determined in early passage ECs. Microarray analysis of primary cells was performed. Cells were reprogrammed with lentiviral vectors expressing Thomson or Yamanaka factors and reprogramming efficiencies were correlated to PDs. Generated iPS clones were karyotyped. Exom sequencing has been performed and detected SNPs will be confirmed by PCR.
RESULTS:
ECs from blood of adult donors could be isolated in a very small portion of samples. Higher proliferation and reprogramming rates in cells from younger sources were observed, these parameters correlate positively. In contrast, reprogramming of cells from aged sources was 10-100 fold less efficient with both lentiviral constructs. Many genes related to cell cycle and genome repair were upregulated in cells with high reprogramming efficiency. Karyotyping revealed chromosomal aberrations in considerable portion of iPSC from elderly patients, whereas iPSC clones from young sources had normal karyotype. Bioinformatic analysis of exom sequencing data is ongoing.
CONCLUSIONS:
Our preliminary data indicate that young cell sources are easier to reprogram and iPSCs derived from juvenile cells may be of superior quality compared to iPSCs from elderly patients. These features make them promising candidates for future therapeutic applications.

Haase A. et al. (2009) Generation of induced pluripotent stem cells from human cord blood. Cell Stem Cell 5(4):434-441.
Busuttil RA, Garcia AM, Reddick RL, Dollé ME, Calder RB, Nelson JF, Vijg J. (2007) Intra-organ variation in age-related mutation accumulation in the mouse. PLoS One 2(9):e876.

Abstract No. P040

The Human Induced Pluripotent Stem Cell (hiPSC) Unit at the HMGU - Reprogramming Techniques and Aims.

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Reprogramming of somatic cells into induced Pluripotent Stem Cells (iPSC) is accomplished by ectopic expression of pluripotency genes and/or treatment with chemical reagents. It has been demonstrated that iPSCs can be instrumental for disease modeling, drug target screenings and cell replacement therapy. The Human Induced Pluripotent Stem (hiPS) Cell Unit, located at the Helmholtz Zentrum Munich, Neuherberg campus was established for enabling the scientific staff and collaborators to benefit from iPSCs as an experimental platform. Activities in the unit include primarily production and characterization of patient iPSC lines and development of differentiation protocols. Primary reprogramming technologies used at the unit are based on incorporation of transgenes, utilizing transposomes, plasmids and artificial mRNA (artRNA), and characterization techniques include: karyotyping, analysis of pluripotency proteins, test of differentiation potential and teratoma assay. The unit's focus currently includes neurodegenerative diseases, diabetes, and motor disorders. Future goals of the unit include developing efficient reprogramming methods based on advances in chemical reprogramming. We would like to introduce the unit's activities and technologies at the GSCN meeting and use this opportunity for reaching out for potential collaborators in Germany.

Abstract No. P041

HIF1α drives the reprogramming to pluripotency through early glycolytic shift and up-regulation of PDK1-3 and PKM2

Alessandro Prigione [1,*], *Nadine Rohwer* [2], *Sheila Hoffman* [1], *Barbara Mlody* [3], *Katharina Drews* [3], *Raul Bukowiecki* [1], *Erich Wanker* [1], *Markus Ralser* [4], *Thorsten Cramer* [2], and *James Adjaye* [5]

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Reprogramming somatic cells to a pluripotent state drastically reconfigures the cellular anabolic requirements, thus potentially inducing cancer-like metabolic transformation. Accordingly, we and others previously showed that somatic mitochondria and bioenergetics are extensively remodeled upon derivation of induced pluripotent stem cells (iPSCs), as the cells transit from oxidative to glycolytic metabolism. In the attempt to identify possible regulatory mechanisms underlying this metabolic restructuring, we investigated the contributing role of hypoxia-inducible factor 1 alpha (HIF1α), a master regulator of energy metabolism, in the induction and maintenance of pluripotency. We discovered that the ablation of HIF1α function in dermal fibroblasts dramatically hampers reprogramming efficiency, while small molecule-based activation of HIF1α significantly improves cell fate conversion. Transcriptional and bioenergetic analysis during reprogramming initiation indicated that the transduction of the four factors is sufficient to up-regulate the HIF1α target pyruvate dehydrogenase kinase (PDK) 1 and set in motion the glycolytic shift. However, additional HIF1α activation appears critical in the early up-regulation of other HIF1α-associated metabolic regulators, including PDK3 and pyruvate kinase (PK) isoform M2 (PKM2), resulting in increased glycolysis and enhanced reprogramming. Accordingly, elevated levels of PDK1, PDK3, and PKM2 and reduced PK activity could be observed in iPSCs and human embryonic stem cells (hESCs) in the undifferentiated state. Overall, the findings suggest that the early induction of HIF1α targets may be instrumental in iPSC derivation via the activation of a glycolytic program. These findings implicate the HIF1α pathway as an enabling regulator of cellular reprogramming.

Prigione A, Fauler B, Lurz R, Lehrach H, Adjaye J. The Senescence-Related Mitochondrial /Oxidative Stress Pathway is Repressed in Human Induced Pluripotent Stem Cells. Stem Cells. 2010 Apr;28(4):721-33
Prigione A, Lichtner B, Kuhl H, Struys EA, Wamelink M, Lehrach H, et al. Human iPSCs Harbor Homoplasmic and Heteroplasmic Mitochondrial DNA Mutations While Maintaining hESC-Like Metabolic Reprogramming. Stem Cells. 2011 Sep;29(9):1338-48.

Abstract No. P042

Modified mRNAs, OSKM+Lin28 reprogramming of human fibroblasts

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To avoid genetic alternations that may interfere with the use of human induced pluripotent stem cell (hiPSCs) for clinical and disease modeling purposes, efforts have been made for developing transgene-free somatic cell reprogramming techniques. One of the most promising approaches is based on synthetic modified mRNAs (mmRNAs) encoding reprogramming factors. We have recently incorporated this technology to the panel of reprogramming methods used by the hiPSC unit at the Helmholtz Center Munich. We utilize mmRNAs encoding for: Oct4, Sox2, cMyc, Klf4 proteins and Lin28. We increase the stability of mmRNAs by substituting structural features of mRNAs, including incorporation of synthetic 5' Cap analogs, 5' and 3' translated regions, polyA tail, and cytidine and uridine analogs. Our protocol consists of transfecting the 5 factor cocktail daily over the course 15 days in the presence of interferon type-I neutralizing protein. We have thus far established hiPSC lines from human foreskin fibroblast, and we are currently adapting the protocol to adult fibroblasts from individuals affected by neurodegenerative and motor diseases, and diabetes.

Abstract No. P043

Modulating Growth Factor Addition to Essential 6™ Media for a Complete Xeno-free Media From Fibroblast Culture to iPSC Generation and Expansion

Alexandria Sams [1], *Shayne Boucher* [1], *Rene Quintanilla* [1], *Andrew Fontes* [1], *Katja Hufschmid* [1,*], and *Uma Lakshmipathy* [1]

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The generation of induced pluripotent stem cells (iPSCs) from somatic cells has the potential to revolutionize cell-based therapeutic applications. Current methods require multiple media systems for various stages of reprogramming. An ideal solution would be a media system that can be used from the original patient sample harvest to expansion of the resulting iPSC clones.

Essential 8™ media has emerged as an easy, simple and cost effective media system for the expansion of pluripotent ESC and iPSCs. Here, we use the basal version without growth factors, termed Essential 6™ media as the base media and optimize the addition of the two key growth factors, bFGF and TGFβ, at various stages during reprogramming. Our results suggest that unlike other xeno-free media systems, Essential 8™ media supports efficient transduction via Sendai virus without toxicity. Furthermore, optimal timing of TGFβ removal a week after transduction supported efficient reprogramming resulting in iPSC colonies that were subsequently expanded on recombinant human vitronectin and Essential 8™ media. Characterization studies indicated a normal karyotype, pluripotency marker expression, and in vitro differentiation into all three lineages after multiple passages. While use of the Essential 8™ media system has been reported for episomal reprogramming, its optimization in other reprogramming methods and workflows enables a modular, easy to use, feeder-free, xeno-free system.

Takahashi K & Yamanaka S. (2006). Cell. 126(4): 663-76.
Chen G, G. D., Hou Z, Bolin JM, Ruotti V, Probasco MD, Smuga-Otto K, Howden SE, Diol NR, Propson NE, Wagner R, Lee GO, Antosiewicz-Bourget J, Teng JM, Thomson JA. (2011). Nat Methods. 8(5): 424-9.

Abstract No. P044

Differentiation of stem-cell based intestinal organoids into functional M-cells

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Microfold cells (M-cells) are specialized intestinal epithelial cells in the follicle associated epithelium overlying the Peyer's Patches. M-cells deliver luminal antigens to immune cells and thereby form a necessary link between the gut lumen and the mucosal immune system. In vivo studies have shown that M-cell differentiation can be induced upon binding of Rank ligand (RankL) to its receptor Rank.
We have used the intestinal organoid culture system to further investigate M-cell differentiation. An intestinal organoid can be grown from a single adult intestinal stem cell and solely involves epithelial cells. We have shown that upon incubation with RankL, small intestinal organoids developed into Annexin V and GP2 expressing M-cells. These cells were able to take up beads, confirming M-cell function. Furthermore, we have shown that RankL-induced M-cell development was dependent on the transcription factor SpiB. Organoids established from SpiB-deficient mice did not develop into functional M-cells upon incubation with RankL despite expression of Rank. These data showed that M-cells can be induced in an epithelial stem cell based culture system without the influence of the intestinal immune system or the microbiota. Induction of SpiB by Rank-RankL interaction was crucial for this M-cell development.
However, we now show that enforced overexpression of SpiB in intestinal organoids did not induce M-cell development, suggesting that SpiB is not operating autonomously. Therefore, we are currently looking into the involvement of another transcription factor in the induction of the M-cell developmental program.

Abstract No. P045

Reprogramming triggers mutagenic endogenous LINE-1 and Alu retrotransposition in human induced pluripotent stem cells

Gerald Schumann [1,*], *Sabine Jung-Klawitter* [1], *Nina Fuchs* [2], *Martin Munoz-Lopez* [3], *Kyle Upton* [4], *Ruchi Shukla* [4], *Jichang Wang* [2], *Attila Sebe* [1], *Anett Witthuhn* [5], *Alexandra Haase* [5], *Ulrich Martin* [5], *Zoltan Ivics* [1], *Jose Garcia-Perez* [3], *Zsuzsanna Izsvak* [2], and *Geoffrey Faulkner* [4]

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OBJECTIVE: The use of human induced pluripotent stem cells (hiPSCs) holds great therapeutic promise for regenerative therapies and disease modeling. However, reprogramming and cultivation of hiPSCs can induce genetic and epigenetic abnormalities that can result in tumorigenic hiPSCs and undermine the use of iPSCs or their derivatives in regenerative medicine. Activation of endogenous mobile retrotransposon families LINE-1 (L1), Alu and SVA can cause such mutations, that were shown to be the cause of genetic disorders and tumor diseases. We investigated if endogenous L1, Alu and SVA elements mobilize in hiPSCs and contribute to their genomic destabilization.

METHODS: To explore if L1 elements are activated in hiPSC lines, we analyzed CpG methylation of L1 promoter regions by bisulfite sequencing, and L1 expression by qRT-PCR and immunoblot analysis in eight iPSC lines and their somatic parental cells. We mapped individual mobilization events that occurred during or after reprogramming by using a novel high-throughput protocol named retrotransposon capture sequencing (RC-seq). New transposition events that were only present in the iPSC lines, were validated as de novo by insertion site PCR and capillary sequencing. Effects of intronic de novo insertions on host gene expression was analyzed by qRT-PCR.

RESULTS: Reprogramming reduced methylation of the L1 promoter significantly, induced full-length L1 mRNA expression by up to 25,000-fold relative to parental cells and mobilized mutagenic L1 retrotransposons. 50% of all de novo transposition events were found in protein-coding genes, including PTPN9 impairing oncogenic growth and invasion of breast cancer cells. L1-mediated mobilization occured during or after reprogramming into iPSCs and during cultivation, amplifying the number of functional, mutagenic L1 elements. These mobilization events can perturb key protein-coding genes with unknown consequences in differentiated cells, questioning biosafety of hiPSCs and their derivatives. Currently, we are also investigating methods to restrict mutagenic L1-mediated retrotransposition triggered by reprogramming.

Abstract No. P046

Generation of induced pluripotent stem cells using the Sleeping Beauty transposon system

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The therapeutic hope represented by human embryonic stem cells has been hampered by ethical and immunological concerns. The induced pluripotent stem (iPS) cells represent a new, powerful alternative which overcomes several limitations of human embryonic stem cells and are promising tools for cell and gene therapy applications. Generation of iPS cells has been achieved successfully by simultaneous overexpression of defined reprogramming transcription factors. iPS cells present a promise for vast application fields, from in vitro disease modeling to pharmaceutical screening and drug development, and are a unique tool for dissecting the molecular events of reprogramming and cellular differentiation, presenting the potential for cellular replacement/transplantation therapies.
We present here the successful reprogramming of human foreskin fibroblasts (HFF-1) using the Sleeping Beauty transposon system: the SB100X transposase in combination with a Sleeping Beauty transposon construct containing Oct3/4, Klf4, Sox2, c-Myc with or without Lin28, driven by a CAG promoter in a single cassette. The reprogramming factors in the construct were linked with viral 2A sequences. The efficiency of iPS cell derivation with SB transposon system was in the range of that obtained with retroviral vectors. The iPS cells obtained were characterized for pluripotency and differentiation potential: iPS cells displayed a stable karyotype, and hallmarks of pluripotency including expression of stem cell markers and the ability to differentiate into embryoid bodies in vitro.
Future work will be focused on using this powerful technique in different disease models.

Abstract No. P047

Dissecting DNA modification pathways in stem cells and neurons using stable isotope tracing and mass spectrometry

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The Tet enzyme family was recently shown to catalyse sequential oxidation of genomic 5-methylcytosine (mC) to hydroxymethyl-, formyl- and carboxyl-cytosine (hmC, fC and caC, respectively). This discovery has not only expanded the number of enzymatically generated DNA modifications in mammalian genomes, but is also providing new insight on epigenetic control of gene expression in pluripotent cells and during specification of germ and somatic cell lineages. mC oxidation is thought to provide modified cytosine states with alternative epigenetic functions as well as a pathway for their ultimate erasure. Several alternative mechanisms for the removal of cytosine modifications have been proposed, but their respective relevance and extent of intersection in distinct cell types and tissues are unknown. We have established mass spectrometry methods to measure the global abundance of modified nucleosides with high accuracy and can resolve identical nucleosides bearing distinct stable isotopes. Upon metabolic labeling of genomic nucleosides with stable isotopes we follow their global modification dynamics and kinetics in defined cell states and during differentiation. In combination with genetic or functional depletion this strategy allows us to define nucleotide (de-)modification pathways. We recently found that Tet enzymes, in addition to 5-modified cytosines, oxidize also thymidine (5-methyluridine) to 5-hydroxymethyluridine in the genome of ESCs. In addition, it is highly controversial whether mC and hmC are deaminated to T and hmU, respectively, as part of enzymatic demethylation pathways. We could detect these reactions in ESCs and are identifying the factors involved. We are also investigating the relevance of these pathways to the differentiation of neural progenitors into neurons.

Abstract No. P048

Reprogramming human somatic cells towards pluripotency and their differentiation to hematopoietic stem and progenitor cells

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*Presenting author

At present, somatic gene therapy of hematopoietic stem and progenitor cells (HSPCs) is based on retroviral vector based complementation of underlying genetic defects. However, this procedure is associated with a significant risk of therapy-associated leukemogenesis due to their random integration into the genome. Induced pluripotent stem cells (iPSC) may represent an alternative to somatic stem cells as they allow for homologous recombination, clonal expansion and differentiation towards HSPCs, in vitro.

For iPSC generation, we transduced human umbilical vein endothelial cells with a lentiviral vector co-expressing the cDNAs for the human Yamanaka factors (OCT4, KLF4, SOX2, MYC) linked to a fluorescent reporter. Flanking Flp-recombinase target sites allow for the removal of the vector by Flp-recombinase thus preventing reactivation of the vector during differentiation. Upcoming iPSC-colonies grew similar to human ES-cell line H1 (huESC-H1). They showed hallmarks of pluripotency such as AP activity, expression of SSEA-4, TRA-1-60/-81 as well as a re-expression of the endogenous transcription factors NANOG, LIN28A and DNMT3B. Global transcriptome analysis by RNAseq revealed significant similarity of two iPSC-clones to the huESC-H1. For excision of the reprogramming vector, Flp-recombinase was introduced into iPSCs by virus particle mediated protein transduction and clones grown from deposited single cells were screened by PCR. So far, one out of 50 iPSC-clones showed successful excision.

As differentiation of human pluripotent stem cells towards HSPCs capable of long-term repopulation in appropriate recipient mice has not been established yet, we wished to optimize the conditions for hematopoietic differentiation. Pluripotent cells were differentiated as embryoid bodies (EBs) in suspension supplemented with appropriate cytokines under normoxic or hypoxic conditions. After 14 days, EBs were dissociated and either evaluated for hematopoietic colony formation and for expression of hematopoietic surface molecules by flow-cytometry. Our results indicate that differentiation in a reduced pO2 atmosphere strongly supports generation of CD34+ hematopoieticnprogenitors.

Abstract No. P049

E2F6 initiates methylation of germ cell gene promoters in undifferentiated embryonic stem cells

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Germ cell gene silencing is the most robust coordinated DNA methylation event during ES cell differentiation and embryonic development. The transcription factor E2F6 may be directly involved in this process as several germ cell genes are ubiquitously expressed in somatic ells of E2F6 -/- mice. We therefore generated knockout mouse ES cells to study the role of E2F6 and its associated atypical polycomb repressive complex PRC1.6 in transcriptional repression of germ cell genes and in establishment of promoter methylation during ES cell differentiation. Micro array analysis identified 82 genes with >2 fold derepression in E2F6-/-ES cells. 23 of the 25 top targets are germ cell genes. They are characterized by promoter proximal E2F6 binding sites and E2F6 dependent basal and differentiation induced DNA-methylation. Re-expression of E2F6 in E2F6 -/- ES cells restores basal and differentiation induced methylation of target gene promoters. Kinetic experiments reveal a close correlation between completion of promoter methylation of E2F6 target genes and down regulation of Tet1 and Tet2 DNA demethylases during ES cell differentiation. In ES cells, these promoters exhibit high levels of the DNA demethylation reaction intermediate hydroxymehylcytosine (hmC) and knock down of Tet1 and Tet2 result in repression of transcription, indicating that Tet1 and Tet2 counteract E2F6 induced DNA-methylation in undifferentiated ES cells. Once established during differentiation, E2F6 induced promoter methylation is maintained in somatic cells of E2F6 -/- mice in the absence of E2F6 protein.
Knock down experiments in ES cells and analysis of GEO datasets reveal that in addition to repression of transcription by precluding binding of the transcriptional activator E2F1, the majority of high confidence E2F6 target genes are co-repressed by subunits of the PRC1.6 complex that controls differentiation programs in ES cells and during early development.

Abstract No. P050
Inhibition of Induced Direct Cell Fate Programming

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Studying cell type conversion in model organisms can provide insight into mechanisms that maintain and protect specific cellular identities. Knowledge about such mechanisms has the potential to facilitate cell fate programming strategies thereby supporting the development of future biomedical approaches. The direct programming of mature somatic cells to a different cell type by ectopically expressed Fate-inducing Transcription Factors (FiTFs) is a promising strategy to generate required tissues in vivo. Although direct programming is successfully being applied [1], it appears that not all cell types can be efficiently transdifferentiated by forced expression of specific FiTFs.
Recent studies provide evidence that inhibitory mechanisms play an important role in restricting cellular reprogramming [2]. Such inhibitory mechanisms are often conveyed by factors that regulate accessibility to chromatin by modifying histones and/or the chromatin structure. Yet, it is not well understood, which specific factors mediate inhibition of direct cell fate programming and importantly, whether they act the same way in different tissue types.
Our group aims to understand mechanisms that restrict FiTF-mediated direct programming. We are using C. elegans as an in vivo genetic model system and apply large-scale forward and reverse genetic screenings with high-throughput techniques. Our ongoing work identified factors involved in inhibiting the FiTF-mediated induction of neuronal or muscle fates in different tissue types in vivo. We have previously identified members of the PRC2 (Polycomb Repressive Complex 2) and histone chaperone complexes in C. elegans as inhibitors of converting germ cells into specific neurons or muscle cells. Our preliminary results suggest that in distinct somatic tissue-types different factors and signaling pathways might be involved in order to maintain cellular identity and counteract the activity of ectopically expressed FiTFs to promote direct cell fate programming.

[1] Ladewig J, Koch P, Brüstle O. Nat Rev Mol Cell Biol 2013;14:225.
[2] Rais Y, et al. Nature 2013:1.

Abstract No. P051
Generation of CNS cell types from PNS stem cells

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The neural crest gives rise to the neurons and glial cells of the peripheral nervous system (PNS). Self-renewing neural crest-derived stem cells (NCSCs) are present in migratory neural crest and various postmigratory locations, including peripheral ganglia. We previously demonstrated that NCSCs from embryonic mouse dorsal root ganglia (DRG) are reprogrammed in neurosphere (NS) cultures in the presence of EGF and FGF. rNCSCs generate exclusively central nervous system (CNS) progeny, both in vitro and upon transplantation into the mouse brain (Binder et al., (2011) J. Neurosci. 31, 6379-91). Here we address the timing and mechanisms underlying the reprogramming. Most of the cells acquire CNS characteristics at passage 2, reaching a stable proportion of >90% of Olig2+ cells at passage 3, which is maintained up to at least passage 10. The PNS marker p75 is completely lacking from passage 3 onwards. Under differentiation conditions 3rd passage NS cells generate Olig2+/O4+ oligodendrocytes, GFAP+ cells with astrocyte morphology and CNS neurons.
Reprogramming of NCSCs is completely blocked in the presence of BMP4 in NS cultures, as shown by the expression of neural crest markers p75 and Sox10. In addition, BMP4 NCSCs generate PNS neurons (TuJ1+/Phox2b+) and Schwann cells (O4+/p75+). These findings imply that the CNS vs PNS identity is controlled by antagonistic functions of FGF and BMP4.
The reprogramming leads to a stable CNS identity as shown by delayed BMP4 application. This result is in agreement with our previous observation that rNCSCs only generate CNS progeny, in particular mature myelinating oligodendrocytes, upon transplantation into embryonic, postnatal and lesioned adult mouse brain. Genome wide gene expression profiling of rNCSC NS demonstrates already in culture a complete switch to CNS identity and identified candidate signaling pathways involved in the reprogramming.

Binder et al., (2011): Peripheral Nervous System Progenitors Can Be Reprogrammed to Produce Myelinating Oligodendrocytes and Repair Brain Lesions. J. Neurosci. 31, 6379-91

Abstract No. P052

Reprogramming of nonhuman primate common marmoset cells towards pluripotency

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Pluripotent stem cells are attractive for regenerative medicine because they can be differentiated towards any somatic cell type and tissues needed for transplantation. To test the medical applicability of this notion in a relevant animal model, we are currently establishing protocols to reprogram dermal fibroblasts of the primate common marmoset (Callithrix jacchus) towards induced pluripotent stem cells (iPSCs).

For this purpose, we employed a lentiviral “four-in-one vector”, co-expressing the cDNAs for human OCT4, KLF4, SOX2 and MYC (Yamanaka factors), which had been successfully used for reprogramming human and mouse cells in our lab. The obtained marmoset colonies expressed alkaline phosphatase (AP), stained positive for SSEA-3/-4 but were negative for TRA-1-81/-60 expression. Furthermore, global transcriptome measurements by RNA-seq and subsequent principle component analysis revealed that the generated pre-iPSCs were much more similar to ESCs than to fibroblasts indicating a partially reprogrammed state.

Because the stoichiometry of the ectopically expressed transcription factors is known to be crucial for complete reprogramming, we transduced each Yamanaka factor together with a different fluorescence protein by separate lentiviral vectors. Using this “rainbow reprogramming” system, the stoichiometric ratios of all four transcription factors can be estimated during different phases of iPSC generation. The upcoming colonies displayed a narrow color range and expressed AP, were positive for SSEA-4 and so far partially positive for SSEA-3, but negative for TRA-1-81/-60 expression. These results suggest that, in contrast to human and mouse cells, marmoset cells strongly inhibit reprogramming by a yet undefined mechanism. Recent publications suggest that the late "maturation" phase plays a crucial role during reprogramming. Particularly, the activity of LIN28 enhances stable maintenance of newly formed iPSCs. We are currently evaluating whether inclusion of LIN28 into the reprogramming cocktail ("5F") allows us to overcome the strong intrinsic block mediated by marmoset cells.

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Yu Zhang

Abstract No. P053

Distinct pluripotency factor expression in non-human primate implantation embryos reveals a wide spatio-temporal primordial germ cell migration window

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Germ line cells are unique. They are the only cells that can transmit genetic information to the next generation, sharply contrasting with somatic cells. Using the mouse, general principles of the embryonic differentiation of primordial germ cells (PGCs) from somatic cells were discovered, including expression of characteristic pluripotency factors. In contrast to the mouse, very little is known about primate PGC development due to the extremely limited access to primate embryos. Here, we analyzed 12 excellently preserved non-human primate (Callithrix jacchus) embryos covering the phase from PGC emergence in the endoderm to the formation of the differentiating gonad. We show that pluripotency factors OCT4A and NANOG specifically mark PGCs. In contrast, SALL4 and LIN28 were first ubiquitously expressed and only later down-regulated in somatic tissues, while continuously expressed in PGCs. This demonstrates differential expression of OCT4A, NANOG, SALL4, and LIN28 during primate embryogenesis. We further show that PGCs are present already in the very primitive monkey gonad, while significantly older embryonal stages still exhibit PGCs at their original endodermal site in the gut epithelium. This unexpectedly wide spatio-temporal distribution of PGCs challenges the “dogma of active PGC migration” from the endoderm to the gonads. We therefore suggest an alternative model based primarily on passive translocation of PGCs from the gut to the prospective gonad through intercalar expansion of the mesenchymal tissue harboring the PGCs. In summary, we show that pluripotency factors are differentially expressed in somatic and germ cells during primate post-implantation embryo development and provide a novel model on embryonic PGC translocation in primates.

Abstract No. P054

EMBRYONIC STEM CELL RESEARCH IN LAW, ETHICS AND SCIENCE: WHEN LAW AND ETHICS RULE, RESEARCH HAS TO PAY?

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The triangle of dependence – law, ethics and science – is strongly linked to the success of (embryonic) stem cell research. Law and ethics regulate embryonic stem cell research in most parts of the world. Public attitudes form policies and legal frameworks. Specifically, the interaction between law and ethics and the use of ethics to justify legal standpoints and legal decisions play a major role when regulating embryonic stem cell research. In this regard, the decision of the European Court of Justice (ECJ) in Brüstle v. Greenpeace e.V. (Case C-34/10) will be compared to the US Court of Appeals for the District of Columbia Circuit case Sherley et. al. v. Sebelius et. al. (610 F.3d 69; 686 F.Supp 2d 1; 644 F.3d 388; 776 F.Supp. 2d). These decisions not only show different legal and ethical approaches of embryonic stem cell research in different jurisdictions, but also demonstrate how law and ethics can influence science itself. To which extend do these decisions influence the national and international stem cell research? Different legal and ethical influences will be emphasized which not only affect embryonic stem cell policy and legislation but also embryonic stem cell research and therapy possibilities itself.

This analysis will be put in relation to the recent discussions in genetic testing fostered by the German National Ethic Council's opinion issued on April 30, 2013 which made a comparison to stem cell research and the US Supreme Court decision in Association for Molecular Pathology et. al. v. Myriad Genetics, Inc. et. al. Can the same arguments be found in apparently two different areas of scientific research and which conclusions can be drawn? Law, ethics and science shape the necessary triangle of dependence in stem cell research and it's dynamic of development.

Abstract No. P055

Lin41 characterization in development and stem cell niches

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Lin41 gene encodes a member of the Trim-NHL protein sub-family, discovered in C. elegans as a master regulator of development. Lin41 is a target of the microRNA let-7, and this regulatory relationship is conserved throughout bilateral animals. Like other Trim-NHL proteins, LIN41 was shown to be an E3 ubiquitin ligase influencing the microRNA biogenesis, although its function in other biological contexts remains largely unknown.

Using a Lin41 gene trap mouse line we characterized the expression pattern of Lin41 during mid-gestational stages of the embryo: from E8.5, when is ubiquitously expressed, through restriction to neuroepithelium and limb buds between E10.5 and E12.5, and its loss at E13.5. In the absence of functional LIN41, mouse embryos fail to properly develop the neural tube and die around day E9.5. We also focused in the adult central nervous system (CNS), and reveal Lin41 expression, restricted to the ependymal cells of the lateral ventricle. Due to the relationship of these cells with the subventricular zone (SVZ) neurogenic niche, the pluripotency-related LIN41 protein might be of relevance for the process of adult neurogenesis. In addition, we established an ependymal primary culture model that shows Lin41 expression, and represent a promising tool in the study of Lin41 in the multicilia differentiation program of these cells.

As a new tool for the study of Lin41 in pluripotency and neural differentiation, mouse embryonic stem (mES) cell lines were derived from the gene trap mouse line. Using Lin41 null ES cells, we uncovered that Lin41 is dispensable for self-renewal and pluripotency factor profile maintenance. We plan to perform differentiation of mES cells towards the neural lineage, to study the consequences of Lin41 loss and locate it within the signaling pathways controlling these events, like Shh and FGF.

Rybak et al. 2009

Abstract No. P056

Overexpression of microRNA 219 in human endometrial stem cells induce expression of Myelin basic protein-messenger RNA (MBP-mRNA)

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Introduction: Human Endometrial-derived stem cells (EnSCs) are the abundant and easy available adult stem cells with low immunological incompatibility, which could be considered for cell replacement therapy in future. miRNAs have a critical role in oligodendrocyte development including cell proliferation, differentiation and myelin formation. MiR-219 is necessary to promote oligodendrocyte differentiation by repressing negative regulators of oligodendrocyte differentiation. Oligodendrocytes are myelinating cells in the central nervous system (CNS) that form the myelin sheath of axons to support rapid nerve conduction.
Materials and Methods: The EnSCs after induction with FGF2, EGF, PDGF-AA, they were infected by miR-219-GFP-expressing lentiviruses. 4days after infection, cells were collected and analyzed for expression MAP2 and NF-L as neural markers, PDGFRa, CNP and MBP as oligodendrocyte markers by Quantitative RT-PCR.
Results: The result showed in the infected cells, expression of PDGFRa, MAP2 and NF-L compared with non infected and control cells significantly decreased and expression other oligodendrocyte markers such as CNP and MBP in the level of mRNA increased in compared with control and non infected cells.
Conclusion: In conclusion, the EnSCs could be programmed into pre-oligodendrocyte cells by overexpression of miR-219, and may convince to consider these cells as safe source for cell replacement therapy of neurodegenerative diseases.

Dugas JC, Notterpek L (2011) MicroRNAs in oligodendrocyte and Schwann cell differentiation. Dev Neurosci 33(1):14–20
Dugas JC, Cuellar TL, Scholze A et al (2010) Dicer1 and miR-219 are required for normal oligodendrocyte differentiation and myelination. Neuron 65(5):597–611

Abstract No. P057

Sustained genetic modification of pluripotent stem cells and their differentiated derivatives

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Patient-specific induced pluripotent stem cells (iPSC) hold great promise for studies on disease related developmental processes and may serve as an autologous cell source for future treatment of many hereditary diseases. Aiming at the knockdown of a disease-causing gene, overexpression of shRNAs by lentiviral vectors is a valuable option, but it is limited by silencing of the knockdown construct upon epigenetic remodeling during differentiation.

To evaluate feasibility and sustainability of lentiviral shRNA knockdown in pluripotent stem cells we targeted the mutated, liver disease-causing Z-form of α-1-antitrypsin (A1AT) in iPSC from severe A1AT deficiency patients. Accumulation and polymerization of Z-A1AT in the endoplasmic reticulum of hepatocytes causes liver cirrhosis and hepatocellular carcinoma. We overexpressed a human miR30-styled shRNA directed against Z-A1AT, which is traceable from clonal iPSC lines to differentiated hepatic progeny by an enhanced green fluorescence protein reporter. Importantly, the cytomegalovirus i/e enhancer chicken β actin (CAG) promoter-driven expression of this construct is sustained without transgene silencing during hepatic differentiation and shows a functional and therapeutic relevant knockdown of Z-A1AT in differentiated hepatic cells in vitro and in vivo.

We furthermore attempted to identify genetic elements inside the CAG promoter which allow for its potent resistance to transgene silencing features in pluripotent stem cells and during differentiation. To this end, the chicken β actin element of the CAG promoter was cloned in front of the silencing prone EF1α-short (EFS) promoter. Murine embryonic stem cells and iPSC transduced with this construct showed more resistance to transgene silencing during differentiation compared to the EFS transduced cells.

In conclusion we successfully established a system for sustained expression of a lentiviral shRNA in pluripotent stem cells and their differentiated derivatives and we identified elements inside the CAG promoter which contribute to resistance against transgene silencing.

Abstract No. P058

Neural stem cells respond to the pattern of chondroitin sulfates in the niche environment

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Chondroitin sulfate proteoglycans (CSPGs) and their sulfation by chondroitin-sulfotransferases (Chsts) appear to play a crucial role for the biology of neural stem cells (NSCs) in the embryonic neural stem cell niche during mouse forebrain development. It has been shown that the inhibition of the sulfation by sodium chlorate or the degradation of the CSPG glycosaminoglycans by chondroitinase ABC leads to less proliferation and altered cell fate decisions of the NSCs
The proliferation and differentiation of cortical neural stem cells from E13.5 mouse embryos upon forced expression of distinct Chst-EGFP constructs as well as the knockdown of one specific Chst was examined by neurosphere forming/proliferation and differentiation assays in vitro. The consequences of forced expression and knockdown of Ust are assessed by in utero electroporation.
The overexpression of distinct Chsts in the NSCs was functional as revealed by an increased signal for the complex sulfated CS-epitope detected by the monoclonal antibody 473HD. In the differentiation assay a significant increase in neurogenesis at the expense of gliogenesis was observed.
In consistence with previous observations, the sulfation of the CSPG, i.e. the CS code, plays a role in the commitment of the NSCs within the neural stem cell niche and could function as a possible communication platform between the NSCs and their extracellular surrounding in the neural stem cell niche.

Abstract No. P059

Functional analysis of the histone demethylases KDM6A and KDM6B in differentiating murine ESCs

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Epigenetic modifications such as histone methylation are critically associated with self-renewal and differentiation of embryonic (ESCs) as well as hematopoietic stem cells (HSCs). So far a number of different histone demethylases (KDMs) were identified, which are potentially linked to gene regulation and to stem cell self-renewal and differentiation. KDM6A/UTX and KDM6B/JMJD3 are H3K27me2/3-specific KDMs, which play central roles in the regulation of HOX gene expression and embryonic patterning. They are ubiquitously expressed in embryonic and adult tissues. However little is known about the contribution of KDM6A or KDM6B to the regulation of local and global chromatin states in the hematopoietic system.

In our study we specifically and stably knocked down KDM6A/B in ESCs. Additionally we analyzed KDM6A knockout ESCs. Our results show that the knockdown of KDM6A or KDM6B had no influence on ESC proliferation, while in vitro ESC hemangioblast/hematopoietic differentiation was reduced. Similar results were obtained with KDM6A KO ESCs. Knockdown of KDM6B in KDM6A knockout ESCs further decreased hemangioblast differentiation. Furthermore, we observed reduced expression of the paralogous UTY and the meso/hematopoietic markers Brachyury, Flk1 and Runx1 under KDM6A KO. ChIP analyses of differentiating EBs revealed comparable enrichment of H3K27me3 at the Bry, Flk1, Tal1 and Runx1 promoters in KDM6A WT and KO cells, indicating a demethylase independent role for KDM6A during early hematopoiesis.

To analyze the contribution of the KDM6A/B demethylase activity in ESCs and during differentiation we employed the specific inhibitor GSK-J4 (Kruidenier et al. 2012). Treatment of ESCs with GSK-J4 had no effect on viability and proliferation. In contrast, ESC differentiation in the presence of GSK-J4 was completely abrogated.

In conclusion our results show that both KDM6A and KDM6B are involved in early ESC hematopoiesis. Furthermore, ESC differentiation is completely blocked in the absence of any H3K27 demethylase activity.

Abstract No. P060

MicroRNA 665 Targets Dishevelled-1 and Affects Osteogenic Differentiation of Murine Embryonic Stem Cells

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A unique feature of vertebrate embryonic development is the formation of the skeletal system. Osteoblast differentiation is a key step in proper skeletal development and acquisition of bone mass. All of the major developmental signals including Wnt, Notch, Hedgehog, and TGFβ signalling, along with an increasing number of transcription factors such as Runx-2 and osterix, have been shown to regulate the differentiation and/or function of osteoblasts. However, the roles of microRNAs during differentiation of functional osteoblasts remain to be determined. In order to elucidate the role of microRNAs in osteogenesis, we have turned to murine embryonic stem cells (mESCs), which are an ideal model to study the earliest events of pre-osteogenic specification. We focused on identifying osteogenic lineage committing microRNAs, which are known molecular regulators of other developmental processes. The results of the miRNA profiling revealed candidates which exhibit a differential regulation during embryonic development and bone formation among them microRNA-665. Overexpression and knock-down studies were then coupled with assessment of calcification potential and expression of osteoblast markers to determine whether microRNA-665 promoted osteoblast differentiation. Indeed, microRNA-665 caused changes in calcium accumulation, alkaline phosphatase activity and osteogenic marker gene expression. Furthermore, bioinformatic predictions of microRNA-665 targets were experimentally followed to identify its mRNA targets in osteogenesis. Thus, the direct microRNA-665 target was selected from a group of six potential targets, which showed altered expression after miR-665 manipulation: Ppp4c, Wisp2, Wnt8a, Dkk4, Dvl1 and Dvl2. Specifically, microRNA-665 overexpression resulted in a reduction of mRNA levels of Dishevelled 1 (Dvl1) protein, which is an important component of the Wnt pathway. The RNAi assay confirmed Dvl1 as a direct target of miR-665. Our analysis revealed that distinct miRNAs can play a functional role in modulating osteogenic differentiation and indicates new mechanisms involved in the complex regulatory schemes that lead to osteoblast specification.

Abstract No. P061

The Role of transcription factor NF-kB in the hair cycle and stem cell biology

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During embryogenesis, the transcription factor NF-kB is needed for the development of epithelial appendages, like hair follicles (HF), teeth and exocrine glands. However, the function of NF-kB during homeostasis and regeneration of these appendages in adult life, which requires stem cell activation and proliferation, is still unknown. Using the HF as a model organ and a NF-kB reporter mouse (k-EGFP) to detect in vivo NF-kB activity in the different phases of the adult hair cycle, we have observed NF-kB activity in the secondary hair germ, which contains TA cells and in the LRIG1+ stem cell niche of the junctional zone. Mice with doxycycline-induced epidermal NF-kB suppression or ubiquitously suppressed NF-kB activity, are used to analyze the murine hair cycle in the absence of NF-kB activity. We show that suppression of epidermal NF-kB activity leads to a delay in anagen induction of all hair types and a significant reduction of guard hair numbers. Our data suggest a role for NF-kB in HF stem cell proliferation..

Unpublished data from our laboratory has shown that there is also strong NF-kB activity in intestinal crypts, and mice with suppressed NF-kB activity show a different intestinal morphology. We have therefore also started to analyse the role of NF-kB in intestinal homeostasis. Using our k-EGFP reporter mice, we have verified NF-kB activity in the intestinal stem cell niche, where NF-kB activity is found in crypt stem cells as well as in Paneth cells. Furthermore, intestinal crypts and organoid cultures from intestinal crypts of mice with suppressed NF-kB activity show strongly decreased proliferation of transit-amplifying cells, suggesting, again, a function of NF-kB in stem cell proliferation.

Abstract No. P062

Impact of cardiotrophin-1 nuclear transport on cardiomyogenesis of mouse embryonic stem cells

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CT-1 is a cardioprotective cytokine which acts through LIF receptor β/glycoprotein 130 (gp130)-coupled signaling pathways. In the present study the effects of CT-1 on the differentiation of cardiac cells from mouse embryonic stem (ES) cells was investigated. Our data show that CT-1 as well gp130 expression was transiently increased during cardiomyogenesis of ES cells. Treatment of differentiating ES cells with CT-1 dose-dependent stimulated cardiomyogenesis, increased the cardiac transcription factors MEF2c and Nkx-2.5, the cardiac structural proteins α-actinin, MLC2a, MYH7, MLC1a and MLC2v as well as HCN4 which is prominently expressed in the pace maker region of the mammalian heart. Furthermore cultivation of ES cells under hypoxic (1% oxygen) conditions significantly increased CT-1 expression. In isolated cells CT-1 elicited a transient calcium response which was abolished in presence of the intracellular calcium chelator BAPTA, AM. Exogenous treatment with CT-1 as well as hypoxia treatment resulted in intracellular CT-1 translocation into the cell nucleus within 2-4 h. The stimulation of cardiomyogenesis by CT-1 as well as nuclear translocation of CT-1 under normoxic and hypoxic conditions was abolished in the presence of BAPTA, AM, the nitric oxide (NO)-synthase inhibitor L-NAME as well as the free radical scavenger N-(2-mercaptopropionyl)-glycine (NMPG). It is concluded that nuclear translocation of CT-1 is central to CT-1-mediated cardiomyogenesis and involves intracellular calcium, NO and reactive oxygen species in CT-1 regulated signal transduction pathways.

Abstract No. P063

Regulation of alternative polyadenylation by hnRNPs in human embryonic stem cells

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Alternative polyadenylation (APA) is increasingly being recognized as an important mechanism to control gene expression with essential role in stem cell differentiation. Over half of all mammalian genes contain multiple polyadenylation sites that lead to different 3'UTRs for a gene. Studies have shown that the APA pattern varies across tissues, and is dynamically regulated in stem cell differentiation and reprograming. However, the factors controlling APA are still poorly understood. We elucidated novel TDP-43/TIA/TIAL functions in APA and its connection to the development.

For this study we developed a high-throughput sequencing method (pA-seq) to specifically identify the polyadenylation sites regulated by TDP-43 and TIA1/L1 RNA binding protein. We further used the individual-nucleotide resolution crosslinking and immunoprecipitation (iCLIP) to study how interactions between TDP-43 and pre-mRNAs regulate APA. We determined nucleotide-resolution RNA maps showing how each protein regulates polyA sites by binding in the vicinity to the repressed polyA site. To directly evaluate the effect of this binding on APA, we designed several minigenes containing 3'UTRs with the regulated alternative poly(A) sites. Quantitative RT-PCR showed that minigenes mimic the polyA site regulation seen in the endogenous genes, and this regulation could be disrupted by mutating the RNA binding sites of TDP-43. A sequence-motif swap experiment in a reporter minigene confirmed that both protein can repress the same polyA site if their binding site is positioned close to the polyA site.

Our results demonstrate that hnRNPs proteins regulate APA in human embryonic stem cells in a position-specific manner and present the functional impact of TDP-43 on the transcriptome heterogenity. We propose that regulation of alternative poly-adenylation is conceptually similar to the repression of alterative exons, where hnRNPs compete with the core splicing machinery.

Abstract No. P064

Influence of the microRNA-375 during pancreatic differentiation of human embryonic stem cells

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Human embryonic stem cells (ESC) are a valuable source for the generation of insulin-producing cells but current protocols for pancreatic in vitro differentiation show low efficacy and pancreatic derivatives show no glucose-dependent insulin secretion. Thus, in vitro formation of fully functional beta cells is not possible.
MicroRNAs (miRs) play an important role in beta cell differentiation and function. We hypothesize that there are significant differences in the expression profile of pancreas-associated miRs during in vivo pancreas formation and the in vitro differentiation. Therefore, we analyzed the expression profile of the pancreas-associated miR-375 during in vitro-differentiation of murine and human ESC. In both, the expression of miR-375 increases during development into definitive endoderm (DE) followed by a continual decrease. MiR-375 is essential for early definitive endoderm development in both species since low expression at this stage leads to ineffective production of pancreatic progenitors. To modulate the miR profile, we transfected human ES cells with a miR-375-inhibitor and analyzed DE development as the first step of pancreatic differentiation. Inhibition of miR-375 results in decreased expression of endodermal marker genes like SOX17 and FOXA2. Moreover, the induction of miR-375 at later differentiation stages leads to increased expression of endogenous pre-miR-375 and more efficient formation of endocrine pancreatic progenitors expressing PDX1 and NGN3. Currently, the experiments are verified with a second human ESC line to show, that the observed effects indicate a general mechanism. In summary, miRs are a powerful tool to direct the developmental potential of the pluripotent cells.

Abstract No. P065

Human pluripotent stem cell-derived radial glia-like cells with stable regional identities

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Key challenges associated with the biomedical application of pluripotent stem cell (PSC)-derived neural cells are their controlled patterning towards distinct regional subtypes and the maintenance of an acquired regional phenotype across multiple passages of in vitro expansion. Previous studies have shown that rosette-type long-term self-renewing neuroepithelial stem cells (lt-NES cells) generated from human PSC undergo gradual posteriorization into an anterior hindbrain phenotype, which might be due to regionalizing effects of the growth factors employed for in vitro proliferation (1). During normal CNS development, early NES cells give rise to radial glia (RG) cells, which represent a major source of regionally determined neurons in the embryonic and fetal brain. Here we explored whether PSCs and primary hindbrain-derived neuroepithelial stem cells (NES cells) can be coaxed into RG-like cells and whether those maintain a stable regional phenotype. Using partially differentiating conditions we were able to establish a NSC population, which exhibits features of multipotent neuro- and gliogenic RG cells. Similar to primary RG-like cells (2), these RG-like NSCs could be expanded for at least 25 passages and expressed classical NSC markers such as Nestin and SOX2 as well as markers typically associated with RG cells including SOX9, CD44, AQP4 and HOP, while the lt-NES cell markers PLZF, DACH1, ZO-1 and MMRN1 were downregulated. We next asked whether these cells retain their regional phenotype during in vitro proliferation. To that end we generated RG-like cells from anterior, hindbrain- and spinal cord-patterned rosette-type NSCs. We found that RG-like cells with those regional identities continue to express transcription factors appropriate for their positional identity across multiple passages of in vitro expansion while maintaining their differentiation potential into neurons and glia. Thus, conversion into RG-like cells may provide a route for conserving the regional identity of pre-patterned early NSCs.

(1) Koch et al., PNAS 106:3225-30, 2009
(2) Su et al., Mol. and Cell. Neurosci., 38:245-258, 2008

Abstract No. P066

Interplay of Trim-NHL miRNA regulators in neural development

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Lin41, a conserved heterochronic gene and member of the Trim-NHL family of Ring E3 ubiquitin ligases, is involved in stem cell differentiation. Highly expressed in stem cells and early embryos, Lin41 is downregulated during CNS development, while expression of the other mammalian Trim-NHL family members Trim2, Trim3 and Trim32 increases. In C. elegans, loss of the Trim32 ortholog NHL-2 rescued the hypomorphic Lin41 phenotype, demonstrating a genetic interaction between them. Furthermore, Lin41 acts as an inhibitor and Trim32 as an enhancer of let-7, a differentiation associated microRNA. To better understand these interactions, we first performed co-staining and co-immunoprecipitation assays. We were able to show colocalization of all three Trim-NHLs with Lin41 in cytoplasmatic foci of transfected cells and in E11.5 neuroepithelium. Co-immunoprecipitation from crude E11.5 head extracts showed an RNA-independent physical interaction of Trim2, Trim3 and Trim32 with Lin41. As all four Trim-NHL proteins are functional Ring E3 ubiquitin ligases, this result suggests that the Trim-NHLs act in a combinatorial fashion. We hypothesize mutual ubiquitination or change of target specificity of Lin41 and Trim32 could provide a switch from the pluripotent (let-7 inactive) to the differentiated (let-7 active) cell state.

Abstract No. P067

Extracellular transfer RNA stimulates vasculogenesis and leukopoiesis of mouse embryonic stem cells

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Cell injury releases nucleic acids to support inflammation and stem cell activation. Here, the impact of extracellular ribonucleic acid, especially transfer (t)RNA, on vasculogenesis and leukopoiesis of mouse embryonic stem (ES) cells was investigated. Extracellular tRNA (ex-tRNA) and whole cell RNA but not DNA increased CD31-positive branching points in embryoid bodies. tRNA treatment increased cell numbers of VEGFR2 (flk-1)+, CD31+ and VE-cadherin+ vascular cells as well as CD18+, CD45+ and CD68+ cells, indicating leukocyte/macrophage differentiation. This was paralleled by mRNA and protein expression of hypoxia-inducible factor-1α (HIF-1α), vascular endothelial growth factor-165 (VEGF-165) and neuropilin -1 (NRP-1) as well as phosphorylation of phosphatidyl inositol 3-kinase (PI3K) and VEGF receptor 2 (VEGFR2). Furthermore, ex-tRNA increased protein expression of the pro-angiogenic semaphorin B4 receptor plexin B1 as well as the EphB4 receptor and ephrinB2 ligand. Ex-tRNA enhanced cell migration, which was inhibited by the VEGFR2 antagonist SU5614 and the PI3K inhibitor LY294002. This likewise abolished the effects of ex-tRNA on vasculogenesis and leukopoiesis of ES cells. Ex-tRNA increased Nox-1, Nox-2 and Nox-4 mRNA and boosted the generation of reactive oxygen species (ROS) which was inhibited in the presence of radical scavengers as well as the NADPH oxidase inhibitors apocynin and VAS2870. The latter abolished the stimulation of vasculogenesis upon ex-tRNA treatment. Our findings indicate that ex-tRNA treatment induces vasculogenesis and leukopoiesis of ES cells via ROS generated by NADPH oxidase, activation of VEGFR2 and PI3K.

Abstract No. P068

High-throughput screening in mesenchymal stem/stromal cell (MSC) emphasizes the role of Wnt signaling in stem cell differentiation

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Stem cell differentiation is crucial in many biological processes such as development, tissue-maintenance and organogenesis as well as in the onset and progression of cancer. Increasing evidence suggests, that a finely balanced system of canonical and non-canonical Wnt signals controls these processes. Therefore, we aimed to further dissect the core machinery which controls stem cell differentiation to obtain valuable insights into cancer development as well as to facilitate future tissue engineering approaches.

To investigate differentiation in stem cells, we analyzed mesenchymal stem/stromal cells (MSCs) isolated from human bone marrow as a model system. MSC are a heterogenous population of multipotent cells that rely on Wnt signals to control their continued proliferation and differentiation into e.g. adipocytes, osteoblasts or chondrocytes. We established a high-throughout RNAi screening method and showed that MSC heterogeneity is in principle no obstacle in the screening of primary adult stem cells. Our results, based on kinome profiling indicate that MSC have distinctly different genetic requirements than primary fibroblast, suggesting that MSC, obtained by current methods are indeed a distinct cell population.

Adapting the established screening protocols for cytometric and microscopy read-outs we screened for factors which influence adipogenesis in MSC. We identified several candidates which alter adipogenesis by modulating Insulin as well as Wnt signaling activity. Using TCF/LEF reporter assays we validated a novel candidate protein which represses canonical Wnt signaling and enhances adipogenesis in MSCs. Previous results, indicated that our candidate acts through the PI3K/AKT signaling pathway and serves as a signaling platform for several Wnt pathway components. In conclusion we show that high-throughput techniques can be successfully applied to dissect differentiation processes in stem cells. We successfully used these techniques to study adipogenesis in MSC and revealed a new crosstalk between Wnt signaling and other pathways that control cell fate decisions in MSC.

Abstract No. P069

HOXB4 cooperates with Runx1 to promote the development of hematopoietic cells at the hemogenic endothelium stage, in vitro

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Hematopoietic differentiation of pluripotent stem cells and expansion of emerging stem and progenitor cells (HSPCs), in vitro, are strongly enhanced by ectopic expression of the homeodomain transcription factor HOXB4. However, the underlying mechanisms are still ill-defined. Therefore, we investigated its influence on major fate decisions during different stages of embryonic stem (ES-) cell differentiation, in vitro. For this purpose, we retrovirally expressed HOXB4 or a Tamoxifen-inducible HOXB4-ERT2 fusion protein in different reporter ESC-lines. These lines were differentiated as embryoid bodies (EBs) for 6 days and, after dissociation, cultured on OP9-stroma cells for different periods of time. During cocultivation on OP9 cells, HOXB4 mediated a significant increase in the number of circular, sheet-like structures which expressed Endothelial Protein C Receptor (CD201), VE-Cadherin (CD144), Tie2 (CD202b), Flk1 (KDR, CD309), PECAM1 (CD31) and were capable of Dil-ac-LDL uptake. When using ESCs containing a Venus reporter gene integrated into one Runx1 allele (Runx1(Venus/+); [1]), we noted that the emerging Runx1/Venus+ CD41+ hematopoietic cells were associated with such sheet-structures, thus representing hemogenic endothelium layers. In this reporter line, HOXB4 did not induce or alter initial Runx1 expression during EB-development. However, during the subsequent OP9-cocultivation phase it promoted the appearance of a subpopulation expressing increased levels of Runx1/Venus, which contained all arising CD45+ cells. Furthermore, use of a Runx1-/- ESC-line allowing for Doxycycline-inducible Runx1 expression (iRunx1; [2]) and additionally expressing Tamoxifen-inducible HOXB4 uncovered that HOXB4 alone strongly promoted the formation and accumulation of hemogenic endothelium structures. Only additional induction of Runx1 expression initiated endothelial to hematopoietic transition (EHT) and expression of transcription factors necessary for hematopoiesis, such as Gfi1, Gfi1b and Pu.1. So far, our results strongly suggest that HOXB4 and Runx1 interdepend during hematopoiesis, particularly at the transition of the hemogenic endothelium to definitive hematopoietic progenitor cells, at least during ESC differentiation, in vitro.

[1] Runx1(Venus/+) ESC-line was kindly provided by S.I. Nishikawa, RIKEN Center for Developmental Biology, Kobe
[2] iRunx1 ESC-line was kindly provided by G. Lacaud, Paterson Institute for Cancer Research, Manchester

Abstract No. P070

Efficiency of retinal cell fate induction from mouse embryonic stem cells and hPax6-GFP reporter expression

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The differentiation of pluripotent stem cells into retinal cell types opens new possibilities for studies on retinogenesis and retinal degeneration. Recently, several protocols for the differentiation of pluripotent stem cells into retina have been developed. Here, we modified a recently developed mouse embryonic stem cell (mESC) three-dimensional differentiation approach (Eiraku et al, 2011), to make it independent of a transgenic eyefield reporter, and investigated the efficiency of mESC derived retinogenesis at all steps of the protocol. Further, we characterized the GFP expression pattern of a novel hPax6-GFP transgenic mESC reporter in retinogenesis.

We achieved efficient eyefield induction (82 ± 12 % of aggregates, N=7), as well as patterning into RPE and neural retina domains. However, optic cup-like structures were not formed. Further, upon randomly cutting aggregates into three evenly sized pieces, the majority (86 ±6 %, N=2) generated big, stratified retinal tissue, reminiscent of early postnatal retina in vivo. Each aggregate retina had 0.41 to 1.9 mm (1.4 ± 0.4 mm) circumferential length. This novel approach makes the generation of mESC derived, stratified retina simpler and independent of an eyefield transgenic fluorescent reporter.

Using this 3D retinal differentiation approach, we found that the hPax6-GFP reporter construct is expressed in a major amacrine population (Pax6+, Brn3-, HuCD+, AP2a+, bHLHb5+, Ebf3+), as well as a retinal progenitor or precursor subpopulation (Pax6+, PH3+, Rx+, Chx10+, Crx+) of the Pax6 lineage. Interestingly, two different mESC clones carrying this reporter construct showed striking differences in GFP expression: GFP expression started later in clone 2 and was detected in less cell types than in clone 1. Therefore, these novel mESC reporter lines will be a useful tool to study lamination, amacrine lineages and subtypes, wiring and patterning and degeneration of amacrine neurons in mESC derived retina.

Eiraku M, Takata N, Ishibashi H, Kawada M, Sakakura E, Okuda S, Sekiguchi K, Adachi T, Sasai Y. (2011). Self-organizing optic-cup morphogenesis in three-dimensional culture. Nature7; 472:51-6.

Abstract No. P071

TBX3 Directs Cell-Fate Decision toward Mesendoderm

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Cell-fate decisions and pluripotency are dependent on networks of key transcriptional regulators. Recent reports demonstrated additional functions of pluripotency-associated factors during early lineage commitment. The T-box transcription factor TBX3 has been implicated in regulating embryonic stem cell self-renewal and cardiogenesis. Here, we show that TBX3 is dynamically expressed during specification of the mesendoderm lineages in differentiating embryonic stem cells (ESCs) in vitro and in developing mouse and Xenopus embryos in vivo. Forced TBX3 expression in ESCs promotes mesendoderm specification by directly activating key lineage specification factors and indirectly by enhancing paracrine Nodal/Smad2 signaling. TBX3 loss-of-function analyses in the Xenopus underline its requirement for mesendoderm lineage commitment. Moreover, we uncovered a functional redundancy between TBX3 and Tbx2 during Xenopus gastrulation. Taken together, we define further facets of TBX3 actions and map TBX3 as an upstream regulator of the mesendoderm transcriptional program during gastrulation.

Abstract No. P072

hMSC Differentiation Towards Endothelial and Smooth Muscle Cells are Regulated via Different P2 Receptors and Their Underlying Signalling Pathways

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Human mesenchymal stem cells (hMSCs) have shown their capacity to differentiate towards endothelial and smooth muscle cells. Therefore the usage of hMSCs for vascular tissue engineering is increasingly attractive. Purinergic 2 (P2) receptors participate in a series of cellular processes e.g. cell proliferation, apoptosis and migration where they bind to extracellular nucleotides. Our previous data revealed for the first time that P2 receptors play a key role during hMSC differentiation towards adipocytes and osteoblasts. However, their functional role in endothelial and smooth muscle cell differentiation and the underlying mechanisms are still largely unclear.
Endothelial and smooth muscle-like cells were differentiated from hMSCs and characterized by specific markers. Several P2 receptor subtypes were differently regulated during these specific lineage commitments such as the Gs-coupled P2Y11 receptor which was up-regulated in endothelial cell differentiation and the Gq-coupled P2Y1 which was down-regulated in smooth muscle cell differentiation. The administration of extracellular nucleotides or P2 receptor artificial ligands had a direct influence on these differentiation processes. As expected, the Gq-downstream p38/CREB and Gs-downstream ERK/c-jun pathway was regulated in endothelial cell differentiation and the pathways via Akt or c-jun were regulated in smooth muscle cell differentiation accordingly as shown by a protein phosphorylation array. Furthermore, apyrase digest showed a feedback loop via exogenous extracellular nucleotides on these particular differentiation processes.
In conclusion, P2 receptors act as vital players during the hMSC differentiation towards endothelial and smooth muscle cells. Both, natural and artificial P2 receptor ligands can influence these specific differentiation lineages by regulating specific P2 receptors and their underlying signalling pathways. These findings provide new insights in understanding the molecular mechanisms of MSC differentiation.
Key word: Human mesenchymal stem cells, endothelial cell differentiation, smooth muscle cell differentiation, purinergic receptors

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P081 MYC Restricts The Stemness of Breast Epithelial Stem Cells via a RGS16/RHOA/YAP Axis
Bjoern von Eyss

Abstract No. P073

Differentiation of human multipotent and pluripotent stem cells to develop a human 3D in vitro blood brain barrier model

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Human induced pluripotent stem cells (hiPSCs) as well as multipotent fetal brain cells pose reliable and effective cell sources to generate differentiated functional brain cells and have the advantage to be independent of postnatal brain tissue biopsies including their variations and limitations during in vitro culture.
The aim of this study is the direct differentiation of hiPSCs and fetal multipotent brain cells into human brain astrocytes and endothelial cells. In particular, we want to develop a 3D in vitro co-culture model, which closely simulates the in vivo situation and can be used as a tool in preclinical research such as in drug transport or infection studies.
We are able to maintain undifferentiated hiPSCs under feeder free conditions shown by positive FACS analyses for the pluripotency markers Nanog, Oct 3/4 and Sox-2 and alkaline phosphatase assay. Furthermore, differentiation protocols are adapted to generate brain endothelial cells as well as human astrocytes from iPSCs mimicking the in vivo embryogenesis. Specific hiPSCs differentiation into functional endothelial cells and astrocytes are performed as described recently (Krencik and Zhang 2011, Lippmann et al. 2012).
Neural progenitor cells were isolated from human fetal brains and were spontaneously differentiated into brain astrocytes by treatment with 10 % FCS for 12 days (Lippmann et al. 2011). The cells were characterized by immunohistological stainings against Nestin and GFAP as well as by PCR analyses using cell-specific primers of the same targets.
Finally, it is our aim to combine both cell types in a co-culture setup in order to closely mimic the microenvironment of the BBB in vivo. Therefore, we developed a 3D scaffold consisting of collagen and the typical basal membrane proteins. Moreover, we constructed a dynamic flow reactor system to simulate the bloodstream, which will be combined with our newly generated cells and biomatrix.

Abstract No. P074

The role of Diazepam Binding Inhibitor (DBI) in adult hippocampal neurogenesis.

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In the mammalian brain there are two areas where adult neurogenesis takes place: the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus. Both internal and external cues have been shown to modulate the generation, integration and survival of postnatally generated neurons (Ming and Song et al.,2011; van Praag et al., 2000). GABA acts at the level of the neural stem cells (NSCs) and fast dividing progenitors, most likely as a feedback signal from the neuroblasts or as an indicator of the circuit activity regulating their proliferation. Diazepam Binding Inhibitor (DBI) is an endogenous protein expressed in the SVZ neural progenitors that acts as a modulator of the GABAA receptor. Gain and loss of function studies showed that DBI enhances the proliferation of neural progenitors in the SVZ by preventing them from exiting the cell cycle (Alfonso et al., 2012). Therefore, DBI plays an important role in regulating adult SVZ neurogenesis by modulating the GABA regulatory signal.
Similar to the SVZ NSCs, SGZ NSCs respond to nonsynaptic GABA signaling by the activation of the GABAA receptor which leads to a reduction in their proliferation (Song et al., 2012). Therefore, we decided to investigate whether DBI plays a role in hippocampal neurogenesis as well, and constitutes a general modulator of the cell cycle machinery in postnatally generated neurons. We found that DBI is strongly expressed in the SGZ NSCs. Furthermore, in vivo knockdown of DBI in the DG via a lentiviral strategy leads to a reduction in the stem cell and in the intermediate progenitor cell populations. Our results suggest that DBI promotes postnatal neural proliferation in both neurogenic niches, regulating the production of both inhibitory (SVZ) and excitatory neurons (SGZ).

Alfonso, J., Le Magueresse, C., Zuccotti, A., Kodosevich, K., and Monyer, H.(2011). Diazepam Binding Inhibitor Promotes Progenitor Proliferation in the Postnatal SVZ by Reducing GABA signaling. Cell Stem Cell 10,1016
Song, J., Zhong C., Bonaguidi, M.A., Sun, G.S., Derek, H., Meletis, K., Huang, J., Ge, S., Enikolopov, G., Deisseroth, K., Luscher, B., Christian, K., Ming, G.L., and Song, H. Neuronal circuitry mechanism regulating adult neural stem cell fate decision.

Abstract No. P075

CXCL12 and CXCR4 in the ependymal spinal cord stem cell niche: activation and migration in spinal cord injury

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Regeneration of injured central nervous system and consequently functional recovery is limited. We have demonstrated in experimental animal studies that local infusion of the chemokine, stromal cell derived factor 1 (SDF-1/CXCL12), to injured spinal cord enhances axonal sprouting. In line with this, infusion of CXCL12 dose-dependently improves motor function recovery following spinal cord injury (SCI). However, the mechanisms underlying these beneficial effects of CXCL12 are still unknown, but likely aside from direct effect on axons include recruitment of endogenous stem and progenitor cells via its receptor CXCR4.

We, therefore, sought for the CXCL12-responsive cells in the spinal cord and detected very strong and predominant immunoreactivity for CXCR4 in the ependymal cells located around the central canal. These ependymal stem/progenitor cells (epSPCs) supply progenitors similar to the ventricular layer in the brain and following SCI they migrate to the injury site, accumulate in the glial scar over time and differentiate into scar-forming glial cells and, to a lesser extent, to oligodendrocytes. Modulation and control of the epSPCs niche would, therefore, be a good therapeutic target in SCI. In the neurogenic niche in the brain the CXCL12/CXCR4 signaling regulates homing and exit of stem cells from the niche, suggesting a similar role in the spinal cord epSPC niche.

To explore this, we are using the FoxJ1-CreER/ROSA mice (Meletis et al., 2008), in which epSPCs are genetically labeled, along with locally infused CXCL12, and in combination with the CXCR4- and CXCL12-floxed mice in which the epSPCs are deficient for CXCR4 and CXCL12, respectively. This knowledge will be valuable to promote the endogenous repair process.

Abstract No. P076

Signals from the injury niche increase neural stem cell's fate potential

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During the development of the central nervous system, multipotent neuronal progenitors follow a precise differentiation process to generate the exquisite variety of neuronal subtypes present in the mature brain. Unlike embryonic progenitors, adult neural stem cells (NSCs) only give rise to restricted neuronal subtypes, namely inhibitory interneurons in the olfactory bulb and glutamatergic granule neurons in the hippocampus. However, after brain injury this fate restriction can be partially overcome. The factors of the 'injury niche' that positively influence neurogenesis and that may be responsible for fate reprogramming remain largely unknown. Here we study the regulation of NSCs plasticity by CD95L, a cytokine of the TNF family that is specifically upregulated after brain injury. Using heterochronic transplantations into the developing brain as a measure of fate potential, we show that only upon CD95L stimulation adult NSCs migrate and differentiate into cortical and hippocampal regions. Genome-wide transcriptional profiling revealed that CD95L selectively regulates genes involved in neuron development and chromatin remodeling, suggesting epigenetic priming. Accordingly, ChIPseq for several histone modifications uncovers that multiple loci gain active chromatin marks after stimulation, probably facilitating transcriptional activation during neuronal subtype-specific differentiation. Altogether, we show that cues from the injury niche promote a permissive chromatin state that endows adult NSCs with increased fate plasticity.

Abstract No. P077

Choroid plexus secreted microRNAs maintain neural stem cells by regulating neurogenic fate determinants

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Neurogenesis in the adult mammalian brain is restricted to specialized niches, the SEZ (subependymal zone) of the lateral ventricle and the SGZ (subgranular zone) of the hippocampus. In the SEZ, neural stem cells (NSCs) generate transit amplifying precursors that divide and give rise to neuroblasts, a transient progenitor population migrating to the olfactory bulb (OB). Numerous intrinsic fate determinants have been proposed to control the differentiation of NSCs towards mature neurons in the OB. A genome-wide transcriptome analysis of prospectively isolated NSCs and their progeny revealed that the mRNA, but not the protein, of neurogenic transcription factors (TF) was present already in the NSCs. As the NSCs are a specialized set of astrocytes, we compared the mRNA levels of the neurogenic fate determinants in postmitotic astrocytes and NSCs, which revealed significantly higher mRNA levels in the NSCs, suggesting the priming of NSCs towards neurogenesis.

Towards a better understanding of these posttranscriptional mechanisms, we screened the 3´UTRs of neurogenic TFs expressed in the NSCs for common binding sides recognized by the same miRNAs. Here, we found four candidate miRNAs, all resulting in a functional regulation in the luciferase assay. We could detect two of them in the population of FACS-purified NSCs. Overexpression in neural progenitors, including NSCs, lead to reduced generation of neurons. These data suggest a role of miRNAs in suppressing neurogenesis from adult NSCs. Furthermore, the expression patterns of mature and pre-miRNA suggest the choroid plexus (CP) as the source of one miRNA that is then transported via the cerebrospinal fluid to the SEZ. Taken together, our results suggest a novel mechanism to maintain NSCs in the specific niche via CP secreted miRNAs that regulate neurogenic fate determinants in the NSCs.

Abstract No. P078

DNA damage in mammalian neural stem cells leads to senescence-associated secretory phenotype and BMP2/JAK-STAT mediated astrocytic differentiation

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Recent research demonstrates the necessity to understand the impact of DNA damage on somatic stem cells. Especially for neural stem cells (NSC), this impact is poorly elucidated. We recently reported that wildtype and gene-deficient embryonic stem cell-derived NSC, when exposed to X-ray induced DNA damage, rapidly entered a novel kind of irreversible cellular senescence, despite a widespread transcriptional downregulation of DNA damage response (DDR) signalling machinery. As we have previously associated this phenotype with the terminal differentiation of NSC into astrocytes, we indeed revealed that irradiated NSC lost their self- renewal potential and downregulated the expression of numerous typical stem cell markers. Moreover, irradiated NSC underwent astrocytic differentiation and upregulated the typical marker GFAP while still cultured in conditions promoting self-renewal. We showed the phenomenon of the senescence-associated cytokine secretion to be the relevant mechanism this process. Of these cytokines, BMP2, but not IL-6 or LIF, was responsible for expression of GFAP in irradiated NSC via its novel non-canonical signalling through JAK-STAT. Moreover, control the DDR gene ATM plays a supportive role in cytokine-induced differentiation, this process can be efficiently potentiated through p53 deficiency. Finally, we recapitulated our observation of irradiation-induced NSC differentiation in adult NSC in vitro and in vivo. Using a Sox2—Cre reporter mouse model for NSC fate tracing, we demonstrated that cranial irradiation results in astrocytic differentiation of Sox2-expressing NSC residing in the adult subventricular zone.

Schneider L. et al., Stem Cell Reports 2013 Aug 6, doi: 10.1016/j.stemcr.2013.06.004

Abstract No. P079

Marked expression of male specific genes during differentiation of human embryonic stem cells into dopaminergic neurons

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The genetic basis of sex differences in the neuron development is quite challenging. Omics and epidemiological evidences suggest that the male gender is one of the risk factors for the development of Parkinson disease (PD). However, molecular evidences in gender-biased neurological disorder are still lacking.. Here, we analyzed the expression of 23 Y chromosome genes and 17 of their X-linked counterparts during hESC differentiation to dopaminergic cells. The transcript and protein levels of target genes were analyzed at 0, 15, 25 days after neural differentiation of hESCs. Of 41 genes analyzed in this study, 12 showed very low or no expression in hESC or differentiated cells. Quantitative real-time PCR showed the expression level of 17 Y and 5 X genes including, UTY, SRY, SMCY (KDM5D), DAZ, NLGN4Y, PRY, PRKY, TMSB4Y, USP9Y, DDX3Y, PCDH11Y, RBMY1A, HSFY, RPS4Y, CYORF15A, CYORF15B, CDY, SMCX (KDM5C), DDX3X, HSFX, TMSB4X,VCX increased between 1.5 to 10 folds in differentiated cells compared to hESC. Furthermore, western blotting and immunofluorescence analyses revealed that DDX3Y, PCDH11Y, RBMY1, RPS4Y1, KDM5D, BPY2, PRY, HSFY1,CYORF15A, USP9Y are expressed at protein level on precursore and mature dopaminergic neurons.

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Abstract No. P080

Male specific genes expression pattern under Androgen treatment during differentiation of NT2 to dopaminergic neurons

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We investigated the expression pattern of human Y chromosome genes during differentiation of NTera2/cloneD1 (NT2) cells to neural cells under three different treatments including Retinoic acid (RA), Dihydroepianostrone (DHEA), and RA+DHEA. Quantitative Real-Time PCR analysis showed that expression of neural progenitor markers such as Pax6 and TUJ1 were significantly up-regulated during differentiation. Furthermore, the expression of PTX3 and TH, as dopaminergic neurons markers, significantly increased particularly in response to DHEA+RA treatment. The expression of several Y chromosome genes including RBMY,1 HSFY1, DDX3Y1, and CDY were detected only during differentiation to neural progenitor . Western blot analysis also confirmed the accumulation of Y chromosome proteins in neural progenitor compared to NT2. Overall, our results suggest a possible contribution of Y chromosome genes in neural development. Studies to elucidate the roles of these genes during neural differentiation are in progress

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Abstract No. P081

MYC Restricts The Stemness of Breast Epithelial Stem Cells via a RGS16/RHOA/YAP Axis

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During the past decades, the MYC oncoprotein has been established as an important driver of several tumor entities. Specifically, it is associated with the highly aggressive basal subtypes in breast cancer. Nevertheless, the specific role of MYC in this particular disease remains unsolved. To gain insights into the affected pathways by deregulated MYC expression we analyzed the effect of MYC overexpression in a non-tumorigenic primary cell line, namely HMLE. This cell line is heterogenous since it consists of a more differentiated CD44low/CD24high subpopulation and a basal/stem cell-enriched CD44high/CD24low subpopulation.

RNA-Seq analysis of the sorted populations revealed two important findings. Firstly, the stem cell-enriched population shows a strong upregulation of YAP target genes, the downstream effector of the Hippo pathway and an important factor for the maintenance of several adult stem cells. Secondly, MYC strongly repressed the expression of YAP target genes in the stem cell-enriched population. Consistent with this, MYC expression leads to severely impaired sphere forming ability of the stem cell-enriched poulation.

Further epistatic and biochemical experiments demonstrate that MYC deploys an inhibition of RHOA to affect the activity of YAP. Surprisingly, this effect of MYC on YAP via RHOA is mediated by a single direct MYC target gene, namely RGS16. RGS16 has already been described as a negative regulator of RHOA signaling. Consistent with RGS16 being MYC's critical mediator, depletion of RGS16 is able to completely eliminate MYC-mediated repression of YAP target genes. Interestingly, the effect of MYC on YAP gene expression is maintained during tumorigenesis of the breast. Here, we verify that MYC is correlated with the basal subtype and that expression of a MYC signature is inversely correlated with a YAP signature. Thus, these findings have not only important implications for adult mammary stem cells but also for treatment of breast cancer.

Stem cells in tissues of meso- and endodermal origin: P082 – P094

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Abstract No. P082

CORD BLOOD DERIVED ENDOTHELIAL COLONY FORMING CELLS EMERGE FROM A CD45dim CD31+ CIRCULATING PRECURSOR

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Within the last decade numerous studies focused on endothelial progenitor cells (EPC) serving as biomarkers or resource for cell-based therapies. However, a variety of different cell types are subsumed under the term EPC. Depending on the protocol culture adapted EPC have now been classified as colony forming unit endothelial cells (CFU-EC), early outgrowth/ proangiogenic (CAC) or late outgrowth/endothelial colony forming cells (ECFC). Especially circulating EPC have been postulated as biomarkers, however their precise phenotypic definition is heavily discussed. Authors propose different combinations of the markers CD34, CD133, VEGFR-2/KDR, CD31 and CD45 debating whether EPC are of hematopoietic origin or not.

To gain insight into the early phases of the differentiation cascade, the phenotypes of uncultivated CD34+ mononuclear cells (CD34+ MNC), ECFC and HUVEC at primary passage (p0) and p1 were analyzed. Already within p0 ECFC underwent a rapid maturation from a CD45+ and CD31+ phenotype to a CD45-negative, and endothelial marker-positive phenotype as defined by flow cytometry and multiphoton imaging. In p0 ECFC colonies of homogenous cobblestone morphology contained subpopulations expressing a mature endothelial phenotype, but also subpopulations co-expressing CD45dim and CD31, but no other hematopoietic marker such as CD14, CD41 or CD62p. Imaging revealed that CD45 was dimly expressed at the cell surface - only in ECFC but not in HUVEC. Interestingly, few ECFC showed perinuclear CD45 aggregation or intracellular CD45 caps. Compared to HUVEC, ECFC showed a less concise expression of CD31 at the cell-cell-contact sites. Finally our data confirm ECFC as a unique cell population exerting high angiogenic and vasculogenic capacity

Our study supports the notion that ECFC emerge from a CD45dim CD31+ progenitor and very rapidly mature in culture. The data strengthen the necessity to identify a set of markers capable of prospectively discriminating endothelial from hematopoietic cells as well as progenitor from mature cells.

Abstract No. P083

Towards vascularized tissue engineered bone for elderly patients

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Introduction

Due to demographical changes, the need for biomaterials to reconstruct bone defects in elderly patients is increasing. Today, common scaffolds do not consider age-specific changes in bones (e.g. osteoporosis). Such changes are considered in this project, by manufacturing ß-tricalcium phosphate scaffolds with different concentrations of strontium. Strontium is known to enhance osteoblastic function and to inhibit osteoclastic activity. Using a novel method of synthesis, strontium atoms can directly be incorporated into the crystal lattice of highly pure ß-tricalcium phosphate scaffolds, without affecting the structure. Tailored 3D-scaffolds with defined pore design and size have been developed. The aim of this project is to reinforce the bioactivity of such biodegradable scaffolds.

Methods

Human mesenchymal stem cells (MSC) undergo osteogenic differentiation while cocultured with human umbilical vein endothelial cells (HUVEC), which should form vessels inside the pores of the scaffold. To induce angiogenic and osteogenic conditions in coculture, two novel media were designed. They were compared with established media for monoculture of MSC (SZM) and HUVEC (EBM). In the 2D coculture morphology, cytocompatibility, proliferation, the ability to support angiogenesis and osteogenic differentiation were analyzed.

Results/ Discussion

The results were compared with the respective monoculture. A system to test osteogenic differentiation of MSC and vessel formation of HUVEC in parallel is established. The new designed media are generating promising results. In the future, the coculture will be established in a 3D bioreactor system, to enhance the osteogenic differentiation via shear stress.

Abstract No. P084

Characterization of human adipose-derived stem cells cultured in autologous serum after subsequent passaging and long term cryopreservation

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Recent scientific achievements in the cell and molecular biology have promoted development of autologous stem cell therapy, offering comprehensive possibilities for the treatment of human diseases and dysfunctions. However, clinical applications require extensive expansion of cells to meet the sufficient amount of cells for a patient. It has been shown that the time of mesenchymal stem cell (MSC) culturing can affect their immunophenotypic, differential, and proteome profile.
The aim of this study was to evaluate human adipose-derived stem cells (ASCs) from passage 2 (P2) to P8 cultured in medium containing 5% autologous serum (AS) after a long-term cryopreservation with regards to their surface marker expression, differentiation potential, and immunosuppressive effect in vitro. Using 8-color flow cytometry analysis we showed that all ASCs express typical mesenchymal stem cell markers CD29, CD44, CD73, CD90, CD105 simultaneously, but do not express such markers as HLA-DR, CD34, CD14, CD19, and CD45. Furthermore, median fluorescence intensity of positive cell surface markers increased with each subsequent passage indicating the accumulation of protein expression. The multilineage differentiation experiment demonstrated the ability of ASCs from P3 and P6 to efficiently differentiate into adipocytes and chondrocytes but their potential of osteogenic differentiation was diminished. Data from co-culture of ASCs and autologous peripheral blood mononuclear cells (PBMNCs) indicated that ASCs from P3, P6, and P9 significantly reduce the proliferation of PBMNCs at ASCs:PBMNCs ratio 1:1 and this suppression is dose dependent.
This study demonstrated that ASCs from P2 to P8, cultured in the presence of AS, represent a highly homogeneous cell population possessing multilineage differentiation ability and significant immunosuppressive properties after double freezing and more than 4 years of cryopreservation.

Abstract No. P085

Specification of human developmental progenitors and production of iPS cells for research of multifactorial diseases

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Purification of the individual types of tissue stem cells that emerge from human pluripotent stem cells (hPSCs) is paramount for regenerative treatments and disease modeling in vitro. We have begun conducting iterative rounds of cell surface marker screening using antibodies, analyzing gene expression patterns and assaying the growth potentials of sorted populations that emerge during early differentiation of hPSCs. We discovered cell surface maker combinations enabling isolation of populations from hPSCs treated with TGF-β ligands, and exhibiting primitive endoderm, mesoderm, and trophoblast progenitor characteristics. We propose that these progenitors are analogous to embryonic precursors of the visceral endoderm, primitive streak and chorion (placenta), respectively, and similarly to primitive streak precursors we show that mesoderm progenitors are capable of functional integration in human fetal heart tissue.
Our group now focuses on defining mesoderm progenitor subpopulations, and analyzing the mechanisms governing commitment of these progenitors from hPSCs. Subsequently, together with the human induced pluripotent stem cell (hiPSC) unit and the Institute of Diabetes and Obesity (IDO), we will purify progenitor populations from hiPSC lines derived from diabetes type 2 patients for studying the early phases of the disease.

Abstract No. P086

AGE-RELATED CHANGES IN THE SYSTEMIC ENVIRONMENT AFFECT MSCS FUNCTION AND ENDOGENOUS BONE REGENRATION VIA THE MITOCHONDRIAL/OXIDATIVE STRESS PATHWAY

Sven Geissler [1,*] , *Martin Textor* [1], *Katharina Schmidt-Bleek* [1], *Oliver Klein* [1], *Mario Thiele* [1], *Dorit Jacobi* [1], *Anke Dienelt* [1], *Grit Kasper* [1], *Patrick Strube* [1], and *Georg Duda* [1]

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Introduction: Even tissues capable of complete regeneration, such as bone, show an age-related reduction in their healing capacity (1,2). We hypothesized that this decline is not only associated with the age-related reduction in cell quantity and function but also results from changes in their systemic environment. Thus, we investigated the influence of serum from young and aged Sprague–Dawley rats on MSCs at cellular and molecular level.

Results: We could demonstrate that age-related changes in the systemic environment negatively affect MSC's survival and differentiation. In particular, cultivation of MSCs in serum of aged animals enhances their apoptosis rate, reduced their proliferation potential, compromised their osteogenic differentiation ability, and promoted their differentiation into the adipogenic lineage. Results of subsequent proteome (2DE MS/MS and Western Blot analysis) and cellular analysis identified enhanced intracellular oxidative stress as the underlying cause for the compromised MSC function in response to the age-altered systemic environment. Serum from aged animals not only changed expression of proteins related to mitochondria, unfolded protein binding and stress response, it also significantly enhanced intracellular ROS production and lead to the accumulation of oxidatively damaged proteins. Conversely, reduction of oxidative stress levels by antioxidant supplementation in-vitro or by oral administration in-vivo markedly improved MSC function and bone regeneration, respectively. In aged animals, the systemic antioxidant treatment significantly improved the mineralization, the microstructure, and the mechanical properties of the regenerated bone tissue.

Discussion/Conclusion: In summary, we propose that the systemic environment crucially contributes to the age-related decline in bone regeneration by increasing intracellular oxidative stress levels, hence compromising viability and function of mesenchymal cells. We conclude that novel therapeutic approaches for the improvement of endogenous (bone) regeneration, especially in elderly patients, should focus on ROS protection and/or the recruitment of progenitor cells to the site of injury, rather than simply on cell differentiation.

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Abstract No. P087

A new comparative microarray analysis unveils the transcriptome of native mesenchymal stem cells

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We know from the early work of Friedenstein and Rombouts that adult bone marrow (BM) harbors true mesenchymal stem cells (MSC) with the ability to generate new stroma in transplanted recipients [1]. However, these qualities are rapidly lost from in vitro cultures, which are better described as mesenchymal stromal cells (MStrC). The prospective isolation of enriched populations of the MSC precursors of MStrC would enable systematic studies of stem/progenitor characteristics in this system.

In recent years, we and others have worked independently on the prospective isolation of MSC from adult murine BM. We have defined a population of Sca-1+ CD45- Lineage- bone adherent, oxygen-sensitive (SL45) cells to be highly enriched in precursors of MStrC. The in vitro derivatives of SL45 cells fulfill all tested hallmarks of MStrC including tri-lineage differentiation potential and prolonged culture in vitro.

After carrying out transcriptome analysis of the purified SL45 cell population, we have developed a novel software (virtualArray [2]) that enables us to compare array data generated using different platforms. We have now used this software to compare transcriptome data of SL45 cells with those of independently isolated putative MSC populations analysed from other groups, together with a wide range of other cell types.

The resulting dataset clearly sets all prospectively isolated putative MSC apart from other known mature and stem cell types (including MStrC), while maintaining clear similarities between the MSC populations. Global analysis using hierarchical clustering, principal component analysis and tests of significance identified a specific profile with properties one would expect from native MSC: The development, organization and maintenance of mesenchymal tissues and extracellular matrix. The dataset generates new insights into native, unmodified MSC and establishes a molecular fingerprint of these cells that can be used for the further refinement of isolation and characterization.

Rombouts et al.: Primary murine MSC show highly efficient homing to the bone marrow but lose homing ability following culture. Leukemia, 2003 Jan;17(1):160-70.
Heider et al.: virtualArray: a R/bioconductor package to merge raw data from different microarray platforms. BMC Bioinformatics, 2013, 14:75

Abstract No. P088

Identification of small molecules inducing pluripotency and endoderm differentiation in the absence of extrinsic factors.

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The forkhead box transcrption factor, Foxa2, is expressed in the endoderm progenitors, in the primitive gut tube, in the pancreas progenitors and in the Insulin-producing beta cells of the pancreas. The underlying rationale is that small molecules inducing Foxa2 expression will have the potential to support endoderm differentiation into pancreas. Moreover Foxa2 is known to have a function in secretion of insulin from adult beta-cells, therefore the identified drugs may be useful for pancreas regeneration.

It has also been shown that Foxa2 plays a significant role in goblet and enteroendocrine cells differentiation in intestinal tissue. Therefore, stimulation of Foxa2 may control mucus secretion as well as expression of GLP1/2 in L- and D-cells, which are important for glucose metabolism.

In the light of recent findings in the field of stem cell biology that differentiated cell types can be reprogrammed into induced pluripotent stem cells (iPS), another key goal of our screen will be to identify small molecules able to induce pluripotency and replace essential pluripotency transcription factors.

Abstract No. P089

Signaling pathways controlling adipose tissue stem cells and metabolic plasticity

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The fascinating plasticity of adult adipose tissue is dependent to a great extent on adipose tissue stem/progenitor cells (ASC), an elusive cell type with mesenchymal stem/stromal cell characteristics. These cells contribute to adipose tissue expansion in diet-induced obesity as well as to the remodeling of white fat to oxidative brown fat upon prolonged cold exposure. However, the local mediators and signaling pathways controlling ASC function during organismal metabolic challenges remain obscure. We have previously shown that adipocyte-derived prostacyclin (PGI2) acts in a paracrine fashion downstream of sympathetic/beta-adrenergic stimulation to activate ASCs and promote their differentiation towards a brown adipocyte phenotype during the white-to-brown tissue remodeling process.
Here we have explored PGI2 treatment of primary mouse ACSs as a model to identify intracellular signaling pathways regulating ASC activation and differentiation. Differential gene expression and transcription factor motif analysis on global gene expression data revealed the biphasic regulation of Stat3 target genes downstream of PGI2, which was dependent on the activity of Janus kinases (Jak). Intriguingly, inhibition of Jak activity by small molecules or RNAi effectively disrupted the PGI2-mediated induction of brown fat gene expression. Our first mechanistic data indicate a requirement for the Jak/Stat pathway in early cell activation and commitment to differentiation. In vivo, the biphasic regulation of the Jak/Stat pathway could be recapitulated during beta-adrenergic stimulation in parallel with the expression of gp130 cytokines, highlighting a general functional requirement for Jak/Stat activation in adipose tissue remodeling.

Vegiopoulos et al., 2010. Cyclooxygenase-2 controls energy homeostasis in mice by de novo recruitment of brown adipocytes. Science 328(5982): 1158-61

Abstract No. P090

Effects of integrin activation via kindlin-2 on derivation of highly functional mesenchymal stem cell out of human iPS cells

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It has been revealed that functionally Kindlin-2 promotes tumor cell proliferation, adhesion, migration, and invasion. Recently, Kindlin 2 was found to induce EMT by activating Wnt signaling. Since Kindlin-2 function in the regulation of EMT, it is warranted to explore whether Kindlin 2 involves in the regulation of differentiation of human iPS cells to mesenchymal derivatives to obtain highly functional and highly competent immunomodulatory mesenchymal stem cells. Here we investigated kindlin-2 gain and loss of function in iPSC differentiation toward mesenchymal-like cells and possibly report the beneficial effects of kindlin-2 in generating super functional mesenchmal stem cells by mechanisms involved in kindlin-2 signaling to avoid inconvenient situations and shortfalls of BM-MSC usage.

Kindlin-2 overexpression and knock down have been done in spontaneously differentiated iPS-derived MSCs with lipofectamin LTX and functional confirmation was performed by qRT-PCR and Western blot. Then we investigated survival, proliferation and apoptosis assays in flag-kindlin-2 and kindlin-2 shRNA with respective controls.

Overall results represented that kindlin-2 over expression could significantly increase some of the kindlin-2 target genes such as Integrin B1 and Integrin B3 however there was no significant change in Talin. Moreover kindlin-2 overexpression ended in significantly higher proliferative stem cell population and also less apoptotic bodies which was accompanied by significantly increased apoptotic bodies in Kindlin-2 shRNA transduced cells. We aimed also to investigate iPSC-derived MSCs effects on suppression of immune response and hematopoiesis support.

In conclusion Kindlin-2 can be an effective integrin activating protein with which mesenchymal status increased and highly proliferative MSCs are generated.

Abstract No. P091

INDUCED DIFFERENTIATION OF ADIPOSE-DERIVED MESENCHYMAL STEM CELLS

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Stem cells are non-specialized body cells at a very early stage of development, which under normal conditions in a given tissue can differentiate into different types of functionally specialized mature cells. Mesenchymal stem cells (MSCs) are attractive candidates for clinical use in the reconstruction of damaged tissue, especially as it can be isolated from various sources and reproduced, and their use does not carry any ethical problems. Isolation methods of MSCs from adipose tissue and dental pulp are based on enzymatic degradation of the obtained materials, and isolation methods from umbilical cord blood are based on separation procedure of mononuclear cell. Terms for cultivation of mesenchymal stem cells are temperature of 37˚C and the partial pressure of CO2 5%. MSCs are cultured in the medium, often in α-MEM medium with 10% or 20% of fetal calf serum. Under these conditions of cultivation adherent cells form colonies in 7-14 days. MSCs are multipotent and able to differentiate in vitro conditions into Mesodermal differentiation, forming osteoblasts, chondrocytes and adipocytes. However, they can be differentiated into cells of ectodermal origin (such as neurons) and cells of endodermal origin (eg, β-cells of the pancreatic islets of Langerhans and hepatocytes). The Laboratory for stem cell of the Department of Biomedical Sciences at the State University of Novi Pazar conducts research of MSCs originating from human adipose tissue. In collaboration with surgeons of Health Center of Novi Pazar, and respecting the norms of the Ethics Committee of both institutions in the period from 01.07.2011. to 01.07.2012. were obtained 22 samples of human subcutaneous adipose tissue of patients aged 18 to 65 years. 15 samples successfully completed the process of isolation and cultivation, and 8 induces mesodermal differentiation.

Abstract No. P092
Clonal heterogeneity within the exocrine pancreas

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The pancreas consists of two functional units: the hormone producing endocrine system and the digestive enzyme producing exocrine pancreas. Much effort has been made to investigate if the endocrine system is organized hierarchically. However the progress made in the past decade concluded that the endocrine system is not organized in a hierarchical way and that all beta cells contribute equally to growth and maintenance. If this is also the case for the exocrine pancreas remains elusive. Here we use multicolor lineage tracing to study the clonal contribution of cells within the acinar cell population, the major compartment of the exocrine pancreas. While examining potential progenitor populations we find substantial differences in the proliferation dynamics of single clones among acinar cells. As a complementary in vitro approach to assess clonal heterogeneity, we examine the organoid-forming capacity of these cells. In this assay, we identified a unique subpopulation of acinar cells with the ability to give rise to organoids. Thus, although the acinar population, similarly to the beta cells among the endocrine cells, is often considered as a homogeneous population we find clonally heterogeneous contribution to growth and maintenance of pancreas homeostasis. Furthermore these data challenge the perception of the pancreas as a quiescent organ and indicate that the exocrine pancreas is maintained unipotent progenitors.

Abstract No. P093

Epigenetic determinants of adult muscle stem cell

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One key property that distinguishes pluri-potent embryonic stem cell (ES) from organ specific adult stem cell is the distinct chromatin configuration. While ES cell contains more loosed and open euchromatin, adult muscle stem cell termed satellite cell harbors more condensed heterochromatin indicating different epigenetic determinants controlling embryonic or adult muscle stem cell identity. Such cell belongs to a rare stem cell population in adult skeletal muscle responsible for muscle maintenance and muscle regeneration upon injury. Using shRNA mediated loss of function screen for satellite cell proliferation in vitro and immunofluroscence screen for different epigenetic modifications hence chromatin organization within satellite cell in vivo we identified two epigenetic modifying enzymes: histone arginine methyltransferase Prmt5 and histone lysine methyltransferase Suv4-20h1 respectively, important for satellite cell maintenance. Extensive in vivo studies using transgenic mice and molecular biological studies using chromatin immunoprecipitation (ChIP) revealed that Prmt5 is essential for satellite cell self-renewal hence muscle regeneration and Suv4-20h1 is indispensable for satellite cell quiescence. Thus our findings unveil novel epigenetic factors regulating muscle stem cell homeostasis and link condensed heterochromatin organization to adult muscle stem cell identity.

Abstract No. P094

Comparative Epigenetic Analysis of Stemness Marker Genes in Mesenchymal Stem Cells derived from Human Chorion and Bone Marrow

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Introduction: Mesenchymal stem cells (MSCs) are a promising cell resource for cell-based therapeutics, because they are not immunogenic and have self renewal ability. In the search for alternative source of MSCs, we found chorion MSCs as an appropriate source of MSCs and compared them with bone marrow(BM) MSCs. In this study histone modification of chorion and BM derived stem cells in promoter of stemness marker genes including OCT4, NANOG, SOX2 and also NESTIN as a neural differentiation marker was compared.
Methods: Chorion and BM derived MSCs were isolated and cultured. Passaged-3 cells were investigated in terms of some surface markers as well as differentiation potential into some mesodermal cell lineages. Then, the expression level in addition to incorporation of H3K9ac and H3K9me2 on the promoter of marker genes were evaluated quantitatively by ChIP Real Time-PCR technique.
Results: MSCs from either chorion or bone marrow tissue tended to be fibroblastic in morphology and majority of both cells expressed mesenchymal cell epitopes. Chorion derived MSCs showed significantly higher expression level of SOX2, NANOG and NESTIN in comparison to bone marrow derived MSCs; in addition, the epigenetic profile was in agreement with the differences observed in expression level of aforementioned genes in these sources of MSCs.
Conclusion: These results suggest that chorion derived MSCs have remarkable proliferation potential in comparison to BM derived MSCs. Also, it is expected that chorion drived MSCs have dramatic potential in differentiation to neural lineages. So, we can use these cells as a suitable source of MSCs for therapeutic purposes.

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HSCs and stem cells in hematopoietic malignancies: P095 – P116

P111 Role of Matrilin-4 in stress-induced HSC Activation and Homeostasis
Hannah Uckelmann

P112 Local and global chromatin changes in ex vivo cultured human cord blood-derived hematopoietic stem cells
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Abstract No. P095

Elucidating functional heterogeneity in haematopoietic progenitor cells: a combined experimental and modelling approach

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Cultures of haematopoietic stem and progenitor cells are often characterised by heterogeneous proliferation, background differentiation and cell death, and the maintenance of self-renewal ability within a small subpopulation of cells only. The mechanisms that maintain the fraction of self-renewing cells in a continuously expanding, intrinsically heterogeneous population are unknown, but are likely relevant to similar homeostatic processes in vivo.

We aim to develop a methodology to investigate the population-intrinsic heterogeneity in haematopoietic cell cultures. Therefore, we obtain experimental results on the heterogeneity of the well-established, multipotent murine progenitor cell line FDCP-Mix and interpret it in the context of a single cell-based mathematical modelling approach. In particular, the turnover of FDCP-Mix cells has been studied at both the population and single cell levels to define the functional heterogeneity. In parallel, by adapting an established single-cell based model of haematopoiesis to accommodate variable probabilities of commitment to two lineages as well as lineage-specific cell cycle times and death rates we provide a modelling framework on which to interpret the experimental results.

We find that measurements of heterogeneity at the population level are consistent with a wide variety of model scenarios, while the experimental observation of single cell fates by long-term cell tracking allows the exclusion of certain scenarios and progressive model refinement. The correlation of cell death events and of shortened or prolonged cell cycle times provides evidence that the observed heterogeneity is best explained by a combination of differential regulation of cellular turnover and apoptosis during differentiation.

The functional implications and regulatory aspects of the observed heterogeneity, e.g. via intrinsic feedback loops, need to be further investigated. In this respect, modelling approaches, as the one presented here, allow to make testable predictions and thus demonstrate the potential complementarity of iterative in vitro and in silico approaches to understanding stem cell systems.

Abstract No. P096

Next generation cell-based assays for high content imaging of apoptosis, autophagy and oxidative stress

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Cell death occurs through multiple pathways from multiple origins. While necessary for natural growth and development, dysregulation of apoptosis has been associated with a variety of diseases, including cancer and neurodegenerative disorders. Increased oxidative stress associated with these diseases has also been shown to lead to apoptosis and autophagy, which are indicators of cell death pathways that ultimately shed light on mechanism. Importantly, cell death can occur through a single pathway, or in concert involving multiple pathways. Staurosporine has been shown to induce apoptosis, while chloroquine is known to promote autophagy. Nefazodone results in both apoptosis and autophagy. Here, we utilized a novel series of cellular probes in high content imaging analysis to differentiate between apoptotic and autophagic cell death after induction by different agonists. To accomplish this, we simultaneously examined levels of reactive oxygen species (ROS), autophagosome formation and apoptosis to determine relationships between oxidative stress, autophagy and apoptotic cell death. We used the fluorogenic CellEvent™ Caspase 3/7 Green Detection Reagent as an indicator of apoptosis together with an antibody specific for LC3B to measure autophagosome formation. We also examined oxidative stress with CellROX™ Deep Red Reagent, a near infrared fluorescent ROS probe developed for multiplexed, live-cell studies of oxidative stress. In addition, loss of mitochondrial health was measured in apoptotic cells with the mitochondrial membrane potential dye TMRM. Using a multi-parametric approach in combination with high content imaging mechanisms of cell death by discriminating between cells which were apoptotic (active caspase-3/7), autophagic (LC3B-postive autophagosomes), or both. This approach provided detailed information at the cellular level enabling correlations between oxidative stress and different cell death mechanisms. CellEvent® Caspase 3/7 Reagent is fully amenable to high throughput, cell based analysis, and can be multiplexed effectively with PrestoBlue® Cell Viability Reagent, allowing flexibility across imaging platforms for research application.

Pankiv (2007) J Biol Chem 282 24131-24145
Kimura et al (2007) Autophagy 3 452-460

Abstract No. P097
Combination of Colour and Barcode: Multiplexing clonality

Kerstin Cornils [1,*], *Lars Thielecke* [2], *Svenja Hüser* [1], *Michael Forgber* [3], *Michael Thomaschewski* [1], *Nadja Kleist* [1], *Kais Hussein* [4], *Kristoffer Riecken* [1], *Tassilo Volz* [1], *Maura Dandri* [1], *Sebastian Gerdes* [2], *Ingmar Glauche* [2], *Andreas Dahl* [2], *Ingo Roeder* [2], and *Boris Fehse* [1]

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Background: Genetic marking of cells by integrating viral vectors is a powerful tool to investigate the fate of cells and their progeny. We recently introduced RGB marking, a technique that facilitates clonal tracking based on individual fluorescent colours and thus relies on stable protein expression. As complementary marking approach we now introduced short, high-complex DNA sequences named genetic barcodes in the vector backbone. Barcodes can easily be identified by PCR and quantified by (next-generation) sequencing.
Methods: Four different barcodes were designed, each specific for one defined fluorescent protein. All barcodes consisted of eight pairs of random nucleotides ("BC16") intersected by triplets of fixed nucleotides. Barcoded vectors were used for RGB marking of HEK293T cells for in vitro follow-up, and murine primary hepatocytes prior to transplantation in uPA mice. Finally, a lentiviral vector encoding the oncogene ΔTrkA and barcoded with GFP-BC16 was used to transduce primary murine haematopoietic stem cells to study clonal development of ΔTrkA-driven leukaemia.
Results: Plasmid libraries and high-titre viral supernatants with a complexity of ca. 5 x 105 barcodes were produced and applied for transduction of target cells. After RGB marking of 293T cells and hepatocytes individual barcodes were shown to correlate with defined colour hues underlining the power of the combined marking strategy. Also we were able to assess the clonal composition of ΔTrkA-driven leukaemia in vivo over time based on next-generation sequencing.
Conclusion: Our novel approach combines the virtues of two clonal marking strategies and thus provides the opportunity to verify the identity of marked cells on both the phenotypic and genomic levels. Moreover, we provide evidence that cellular barcoding facilitates the investigation of clonal leukaemia development. Finally, our novel barcodes ciphering for individual fluorescent colours will be useful for studying engraftment of different cell populations in competitive transplantation experiments.

Abstract No. P098
'Targeting quiescent leukemic stem cells through combined treatment strategies'

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Chronic myeloid leukemia was one of the first malignancies suggested to be driven by leukemic stem cells. The constitutively active BCR-ABL kinase is the driving force behind this leukemia and is thus an ideal target for drug development. This led to the development of the tyrosine kinase inhibitor Imatinib, which selectively and potently inhibits the BCR-ABL kinase, leading to a rapid hematologic and cytogenetic response in CML patients. Nonetheless, after years of treatment with Imatinib, residual leukemic cells remain, which can lead to a relapse of the disease on cessation of treatment. The reason for the resistance of CML stem cells is highly debated, one of the mechanisms suggested being dormancy of the leukemic stem cells.
Recently, our group has shown that the cytokine IFNα can very efficiently drive dormant HSCs into an active cell cycle. Here, we are investigating the effect of IFNα on LSCs and analyzing whether IFNα is capable of activating dormant LSCs in a similar way. For this we use a mouse model for CML which inducible and reversibly expresses BCR-ABL under the control of the 3'enhancer of the murine stem cell leukemia gene, thus targeting BCR-ABL expression mainly to the HSC population. To mimic more closely the scenario in a CML patient where both healthy and CML cell populations coexist at the same time, we have generated mixed BM chimeras by mixing LSCs from the transgenic mouse and wild-type HSCs from healthy mice. Our preliminary results suggest that IFNα is indeed able to activate LSCs in a similar way as wild-type HSCs. Currently, we are testing the hypothesis of a combined chemotherapy strategy to eliminate the LSCs as well as the bulk of the tumor by combining TKI treatment with the potential of IFNα to drive LSCs into proliferation, making them susceptible to TKI treatment.

Abstract No. P099

Stem cell survival in the face of genomic instability.

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Genomic instability is a common feature of many cancers. However, since the ongoing acquisition of DNA damage frequently leads to cellular attrition in normal cells, the cancer stem cell must have evolved mechanisms to evade apoptosis in order to propagate itself.
To explore these mechanisms in the context of genomic instability, we have carried out a functional forward genetic screen using the Fanconi Anemia (FA) DNA repair disease model. FA patients display an early onset of leukemia and are predisposed towards some solid tumors. Hematopoietic stem cells (HSCs) isolated from FA knockout mouse models are genetically unstable and demonstrate a severe engraftment defect following in vivo transplantation. We employed retroviral insertional mutagenesis to screen for HSC clones that survive in the face of genomic instability, correlating with a correction of their engraftment defect. Cells with such gain-of-function mutations become over-represented and are able to serially repopulate irradiated recipients.
The emergence of these dominant clones is an infrequent event (1 in 2,640,000 cells), suggesting that only very few genes can compensate for the engraftment defect. We are able to determine which cellular genes are mutated in these dominant clones and predict that they will regulate the cell fate response to DNA damage in stem cells. As the FA signaling pathway is non-functional in several tumor types of non-FA patients, the candidate genes might also be deregulated as a mechanism of tumor cell survival. Hence, these genes are potential synthetic lethal interaction partners of the FA pathway that one could pharmacologically target.

Abstract No. P100

High asymmetric cell division frequency of multipotent progenitors reflects the separation of lympho-myeloid and erythro-myeloid lineages

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Hematopoietic stem cells contain life-long potentials to self-renew and to create progenitors of all mature blood cells. Even though HSCs have been used in clinic practice for several decades now, the knowledge of the mechanisms controlling whether HSC self-renew or become committed to differentiate remains sparse. Apart of hematopoietic stem cell niches, processes of asymmetric cell division seem to control lineages specification and HSC homeostasis. Studying the proliferation kinetics of human CD133+CD34+ hematopoietic progenitors, we demonstrated that their progeny splits up into a CD133+CD34+ and CD133low/-CD34+ cell fraction. Comparing the developmental potential of arising CD133+CD34+ and CD133low/-CD34+ progenitors, we recently showed that multipotent progenitors (MPPs) reside within the sub-population of CD133+CD34+CD45RA- cells. Cells of this population create CD133+CD34+CD45RA+ lymphoid primed multipotent progenitors (LMPPs) and CD133low/-CD34+CD45RA- erythro-myeloid progenitors (EMPs). The observation that LMPPs exclusively inherit the potential to form neutrophils, and EMPs exclusively that to form eosinophils and basophils was unexpected. It provided new lineage relationships and challenges the existence of common myeloid progenitors (CMPs) (1).
Now, we have qualified the novel HC7 anti-CD133 antibody as helpful tool for live cell analyses. We show that the binding of this antibody does neither alter cell biological features of human HSPCs nor influence their cell fate specification processes in vitro and in vivo. In contrast to the AC133 epitopes, the HC7 epitopes were found to segregate asymmetrically in a proportion of dividing CD133+CD34+ cells. In our on-going studies we use the HC7 antibody to record and compare asymmetric cell division rates in different CD133+CD34+ HSPC subsets, i.e. in MPP-, LMPP- and GMP- enriched fractions. Up to now we found that with respect to their CD133 distribution approx. 70% of the cells of the MPP fraction divide asymmetrically.

(1) Görgens et al. (2013) Cell Reports 3(5), 1539-1552.

Abstract No. P101

Complementary roles of the histone methyltransferases Setd1a and Setd1b in mouse development and adult homeostasis

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Epigenetic modifications of chromatin are of biological importance as they effectively determine chromatin structure and stability that in turn establishes and maintains gene transcription. The two mammalian Setd1 proteins namely Setd1a and Setd1b are two out of six histone methyltransferases conducting proper methylation on histone H3 lysine 4 (H3K4). They arose through gene duplication and hence are closely related proteins, which share several conserved domains including the catalytic SET domain. In order to decipher the individual functions of these two proteins, we generated mice lacking either Setd1a or Setd1b protein via homologous recombination. Here we report that Setd1a and Setd1b have essential but distinct functions during embryonic development. While the loss of Setd1a causes embryonic death precisely at embryonic day 7.5 dpc, Setd1b deficient embryos are developmentally retarded from 7.5 dpc until 11.5 dpc, but do not survive beyond. To further test the requirement of Setd1 protein in adult mice, we employed the tamoxifen-inducible Rosa26CreERT2 mouse strain. Deletion of Setd1a in adult mice leads to rapid death between day 12 and 17 after the last tamoxifen gavage. In contrast, Setd1b deletion in adult mice has no effect on the survival shortly after tamoxifen gavage. To determine the role of Setd1 during adult steady-state hematopoiesis we analysed lineage depleted bone marrow cells. In mice, most if not all long-term repopulating activity resides within the cKit+/Lineage-/Sca1+ fraction. FACS analysis reveals a defect in the HSC/progenitor pool in the absence of Setd1a but not Setd1b. Erythropoiesis is impaired going along with a decreased hematocrit. We assume that the rapid death after conditional deletion of Setd1a is caused by bone marrow cytopenia. This assumption will be validated by bone marrow transplantations. Furthermore for conditional mutagenesis in the hematopoietic lineage we plan to cross the Setd1a conditional mouse line into Vav-Cre and Scl-CreERT2 deleters.

Abstract No. P102

Visualizing internalized cargo acidification in live cells with novel pH-sensing fluorescent dyes

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The basic cellular internalization processes of endocytosis and phagocytosis are important to many areas of cell biology including receptor internalization, pathogen response, and apoptotic cell clearance. However, the ability to study these processes has historically been limited by the lack of quality tools to directly monitor the internalization and acidification of cargo. Here we present an assortment of tools to enable research into cellular internalization pathways with particular relevance to immunology. Two fluorogenic, pH-sensitive probes, one green and one red, are presented. These dyes are non-fluorescent at the neutral pH typically found in the cytosol and extracellular environment, but become brightly fluorescent in acidic cellular compartments, permitting direct monitoring of internalization and acidification processes. We present experimental data in which these dyes were used to label microorganisms including yeast and gram-positive and -negative bacteria. Live cell imaging and flow cytometry were then used to monitor the phagocytosis and acidification of these labeled particles by cultured macrophages over time. We anticipate that these tools will enable additional research into the fields of receptor trafficking, drug delivery, host-pathogen response, and clearance of apoptotic cells.

Current Prot. Cytometry. 2002;9.19.1
J Biol Chem. 2009;284:35926-38

Abstract No. P103

MDS-Derived Stromal Cells Exhibit Altered Gene Expression and Support the Engraftment of Disease-Initiating Stem Cells in a Xenograft Model of MDS.

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Myelodysplastic syndromes (MDS) are clonal hematologic disorders characterized by ineffective hematopoiesis, dysplasia and increased risk of progression to acute myeloid leukemia. The development of targeted therapies for MDS has been lagging behind and remains a key clinical challenge that has been hampered, at least in part, by difficulties to establish in vivo model systems that recapitulate disease heterogeneity and complexity.

We show that co-transplantation of CD34+ cells from low risk MDS together with patient-derived mesenchymal stromal cells (MSC) results in robust long-term engraftment in immuno-compromised mice (NSG and NSGS), providing the first in vivo model of low risk MDS. The engrafted cells exhibit clear signs of dysplasia, one of the main clinical features of MDS. The disease initiating cells display a lin-CD34+CD38- phenotype and harbor long-term self-renewal activity in vivo. Mutational tracking show that human grafts maintain the initial patient ratio between healthy and MDS cells. RNA-sequencing comparing MDS-MSCs with healthy MSCs revealed altered expression of key factors involved in cellular adhesion, extra-cellular matrix (ECM) remodeling and cell-cell signalling in diseased MSCs, suggesting a complex interplay between MDS stem cells and their MSC-microenvironment.

In this study we have identified patient-derived MSCs as a critical functional component of lower risk MDS that allow the propagation of the disease in a xenograft recipient. The striking alterations observed in diseased MSCs include cytokine-cytokine receptor interaction, cellular adhesion, ECM remodeling and hypoxia which further suggests that diseased MDS cells alter the function of the normal HSC niches in order to form a functional disease unit. Further exploration of the interaction of MDS stem cells with MSCs at the cellular and molecular level will provide a platform for unraveling the molecular basis of clonal dominance in MDS as well as allow the design of targeted strategies aimed to disrupt the MDS stem cell-MSC niche interactions

Abstract No. P104

The role of the bone marrow niche in IFNα stimulation of hematopoietic stem cells

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Hematopoietic stem cells (HSCs) are maintained in a quiescent state in the bone marrow in a cell environment termed the 'niche'. The bone-marrow hematopoietic-stem-cell niche is a cellular and molecular microenvironment that regulates stem cell function, influence stem cell fate and is also responsible for the engagement of specific programs in response to stress. Severe loss of hematopoietic cells is thought to stimulate HSCs to undergo self renewal in order to replenish hematopoietic cell populations and maintain homeostasis. Interferons (IFNs) are widely expressed cytokines that play key roles in mediating antiviral and immune responses. In contrast to the anti-proliferative effect of interferon α (IFNα) on HSCs in vitro, in vivo, IFNα induces cell cycle entry of HSCs. Given the contrasting response of in vitro and in vivo HSCs to IFNα, it is likely that niche components and maintenance signals from the niche are required. To investigate the role of the niche in the stimulation of HSCs, the current study involves modeling of the stem cell niche in vitro to test stimulation of HSCs under various conditions. The effect of IFNα treatment on niche cell relocation in vivo is characterised by the identification of niche cell components in bone sections using cell specific antibodies. The contrasting effect of IFNα on HSCs in vitro and in vivo is a novel model with which to comprehensively study the role of the stem cell niche itself in stress induced activation of HSCs, and also the response of the HSC niche to stress.

Abstract No. P105

High Resolution Proteomics and Transcriptomics Identifies Novel Markers of Human Acute Myeloid Leukemia Stem Cells

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Acute Myeloid Leukemia (AML) is hierarchically organized with leukemic stem cells (LSC) at the apex. LSC are considered the source of relapse and thus further strategies to eradicate LSC are pivotal to improve patient outcomes. Currently it is neither possible to prospectively isolate pure functional LSC nor distinguish them reliably from normal hematopoietic stem cells (HSC). To search for novel LSC-specific markers, we applied state-of-the-art proteomics and gene expression profiling by next-generation sequencing (RNA-Seq) to LSC-containing and LSC-free cell populations from primary AML patients. To define functional LSC, we FACS-sorted primary cells according to surface expression of CD34 and CD38 and transplanted the resulting sub-fractions into NSG recipients. Sixteen AMLs showed leukemic xeno-engraftment in at least one of the sub-fractions, discriminating LSC-containing and LSC-free sub-populations within the individual patient. Healthy age matched control HSC were isolated from humans without hematological conditions. We performed quantitative proteomic analysis by employing tandem-mass-tag labeling and high-resolution mass spectrometry. Approximately 7,000 proteins were quantified from 18 sub-fractions of six individual AML patients of two different subtypes. Changes in protein expression patterns between different AML subtypes were more pronounced than between individual patients within the same subtype, highlighting the importance of subtype specific analyses. This result was confirmed by the RNA-Seq analysis, which was performed on corresponding cell fractions. Gene Set Enrichment Analysis of the RNA-Seq data sets showed enrichment of known LSC- and other stem cell gene sets in the LSC-containing fractions. Comparison of the gene expression pattern of LSC-containing fractions with healthy HSC revealed distinct expression of previously proposed LSC markers including CD47, TIM-3 and CD99. Ongoing bioinformatic analyses will integrate both data sets to generate a comprehensive expression signature of normal and leukemic stem cells in order to identify surface markers and targetable pathways in LSC of different AML subtypes.

Abstract No. P106

The organotypic epigenetic signature of human stromal progenitors corresponds to their bone and marrow niche-forming capacity in vivo

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Mesenchymal stem/progenitor cells (MSPCs) from various tissues are currently tested in clinical trials despite limited understanding of their in vivo behavior. Here, we determined bone and marrow niche formation by therapeutic-grade MSPCs from bone marrow (BM), white adipose tissue (WAT), umbilical cord (UC) and skin fibroblasts in an ectopic human bone and marrow niche (HuNiche) formation mouse model (Blood 119:4971) to select appropriate cells for bone regeneration. DNA methylation (450K-CpG-methylation array) and gene expression profiling was compared to in vivo osteogenesis and hematopoietic niche establishment.

CpG-methylation revealed tissue-specific epigenetic signatures corresponding to the in vivo differentiation potential. Principal component analysis separated methylation profiles according to tissue of origin. Comparing BM-derived with non-BM-MSPCs we found 4,721 (0.97%) significant differentially methylated CpG-sites (52% hypermethylated, 48% hypomethylated) including >200 CpGs corresponding to genes involved in cartilage and bone formation. DNA-methylation within CpG-sites of RUNX3 and BGLAP/osteocalcin inversely correlated with transcription as confirmed by qRT-PCR and significant differences in protein expression.

Ectopic subcutaneous MSPC transplantation into NSG mice testing for bone and marrow niche formation revealed that BM-MSPCs developed mature human bone (17/17 donors) through endochondral ossification with subsequent murine marrow attraction (10/17 donors) shown by osteosensitive near-infrared imaging, computed tomography and pentachromatic histopathology. Newly formed niches attracted complete mouse hematopoiesis including lineage-negative, Sca-1+/c-kit+ (LSK) HSPCs. Non-BM-derived MSPCs lacked bone and marrow niche-forming potential and did not attract hematopoiesis (0/9 donors) in this model. Induction of human hematopoietic chimerism by transplantation of CD34+ HSPCs before HuNiche formation resulted in immigration of re-transplantable complete human hematopoiesis (LSK; erythrocytes, megacaryocytes, monocytes, granulocytes, CD19 B-lymphocytes, CD4+CD8 T-lymphocytes including FoxP3 Tregs).

These data indicate epigenetically imprinted endochondral bone and marrow niche-forming capability and favor BM-MSPCs for skeletal regeneration. The HuNiche model also allows for studying normal (this study) and malignant (Blood 122:357) hematopoiesis.

Blood 119:4971
Blood 122:357

Abstract No. P107

The role of Setd1b in myeloid neoplasia

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Perturbations of epigenetic regulation are profoundly linked with tumorigenesis. The enzymes that methylate histone tails are central to epigenetic regulation and frequently mutated in many cancers. All expressed euchromatic genes are trimethylated at histone 3 lysine 4 (H3K4me3) on promoter nucleosomes. The responsible enzymatic machinery belongs to the trithorax group (TrxG) of proteins. Six Set1/Trithorax-related H3K4 methyltransferases have been identified in the mouse genome: Mll1, Mll2, Mll3, Mll4, Setd1a and Setd1b (Glaser et al, 2006).
Here, we want to address the function of Setd1b by making use of a conditional knockout mouse that has been generated according to the multi-purpose allele design (Testa et al., 2004). We found that the constitutive loss of Setd1b, which is ubiquitously expressed during murine development, results in embryonic lethality before embryonic day 11.5. In adult mice, conditional Setd1b deletion produces myeloid neoplasia with features of human chronic myelomonocytic leukemia (CMML). Mice die on average 14 weeks after tamoxifen-mediated induction of the knockout due to failure of hematopoiesis. This is reflected by substantial anemia, thrombocytopenia, lymphocytopenia and monocytosis. Consistent with extramedullary hematopoiesis myeloid cells and hematopoietic stem cells (HSCs) are highly enriched in the spleen and in peripheral blood. In the bone marrow of mice deficient for Setd1b the HSC pool is severely increased and differentiation is shifted towards the myeloid lineage.
In summary, the Setd1b conditional knockout mouse serves as a new model for studying the epigenetic mechanisms underlying oncogenic transformations in myeloid neoplasia.

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Abstract No. P108

Laminins in the Hematopoietic Stem Cell Niche Impair Erythroid Differentiation

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Background and Objectives: The fate of hematopoietic stem cells in the bone marrow is controlled by interactions with a specialized microenvironment, the stem cell niches. Although different non-hematopoietic cell types have been defined as essential niche cells, the composition of different niches has not been fully identified. Especially the role of the extracellular matrix in the niches is still poorly defined. In this study we have characterized the expression and function of laminin isoforms in the endosteal stem cell niche.

Methods: The expression pattern of the different laminin chains in primary osteoblasts and mesenchymal stem cells (MSC) was determined by RT-PCR analysis, Western blotting, immunofluorescence staining and immunoprecipitation. For functional adhesion, proliferation and differentiation assays, CD34+ hematopoietic stem and progenitor cells (HSPC) isolated from umbilical cord blood were analyzed in combination with different human recombinant laminin isoforms.

Results: Osteoblasts and MSC mainly synthesize the laminin alpha4 and alpha5 chains. Both cell types secrete the isoforms LM-411 and LM-511 as shown by immunoprecipitation, but they do not deposit a basement membrane. LM-511 had the strongest anti-proliferative effect when added to CD34+ HSPC. This effect was mainly mediated by the integrin alpha6beta1. Stem cell differentiation into myeloid and erythroid colonies was also affected by laminins. The strongest inhibition of erythroid differentiation was observed for LM-511. The expression of E-cadherin, an erythroid differentiation marker, was drastically reduced by the addition of laminins.

Conclusions: Endosteal niche cells secrete LM-411 and LM-511, although both isoforms do not seem to be deposited in a structured extracellular matrix network. The isoforms can impair stem cell proliferation which may help to keep the stem cells in a quiescent state. Developing erythrocytes are commonly not found close to the endosteum which might be explained by the secretion of laminins which strongly impair erythroid differentiation.

Abstract No. P109

Lipopolysaccharide leads to activation of HSCs through IFN and TNFα signaling in vivo

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In the blood system dormant haematopoietic stem cells (HSCs) with life long self-renewal capacity are at the top of the hierarchy. They give rise to actively cycling HSCs that control the blood cell production during homeostasis. Under stress conditions large amounts of mature blood and progenitor cells are lost. This leads to a feedback signal and thus to activation of the dormant HSCs, which start to proliferate and produce new progenitors and mature blood cells. The molecular and cellular mechanisms of these feedback loops remain largely unknown.

We recently demonstrated, that the cytokine IFNα, which is produced upon viral infection, is able to activate the entire HSC pool including dormant HSCs to proliferate and up-regulate Stem Cell Antigen 1 (Sca-1). To explain this surprising effect of IFNα on HSCs, we are exploring whether IFNα is part of a feedback loop leading to the activation of HSCs in response to stress. Interestingly, injection of mice with lipopolysaccharide (LPS) leads to increased IFNα production, followed by a TLR4- and Sca-1-dependent activation of quiescent HSCs. However, though IFNα has a direct effect on HSCs, LPS has an indirect effect on HSCs via CD11b+ myeloid cells in the bone marrow. Unraveling the mechanism underlying the indirect activation of HSCs in response to LPS revealed not only a role for IFN signaling but also the importance of TNFα signaling. In fact, in vivo TNFα treatment alone leads to activation of HSCs. Furthermore, in vivo inhibition of TNFα signaling in mice lacking both IFN receptors showed that both IFN and TNFα signaling are mediating the LPS induced activation of HSCs.
Together these data increase our knowledge on the mechanism of stress-induced activation of HSCs by both IFNα and TNFα signaling. Furthermore, the data unravel a role for the myeloid compartment in stress induced HSC activation.

Abstract No. P110

Cytokine-regulated Gadd45γ induces differentiation and lineage selection in hematopoietic stem cells

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The balance of self-renewal and differentiation in long-term repopulating hematopoietic stem cells (LT-HSC) must be strictly controlled to maintain blood homeostasis and to prevent leukemogenesis. Cytokines can induce differentiation in LT-HSCs, but the molecular mechanism orchestrating this delicate balance requires further elucidation.
We identified the tumor suppressor Gadd45γ as an instructor of LT-HSC differentiation under the control of differentiation-promoting cytokine receptor signaling via STAT5A/B. Gadd45γ immediately induces and accelerates differentiation in LT-HSCs, and overrides the self-renewal program in vitro and in vivo by specifically activating MAP3K4-mediated p38 MAPK. Conversely, the absence of Gadd45γ enhances the self-renewal potential of LT-HSCs. Videomicroscopy-based tracking of single LT-HSCs revealed that, once Gadd45γ is expressed, the development into lineage-committed progeny occurred within 32h, and uncovered a selective lineage choice with a severe reduction in megakaryocytic-erythroid cells. We discovered Gadd45γ as a central molecular linker of extrinsic cytokine differentiation and lineage choice control in hematopoiesis.

Abstract No. P111

Role of Matrilin-4 in stress-induced HSC Activation and Homeostasis

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The life-long maintenance of the blood system is accomplished by a pool of self-renewing and pluripotent hematopoietic stem cells (HSCs). Adult HSCs are found in a dormant state for most of their lifetime, entering cell cycle only to maintain homeostatic blood supply. Upon loss of differentiated cells due to injury or toxic insult, HSCs are stimulated to proliferate. The molecular mechanisms underlying the processes of activation of HSCs are still largely unknown.
Our group has previously shown that interferon α (IFNα), a cytokine produced during viral infection, can efficiently induce HSCs proliferation in mice in vivo. This response is dependent on signaling via the IFNα receptor (IFNAR) and STAT1 leading to downstream induction of IFNα target gene expression (Essers et al., 2009). To gain insight into the mechanism of activation, we compared the transcriptional response of wildtype HSCs treated with PBS or IFNα by microarray expression analysis. Interestingly, we discovered several cell cycle inhibitors, such as p57 to be down regulated upon IFNα treatment, possibly explaining the cell cycle entry of these cells. Even more striking is the downregulation of the extracellular matrix protein Matrilin-4. Matrilin-4 is a member of the von Willebrand factor A-containing family of extracellular adapter proteins, which form filamentous structures outside of cells. We found Matrilin-4 to be highly expressed in long-term HSCs compared to short-term HSCs or committed progenitors during homeostasis and it is almost completely depleted upon in vivo treatment with IFNα or other inflammatory cytokines. These data suggest that Matrilin-4 plays an essential role in niche remodeling following stress. Here we present our data on the investigation of the function of Matrilin-4 in HSC activation and homeostasis using Matrilin KO mice and in vivo retroviral overexpression.

Abstract No. P112

Local and global chromatin changes in ex vivo cultured human cord blood-derived hematopoietic stem cells

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Cord blood hematopoietic stem cells (CB-HSCs) are an outstanding source for therapeutic transplantation approaches. However, the amount of cells per donor is limited. In order to make sufficient numbers of CB-HSCs available for adult patients, expansion is required. Different protocols were described for HSC expansion but little is known about the molecular mechanisms leading to the culture-induced loss of engrafting potential. Epigenetic mechanisms play essential roles in controlling stem cell potential and fate decisions. Therefore, we started to characterize global and local epigenotypes and expression of critical stem cell genes during CB-HSC expansion. CB-CD34+ cells were MACS-isolated and cultured in StemSpan serum-free medium together with heparin, SCF, TPO, FGF, with/without IGFBP2 and ANGPTL5 (Zhang et al., Blood, 2008). Subsequently the NOD/SCID mouse model was used to study the engraftment potential of expanded cells. To explore global chromatin changes in freshly isolated and expanded cells, levels of the bivalent histone marks H3K4me3 and H3K27me3 were determined by chromatin flow cytometry and Western blot analyses. For analysis of genome wide chromatin changes after ex vivo expansion, we performed transcriptome profiling by microarray and chromatin immunoprecipitation combined with deep sequencing (ChIP-seq). Additionally, local chromatin transitions were monitored by ChIP analysis on promoter regions of developmental and self-renewal factors. On global levels, freshly isolated CD34+ and CD34- cells differ in H3K4me3 (active) and H3K27me3 (repressive) levels. After 7 days of expansion CD34+ and CD34- cells adopt similar levels of active and repressive marks. However, depending on the cytokine cocktail, the expanded cells show different H3K4me3 and H3K27me3 levels. Expanding the cells without IGFBP2 and ANGPTL5 leads to a higher global H3K27me3 level. Also the ChIP-seq data reveal cytokine cocktail-dependent histone modification profiles upon expansion. Taken together our data provide a better molecular understanding of chromatin changes upon expansion of CB-HSC.

Abstract No. P113

Connective Tissue Growth Factor (Ctgf/Ccn2) Is a Novel Extrinsic Niche-Derived Regulator Of Hematopoietic Stem Cells

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Hematopoietic stem cells (HSC) are regulated by an interplay of intrinsic and extrinsic signals. We studied the dynamic interaction of HSC and niche stromal cells, using co-cultures of HSC (lineage-negative Sca-1+ c-Kit+: LSK) cells and HSC-maintaining UG26-1B6 stromal cells. Microarray analyses from cells prior to co-culture and cells sorted separately from the cultures revealed that most changes in gene expression take place in the first 24 hours of co-culture. Analyses using STEM clustering, LIMMA, and ToppGene databases identified connective tissue growth factor (Ctgf) to be strongly upregulated in both stromal and LSK cells. To study Ctgf as a stromal mediator, LSK cells were co-cultured with siCTGF knockdown stromal cells. We showed that short-term HSC activity was unchanged. But, siCtgf-stromal cells were unable to sustain long-term repopulating ability. A Boolean model simulating possible signaling mechanisms was extracted from the micro array data. We validated this model by assessing protein levels and phosphorylation using immunocytofluorescence in LSK cells from co-cultures with siCtgf stromal cells. In the absence of extrinsic Ctgf, Pten was increased in LSK cells but Akt phosphorylation (both p308, and p473) and Erk were unchanged. In contrast, canonical Wnt signaling (LRP6, Gsk3b, b-catenin) was turned off, whereas Tgf signaling (Smad2/3) signaling were was turned on. This resulted in a downregulation of G1 transition (downregulation of Cyclin D1, upregulation of p27Kip1 and modulations in the phosphoryalation of both Rb and p53). In addition, we found that extrinsic Ctgf auto-induces Ctgf in HSC, demonstrating intrinsic-extrinsic feedback in both stromal cells and LSK cells. In summary, co-culture of LSK cells with stromal cells affects Tgf and canonical Wnt signaling pathways in both HSC and the niche. Our studies show the dynamics of reciprocal signaling between HSC and niche stromal cells and give insights how the niche regulates early regenerative responses in hematopoiesis.

Abstract No. P114
Molecular characterization of dormancy in HSCs

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Adult stem cells are required to maintain highly regenerative tissues such as the skin, the intestinal epithelium and the hematopoietic system. Mouse hematopoietic stem cells (HSCs) are the most well characterized somatic stem cells to date. Recently a population of dormant HSCs has been identified within the LSK CD150+CD48-CD135-CD34- population by label retaining assays. Computational modelling suggested a proliferation frequency during homeostasis of about 5 divisions per lifetime. While these dormant HSCs form a silent reservoir of the most potent HSCs during homeostasis, they are efficiently activated to self-renew in response to bone marrow injury. This allows the fast production of progenitors and mature cells ensuring repair and survival. After re-establishment of homeostasis, activated HSCs return to dormancy, suggesting that HSCs are not stochastically entering the cell cycle but reversibly switch from dormancy to self-renewal under conditions of hematopoietic stress.
To identify the molecular mechanisms underlying dormancy we have compared the mRNA expression profile of dormant and active HSCs within the LSKCD150+CD48-CD34- population using the H2B-GFP label retaining assay. The analysis revealed differential expression of approximately two hundred genes. In particular, genes promoting proliferation and transcripts encoding a variety of histone proteins were up-regulated in active HSCs. Strikingly, a set of genes involved in megakaryopoiesis and platelet function were up-regulated in these cells. In contrast, dormant HSCs are characterized by high expression of transcripts involved in cell cycle inhibition and inhibition of apoptosis as well as transcripts of proteins involved in cell adhesion.

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(3) Trumpp et al., (2010). Awakening dormant haematopoietic stem cells. Nat Rev Immunol. 2010 Mar;10(3):201-9.

Abstract No. P115

HSC Exit From Dormancy Provokes De Novo DNA Damage, Leading To Bone Marrow Failure If Unresolved By The Fanconi Anemia Pathway

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Long-term quiescence has been proposed to preserve the genomic stability of hematopoietic stem cells (HSCs) during aging. Current models of HSC aging are limited in their ability to observe DNA damage in vivo and the consequences of this damage upon hematopoiesis. We sought to identify whether physiologic mediators of HSC activation could be used as agonists to provoke DNA damage and HSC attrition in vivo.

Mice were treated with a range of agonists that promote the in vivo exit of HSC from a dormant state into active cycling. HSC demonstrated a rapid 3-5-fold induction of DNA damage after treatment with all agonists ($p<0.01$). Stress-induced exit from quiescence correlated with increased mitochondrial metabolism in HSC, as evaluated by elevated mitochondrial membrane potential and superoxide levels. Additionally, we could directly observe a 1.4-fold increase in reactive oxygen species-induced 8-Oxo-dG lesions in activated HSC ($p<0.05$). At 48 h post-treatment, γ-H2AX levels began to decrease and this repair was concomitant with an induction of the Fanconi anemia (FA) DNA repair pathway (4-fold induction, $p<0.01$).

Treatment of FA knockout mice (Fanca-/-) with polyI:polyC led to a HSC proliferative response comparable to wild type (WT) mice but resulted in a 2-fold higher level of activation-induced DNA damage ($p<0.05$), directly implicating the FA repair pathway in resolving activation-induced DNA damage. Four rounds of serial in vivo activation led to a permanent 4-fold depletion of functional HSC ($p<0.01$). Subsequent rounds of HSC activation resulted in the onset of a severe aplastic anemia (SAA) in 33% of treated Fanca-/- mice but not in WT controls.

Taken together, these data demonstrate that exit from dormancy in vivo leads to de novo DNA damage in HSC and that this may be a major driver of the SAA that is observed in patients who have an inactive FA repair pathway.

Abstract No. P116

Hyperinflammation leads to exhaustion of the hematopoietic stem cell pool in Nox2 deficient mice

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NADPH oxidase 2 (Nox2) deficiency is associated with an increased susceptibility to bacterial and fungal infections, but also with sustained inflammation. Little is known about the role of Nox2 in hematopoietic stem and progenitor cells. In our studies, we demonstrate that Nox2 k.o. mice harbor a defect in their hematopoietic stem cell (HSC) compartment that is associated with the persistent presence of inflammatory cytokines.

Competitive repopulating unit assays revealed a reduced frequency of long-term repopulating cells in Nox2 k.o. mice. Direct competition experiments with wild type and Nox2 deficient cells transplanted into the same recipient mouse, showed that Nox2 k.o. cells were outcompeted during long-term reconstitution by their wild type counterparts. Consistently, the bone marrow from Nox2 k.o. mice displayed faster exhaustion in in vitro assays. We found that HSCs from Nox2 k.o. mice enter the cell cycle more frequently than wild type cells. This leads to an expansion of the Nox2 y/- hematopoietic progenitor cell (HPC) pool.

To decipher the physiological background of these findings, we performed cytokine arrays with bone lavage and found increased levels of a variety of proinflammatory cytokines in the samples of Nox2 y/- mice. One of these cytokines, interleukin-1 beta (IL-1b), induced the expansion of the HPC pool in vitro and in vivo during short-term application. Ongoing experiments focus on the effects of chronic IL-1b stimulation on HSC/HPC self-renewal.

In summary we propose a model where persistently increased cytokine levels in Nox2 deficient mice lead to proliferative stress of HSCs, expansion of HPCs and the exhaustion of repopulating HSCs, which accounts for the functional deficit of Nox2 k.o. bone marrow.

Cancer stem cells in solid tumors: P117 – P128

Abstract No. P117

Role of REST in the control of self-renewal and Tumorigenic Competence of Human Glioblastoma StemCells

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REST (Repressor Element 1 Silencing Transcription factor) is a master repressor of neuronal programs in non-neuronal lineages shown to play a central regulatory role of developmental programs and stem cell physiology. Aberrant REST function has been associated with a number of pathological conditions. In cancer biology, REST has been shown to play a tumor suppressor activity in epithelial cancers but an oncogenic role in brain childhood malignancies such as neuroblastoma and medulloblastoma. Here we focussed our attention on REST expression in human glioblastoma multiforme (GBM) specimens and its role in human GBM cells carrying self-renewal and tumorigenic competence. Our results indicate that REST is expressed in GBM specimens where its presence is particularly enriched in tumor cells in the perivascular compartment. REST is highly expressed in self-renewing tumorigenic-competent GBM stem cells and its knock down strongly reduces their self-renewal in vitro and tumor-initiating capacity in vivo and affects levels of miR-124 and its downstream targets. We also show that REST controls a wide set of miRNAs in GBM stem cells. These results indicate that REST contributes to GBM maintenance by affecting the self-renewing and tumorigenic competence of stem cell compartment. We can predict that a better understanding of these circuitries in these cells might lead to new exploitable therapeutic targets.

Conti L, Crisafulli L, Caldera V, Tortoreto M, Brilli E, et al. (2012) REST Controls Self-Renewal and Tumorigenic Competence of Human Glioblastoma Cells. PLoS ONE 7(6): e38486.

Abstract No. P118

Glioma-initiating cell induced Interleukin-6 production is mediated by Toll-like receptor 4 in microglia

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Malignant gliomas are the most frequent primary tumors of the brain with poor clinical prognosis. Infiltrating peripheral macrophages and resident microglia that constitute the dominant tumor-infiltrating cells in glioblastoma are induced by the glioma cells to become immunosuppressive and tumor supportive. Glioma-initiating cells (GIC) could potentially promote this pro-tumorigenic phenotype. Exploring the interaction between GIC and glioma associated microglia/macrophages (GAM) may offer us an opportunity to further understand the cellular and molecular features of the GIC niche. We here investigate the potential of GIC versus bulk cells to induce a pro-tumorigenic microglial cytokine profile.

In the present study we stimulated primary cultured microglia with glioma conditioned medium (GCM) from GICs enriched or depleted GL261 cells and cytokine levels were determined by FlowCytomix. An almost 4-fold upregulation in microglial IL-6 secretion was observed using GCM from GICs while the secretion was unchanged with GCM from GICs depleted GL261 cells. Since Toll-like receptors are pattern recognition receptors that are responsible for pro-inflammatory cytokines release, we screened through all the TLRs and identified TLR4 as the main TLR controlling microglial IL-6 secretion. IL-6Rα and gp130 are highly expressed in GICs but not in microglial cells. The implantation of GL261-EGFP cells into IL-6 -/- mice resulted in significantly smaller tumors as compared to wild-type control mice. IL-6 and IL-6Rα are also expressed in human gliomas (which contain up to 30% microglia/macrophages) and inversely correlates with patient survival. Our results show that GICs, but not the bulk glioma cells initiate microglial IL-6 secretion. IL-6 in turn promotes glioma cell growth and invasion.

Abstract No. P119

Novel small-molecule antagonist of β-catenin/TCF4 interaction: interference with the self-renewal of cancer stem cells

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The Wnt/β-catenin signaling pathway is an evolutionary conserved signaling cascade that is essential for embryonic development and tissue homeostasis. Wnt/β-catenin signaling plays pivotal roles in the recognition of extracellular signals and subsequent transfer of information to the nucleus, where transcription through the interaction between β-catenin and the TCF/LEF transcription factors is regulated. Deregulation of Wnt/β-catenin signaling can initiate and promote various human cancers, among them colon cancers. Therefore, Wnt/β-catenin signaling presents an attractive target for cancer therapy; nevertheless no rationally developed compound that targets Wnt signaling is yet in clinical use. To develop small-molecule inhibitors of Wnt/β-catenin signaling, a high-throughput screening that interferes with the critical interaction between β-catenin and TCF4 was performed, using recombinant purified proteins and advanced AlphaScreen and ELISA technologies.
Compound LF3, a 4-thioureido-benzenesulfonamide derivative, was identified from high-throughput screening, which showed potent inhibition of the TCF4/β-catenin interaction. LF3 inhibited Wnt/β-catenin signaling in cells that carried exogenous reporters and in colon cancer cells that exhibited endogenously high Wnt activity. Limited medicinal chemistry identified an essential core structure of LF3 and residues that could not be exchanged. LF3 attenuated Wnt-related characteristics of cancer cells, like high cell motility and hyper-proliferation by inducing cell cycle arrest in the G1 phase. LF3 does not cause apoptosis or cell death, nor does it interfere with cadherin-mediated cell-cell adhesion. Remarkably, the self-renewal capacity of cancer stem cells was blocked by LF3 in concentration-dependent fashion, as suggested by reduced sphere formation of colon and salivary gland cancer stem cells in serum-free and non-attached conditions. In vivo, LF3 reduces tumor growth and induces differentiation of colon cancer stem cells in mouse xenograft. Taken together, the data presented in this study indicate that LF3 is a specific inhibitor of canonical Wnt signaling, and has potential to be further developed for preclinical and clinical studies.

Wnt signaling in stem and cancer stem cells. Curr Opin Cell Biol. 2013 Apr;25(2):254-64.
WNT signalling pathways as therapeutic targets in cancer. Nat Rev Cancer. 2013 Jan;13(1):11-26.

Abstract No. P120

MACC1 in colon cancer stem cells: Differential expression in side populations and a putative interaction with Oct4

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The gene MACC1(Metastasis-Associated in Colon Cancer 1) has been affirmed as a biomarker for metastasis in colorectal cancer (CRC). However, only a subset of cells within a tumor is endowed with the potential to propagate the latter and concomitantly drives metastasis. Following this cancer stem cell hypothesis, MACC1's role at the level of the stem cell is therefore of crucial importance and investigated herein.

We seek to analyze the expression of MACC1 directly in the stem cell population of CRC patient material, cell lines as well as mouse models available in our lab. At the cell line level, we could already show that the sorting of SW620 cells via CD44 enables differential enrichment of the intestinal stem cell marker Lgr5 together with MACC1. In parallel, we aim to elucidate MACC1's role in stem cell signaling. Of note, we found that MACC1 levels modulate the expression of Oct4 in CRC cell lines. Overexpression of MACC1 in CaCo2 and SW480 cells was related to an increase in Oct4 mRNA and protein expression. Consistently, knockdown of MACC1 in CaCo2 and SW620 cells resulted in decreased levels of Oct4.

In conclusion, we provide here the first molecular link between MACC1 and stem cells. Additional studies will help clarify the nature of the MACC1 and Oct4 interaction and its functional relevance. The finding that the expression of MACC1 is indeed focused in cancer stem cells might further increase its prognostic value and accentuate the importance of MACC1 in carcinogenesis.

Abstract No. P121

SOX2 expressing cancer stem cells mediate therapy resistance in ovarian carcinoma

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The SOX2 gene has been mainly studied in self-renewal of embryonic cells and somatic cell reprogramming to pluripotency. Recently, SOX2 expression was reported in several tumor types including ovarian carcinoma, where it has been associated with poor prognosis. Induction of SOX2 was shown to impose cancer stem cell (CSC) properties on human serous ovarian carcinoma cells (SOC) enhancing their tumor initiating capacity in murine xenotransplantation models. Here, we use a tdTomato lentiviral reporter system recognizing a human SOX2 enhancer element to isolate putative ovarian CSCs. Indeed, spheres cultures enriching for CSCs showed much higher percentages of SOX2-positive cells than 2D-cultures. SOX2-positive but not -negative or -low cells isolated by FACS possessed serial replating potential up to quaternary spheres. On the molecular level, qRT-PCR analysis revealed that stem cell associated genes (NANOG, OCT4 variant and LIN28) were highly enriched in SOX2-positive cells. Importantly, treatment with platin-based chemotherapies or paclitaxel strongly enhanced the percentages of SOX2-positive cells, indicating a selective potential to survive conventional chemotherapy, which is in line with their proposed role as ovarian CSCs.
To further explore the role of SOX2 in ovarian CSCs, we next established a model for xenotransplantation of human SOC cells into zebrafish larvae. Zebrafish xenografts are amenable for the assessment of tumor cell invasiveness and metastasis as well as neoangiogenesis, and can be used for small molecule screens. Indeed, zebrafish larvae injected with down to single SOX2 overexpressing cells showed tumors at much higher frequency and of larger sizes than embryos injected with control cells, which mimics the data collected in murine xenografts. Together, our results indicate that SOX2-positive CSCs mediate chemotherapy resistance in SOC and suggest zebrafish xenograft assays as a novel model for studies on overcoming therapy resistance.

Abstract No. P122

Mammary Gland Cancer Stem Cells in Mice Require Shp2 Signaling for Self-renewal and Suppression of Senescence

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Human breast cancer is a complex disease, and currently only limited options exist for therapies. The protein tyrosine phosphatase Shp2 (encoded by the PTPN11 gene) is a critical regulator of receptor tyrosine kinase and cytokine receptor signaling, and is upregulated in human breast cancers. Here we employed the MMTV-polyomavirus middle T (MMTV-PyMT) mouse tumor model to evaluate the role of Shp2 in mammary gland cancer development. Conditional ablation or pharmacological inhibition of Shp2 blocks mammary gland tumors and metastases. Shp2 deficiency induces cell cycle arrest and senescence of mammary gland cancer stem cells (CSCs), which leads to the inhibition of CSC self-renewal. These data demonstrate that Shp2-dependent signaling is crucial for mammary gland tumor formation and progression in this well-established mouse model. We provide evidence that Shp2 stimulates downstream signaling of Src, Fak, Mek1 and Notch in mammary gland CSCs. Inhibition of these signaling systems by specific small-molecule inhibitors prevents self-renewal and induces senescence, thereby mimicking Shp2 deficiency. Specific gene expression programs depend on Shp2 in mammary gland CSCs: Aurka/b-, Skp2- and Hey1-mediated repression of p15 and p21, and Mek1-mediated repression of Runx2 and Gadd45g. Shp2-dependent gene expression signatures that are important for cell cycle progression and senescence prevention predict the outcome of human breast cancer patients. Our findings indicate that Shp2 inhibition may be useful for the therapy of human breast cancer.

Aceto N, et al. (2012) Tyrosine phosphatase SHP2 promotes breast cancer progression and maintains tumor-initiating cells via activation of key transcription factors and a positive feedback signaling loop. Nat Med 18: 529-537.
Grossmann KS, Rosario M, Birchmeier C, Birchmeier W (2010) The tyrosine phosphatase Shp2 in development and cancer. Adv Cancer Res 106: 53-89.

Abstract No. P123

The in vitro differentiation ability of hybrid cells derived from cell fusion events between murine breast cancer cells and bone marrow-derived cells

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With relation to the importance of cell fusion in tumour tissue heterogeneity we have previously shown that murine breast cancer cell lines can fuse spontaneously with murine bone marrow-derived cells (mBMDCs) in vitro. Hybrid cell clones emerged from such spontaneous fusion events in a co-culture model showed genetic alterations and marked heterogeneity compared to their parental cell lines. The need of adaption processes in tumour progression and dissemination led to the question if hybrid cell clones could maintain the capacity to differentiate into the neuronal, adipogenic and osteogenic lineage.

By cultivating the hybrid cell clones and parental cell lines under appropriate conditions to established differentiation protocols, differentiation was performed and tissue specific markers were used for validation of the differentiation state. Incubation of cells in neuronal differentiation medium was associated with an up-regulation of neurofilament M and class III β-tubulin in both hybrid cell clones and parental cell lines. PCR data were validated by immunocytochemical staining.
After induction of adipogenic differentiation elevated levels of intracellular lipid storage vesicles could be observed in all cells and demonstrated by Oil Red O staining. Low levels of adipose tissue markers aP2 and PPARγ were already detectable in undifferentiated cells whereas differentiation led to a marked increase.
Osteogenic differentiation was accompanied by increased alkaline phosphatase activity in all cells. Moreover, expression of bone sialoprotein II could also be detected in all differentiated cell lines, while collagen I α 1 was only found in mBMDCs and hybrid cell clone 3.

In conclusion, our data indicate that hybrid cell clones are able to differentiate into specialized cells from either neuronal, adipogenic or osteogenic lineages. These stem cell properties show that hybrid cell clones derived from cell fusion events could adopt specific tissue properties through differentiation and thereby adapt better to new environments.

Abstract No. P124

A patient-derived renal cell carcinoma model as a platform for the identification of novel diagnostic markers and therapeutic targets

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Clear-cell renal cell carcinoma (ccRCC) is highly resistant to conventional therapies. Pre-clinical models, accurately mimicking this disease, are critical for the development of novel effective therapies. Previous attempts to culture ccRCC cells have been proven difficult and thus, only few cell lines are available. This might explain why in contrast to other tumor entities, tumor-initiating cell (TIC) subpopulations have so far not been reported for ccRCC. TICs have been associated with tumor progression, metastasis and drug-resistance and have consequently also been named cancer stem cells (CSCs). Therefore, the development and characterization of clinically relevant patient derived ccRCC-models would provide a basis to study the complex mechanisms underlying ccRCC.

Hence, we established a patient-derived serum-free culture system that can be used to propagate primary ccRCC cells. These cells retain their tumor-initiating potential and mimic the human malignancy upon orthotopic injection into immunodeficient mice. They not only mirror the histological properties but also the molecular features of the parental tumor. The established ccRCC cultures were then used as a screening platform for the identification of surface proteins, which are heterogeneously expressed among tumor cells. Functional in vitro and in vivo assays showed that subpopulations defined by expression of these markers also displayed distinct functional characteristics. Gene Set Enrichment Analysis (GSEA) revealed differences in the signaling networks active in these sub-populations. In addition, molecular and cellular assays addressed the significance of these pathways for the maintenance of ccRCC malignancy. Currently the identified surface markers and underlying pathways are validated as potential novel diagnostic and therapeutic targets.

Therefore, our novel patient-derived serum-free spheroid culture system serves as a platform to expand patient derived ccRCCs. It further allows the analysis and characterization of different subpopulations, which may ultimately contribute to the understanding of the molecular basis underlying progression, therapy-resistance and metastasis of this disease.

Abstract No. P125

Identification of circulating metastasis-initiating stem cells in breast cancer

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Despite the latest advances in preclinical research, metastatic cancer is still reported as the main cause of cancer-related death. Currently, increasing interest has emerged in the clinical evaluation of Circulating Tumor Cells (CTCs) as a liquid biopsy for metastatic tumors. Moreover, advancements of the cancer stem cell theory have led to speculate that certain CTCs might display stem cell-like traits, enabling those cells to initiate secondary tumors at distant sites. Our lab has recently been able to identify a subset of CTCs from breast cancer patients having the capacity to initiate new metastases in bones, lungs and liver after transplantation into highly immuno-compromised mice. A subset of EpCAM+ circulating cells isolated from the blood of metastatic ER+ breast cancer patients displayed a CD44+ MET+ CD47+ phenotype. We here propose that this surface marker-defined subset might contain metastasis-initiating cells. Hence we present new patient-derived experimental platforms that could allow functional studying of the metastatic potential of these cells at the patient level.

Baccelli I. et al., Identification of a population of blood circulating tumor cells from breast cancer patients that initiates metastasis in a xenograft assay, Nature Biotechnology 31, 539–544 (2013), doi:10.1038/nbt.2576
Baccelli I. and Trumpp A., The evolving concept of cancer and metastasis stem cells, J. Cell. Biol. 198(3):281-93 (2012), doi: 10.1083/jcb.201202014

Abstract No. P126
Molecular links between EMT and stemness

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Epithelial-Mesenchymal Transition (EMT) has been shown to promote tumor dissemination as well as tumorigenicity. Previously, we identified autocrine loops engaging Wnt and TGF-beta pathways that are induced by EMT. Interestingly, establishment of these autocrine loops required the repression of several endogenous inhibitors (DKK1, SFRP1 and BMP4). Consequently, exposure of breast cancer cells to recombinant SFRP1 and BMP4 inhibited tumorigenicity and metastasis, suggesting dependence on continuous autocrine signaling. Further, a similar set of signals appeared to control the interconversion of stem/progenitor cell-containing basal and lineage-restricted luminal human breast epithelial cells (Scheel C., et al., 2011).
While these findings might be of great utility to target aggressive breast cancer cells by blocking autocrine signals, we determined that cellular heterogeneity poses a major obstacle to resolve the temporal dynamics of signaling networks governing the induction and maintenance of EMT-associated functional traits at a molecular level. Specifically, we observed that induction of an EMT in immortalized human breast epithelial cells elicits a highly heterogeneous and protracted transition and imposes strong selection pressures. Moreover, we noted that in response to a multitude of EMT-inducing stimuli, a greater proportion of cells adopts mesenchymal compared to stem cell-like traits. Together, these observations suggest that, at least in certain contexts, the EMT program is not sufficient to generate stem cell-like traits. To address these issues, we isolated subpopulations within breast cancer cell lines that are either enriched or depleted in stem-cell like characteristics following induction of an EMT. Since these subpopulations can be sorted prospectively based on cell surface markers, we propose that a pre-existing cellular state determines whether an EMT-inducing stimulus, such as expression of the EMT-TF Twist or Snail, results in mesenchymal transdifferentiation only, or in the acquisition of additional, stem-cell like traits.

Scheel, C., et al. (2011). Paracrine and autocrine signals induce and maintain mesenchymal and stem cell States in the breast. Cell 145, 926-940.

Abstract No. P127

GDF3 in cancer stem cell biology

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Misexpression of growth factors, particularly those related to stem cell-like phenotype, is often observed in several cancer types. It has been found to influence parameters of disease progression like cell proliferation, differentiation, maintenance of undifferentiated phenotype and modulation of the immune system. GDF3 is a TGFB family member associated with pluripotency and differentiation during embryonic development that has been previously reported to be re-expressed in a number of cancer types. However, its role in tumor development and progression has not been clarified yet.
In this study we decipher the role of GDF3 in an in vitro model of cancer stem cells, NCCIT cells. By classical approach to study protein function combined with high-throughput technique for transcriptome analysis and differentiation assays we evaluated GDF3 as a potential therapeutic target.
We observed that GDF3 robustly induces a panel of genes related to differentiation, including several potent tumor suppressors, without impacting the proliferative capacity. Moreover, we report for the first time the protective effect of GDF3 against retinoic acid-induced apoptosis in cells with stem cell-like properties. Our study implies that blocking of GDF3 combined with retinoic acid-treatment of solid cancers is a compelling direction for further investigations, which can lead to re-design of cancer differentiation therapies.

Abstract No. P128

Advanced Model System for Serous Ovarian Carcinoma revealing Novel Drug Targets and Cancer Stem Cell Markers

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Ovarian cancer is the most lethal form of gynaecological cancers. Despite advances in therapy, only 30% of patients with advanced-stage ovarian cancer survive five years after diagnosis, exemplifying the need for improved therapeutic and diagnostic tools. Still the common model systems for drug screening and functional studies are long-term FCS-cultured ovarian cancer cell lines. The resulting cell lines frequently do not resemble the original malignancies and drug screens performed with those cell lines might yield hits of limited relevance for patient-tumours. Further, phenotypic heterogeneity is lost and with it the possible presence of putative 'cancer stem cells'. This 'CSC' population might play a role in drug-resistance, recurrence and metastasis. For ovarian cancer however, no CSC subpopulation has been conclusively described so far.

We developed advanced in vitro and in vivo models for ovarian cancer, which show a high degree of genetic and histological identity with the original patient tumor. Unlike conventional FCS-based cultures this system retains the molecular heterogeneity of the human malignancy. Xenografts grown in immune-compromised mice were classified as serous ovarian carcinoma by histo-morphology and expression of ovarian cancer specific markers CA125 and WT1. The in vivo models also recapitulate the patient disease by metastatic spread to liver and diaphragm and the development of ascites. Our advanced models facilitate screening for novel diagnostic and therapeutic targets in serous ovarian carcinoma, not accessible with conventional cell lines.

In the past, model systems for ovarian cancer have been incapable of accurate mimic the human disease. Here we present a model system, which combines CSC culture with xenotransplantation and models the human disease more faithful. This model allows studying metastasis development and drug resistance in serous ovarian cancer, uncovering potential therapeutic targets previously not accessible.

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Abstract No. P129

Impact of micro- and nano-structured biomaterials on proliferation and differentiation of mesenchymal stem cells.

Giulio Abagnale [1,*] , *Michael Steger* [2], *Vu Hoa Nguyen* [3], *Uwe Schnakenberg* [3], *Arnold Gillner* [2], and *Wolfgang Wagner* [1]

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Surface topography influences formation of focal adhesions and may thereby impact on cellular behavior. In this study, we analyzed how mesenchymal stem cells (MSCs) are affected by various line-and-space patterns, particularly with regard to morphology, proliferation, and in vitro differentiation. These patterns were generated on polyimide (PI) which is biocompatible, flexible, easy to handle, and shows low friction. Microstructures were developed using optical lithography followed by reactive ion etching technology in 25 different combinations of width (ranging from 2 µm to 15 µm) and spacing (2 µm to 15 µm). These substrates were assembled in a chess-board layout for comparative analysis and validated by scanning electron microscopy. Furthermore, we produced a PI foil with defined circular spots arranged in a hexagonal symmetry; each spot is structured with grooves of 650 nm periodicity using laser multi-beam interference technology and the patterning was validated by atomic force microscopy. MSCs from adipose tissue aligned in parallel to micro-structured grooves. Proliferation – with regard to population doublings within 1 week – was similar on PI as compared to conventional tissue culture plastic (TCP) and it was moderately affected by microstructures. Adipogenic and osteogenic differentiation were increased by smaller and bigger width, respectively. Even on nanostructured PI the cells clearly aligned along the grooves. Osteogenic and adipogenic differentiation were significantly increased by nanostructured grooves in comparison to unstructured PI or TCP. These results support the notion, that growth and lineage-specificity of MSCs can be directed by defined surface patterns in biomaterials.

Abstract No. P130

Endogenous stem cells in the adult brain react to stroke: long-term observation with bioluminescence

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Introduction: The adult brain contains neural stem cells (NSCs), which show regenerative behavior in response to neurological disorders. Following stroke, increased proliferation of these endogenous NSCs and directed migration into the ischemic striatum (Thored et al. 2006) was observed excitingly. This spontaneous regeneration is not sufficient to recover brain function, however, these endogenous NSCs have high potential to be functionalized in regeneration therapy. We report here the successful application of a DCX-luciferase reporter mouse (Couillard-Despres et al. 2008) for quantitative and long-term characterization of the endogenous NSCs proliferation after stroke.
Methods: 13 male adult DCX-luc mice received a middle cerebral artery occlusion (MCAO) or sham surgery. Bioluminescence (detection of NSCs) and magnetic resonance imaging (visualization of stroke) was performed before, 7 and 14 days after MCAO. Animals received BrdU (50 mg/kg i.p. 2/d) between day 3 and 7. Animals were sacrificed at 7 or 14 days post MCAO. Brain tissue was processed for immunohistochemistry.
Results: Before MCAO photon emission (PE) was equal on both hemispheres. Stroke resulted in a visible increase in PE over the ischemic hemisphere at 7 and 14 days after MCAO, while sham surgery did not lead to intensity change. Quantification of the PE results in a rise of 4% compared to the intact hemisphere. Preliminary immunohistochemical analysis of DCX+ cells in the SVZ indicates a slightly thicker appearance of the rostral end.
Conclusions: Increased proliferation of endogenous NSCs can be followed over time in response to stroke and is quantifiable in living mice using this mouse model with a neurogenesis-specific molecular reporter. This approach will be highly valuable for the investigation of regenerative therapies for stroke. Therapeutic compounds aiming to enhance endogenous neurogenesis can be quickly tested for their effectiveness without the need of tedious immunohistochemical quantifications.

Thored et al. Stem Cell. 2006). 24:739 –747
Couillard-Despres et al. Molecular Imaging. 2008. 7(1):28–34

Abstract No. P131

Calcineurin regulates coordinated multi-tissue progenitor cell growth during fish fin regeneration to determine appendage size

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Vertebrates develop organs and appendages from stem and progenitor cells by controlling their growth in a proportionally coordinated manner, and those animals that regenerate do so by recreating the lost structures to the original dimensions. It is unclear which molecular mechanisms execute this coordinated growth control. We show that the phosphatase calcineurin regulates coordinated outgrowth of the multi-tissue progenitor cells of the blastemas regenerating zebrafish appendages. Calcineurin inhibition with FK506 (Tacrolimus) or with Cyclosporine A results in continued regeneration of the fins well beyond their original dimensions. Despite the enhanced regenerative growth, the treated fins do not form tumors. Treatment with other immunosuppressants does not enhance regenerative outgrowth. Congruent with our results, we show that during the typical regeneration progression, calcineurin activity is reduced when blastema outgrowth is highest and its activity increases as the rate of regenerative outgrowth decreases. From measurements of growth rates and morphometric analysis of proximodistal asymmetry, we show that calcineurin inhibition shifts regenerative growth from the distal isometric growth to an allometric growth that operates during the formation and regeneration of proximal fin architecture. This shift is associated with the upregulation of retinoic acid signaling, a signal transduction program known to proximalize developing and regenerating tetrapod limbs. In summary, we have identified a calcineurin-mediated mechanism that operates as a molecular switch between distal isometric growth and proximal allometric growth.

Abstract No. P132

Imaging the glial fate by a novel dual reporter approach

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Objectives
Neural stem cells (NSCs) retain their potential to differentiate into neurons, astroglia and oligodendroglia upon intracerebral transplantation. We established a novel imaging reporter system for quantitative visualization of the neural fate in vitro and in vivo.
Material and Methods
A GFAP- radial glia-like NSC line was transduced in two steps. The lentiviral vectors pcdh-EF1-hRluc-T2A-copGFP and pcdh-GFAP-Luc2-T2A-mCherry-SV40-Zeo were generated to introduce two different luciferases and fluorescent proteins. The constitutive EF1 promoter and the cell-specific GFAP promoter provided selective imaging reporter expression. Glial differentiation was induced by withdrawal of growth factors and the addition of 1% FCS. 300,000 double transduced cells and a control cell line were stereotactically transplanted into the right and left striatum of nude mice (n=4). Dual reporter BLI was performed for up to 7 days post transplantation. The cell-specific Luc2 signal is normalized to the constitutive hRluc signal at each time point. Immunohistochemistry (IHC) was used to verify the imaging reporter pattern specific for astrocytes.
Results
A significant 24-fold increase in Luc2 bioluminescence was observed from day 1 to 4 of differentiation and down-regulation of the constitutive copGFP reporter was excluded. The mCherry fluorescence onset was monitored after 7 days of differentiation. The differentiation state is verified by GFAP IHC revealing the difference between the native, proliferative NSC state (copGFP+/ mCherry-/GFAP-IHC-) and the glial state (copGFP+/mCherry+/GFAP-IHC+). Glial differentiation in vivo could be monitored by an increase in Luc2 signal and is currently verified by IHC.
Conclusions
The new dual reporter system allows monitoring of NSC differentiation into astrocytes in vivo, quantitatively, and without the need of additional histology.
Acknowledgements
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Abstract No. P133

Human placenta derived mesenchymal-like adherent stromal cells for the treatment of skeletal muscle injury in rats

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Cell therapy is a promising tool in muscle regeneration. We could show that the transplantation of autologous mesenchymal stromal cells (MSC) improved the regeneration of traumatized muscle. The disadvantages of the autologous approach are the time-intense isolation and expansion procedures of the MSC. Allogeneic cells are used as an “off-the-shelf” product. Another limitation of the autologous transplantation is it's accessibility only for patients with MSC-pool, which has its complete regenerative potential. Therefore we investigated the efficacy of PLX cells, which are mesenchymal-like adherent stromal cells (ASCs) derived from placenta and expanded under 3D growth-conditions in a rat skeletal muscle injury model.

40 female Sprague Dawley rats were used for this study. The left soleus muscles were traumatized with a curved forceps. Two groups were treated with 2x106 PLX cells either immediately after muscle injury (group 1) or a week later (group 2), both control groups (group 3 and 4) received sham treatment with 20µl saline at the respective time points. In vivo functional muscle testing and a histological analysis were performed four weeks after trauma.

Administration of 2x106 PLX immediately after trauma resulted in a significant improvement of contraction force after fast-twitch (FT) stimulation (0.7±0.29) compared to the sham-treated control muscles (0,39±0,2, p=0.01). Delayed administration of 2x106 PLX treatment improved muscle contraction force significantly (PLX-treated 0.75±0.29, sham-treated 0.44±0.2, p=0.02). Muscles treated immediately or delayed showed no difference in contraction forces. The histological analysis indicated that after both time points, PLX could be found in the interstitium of the traumatized muscles.

This data demonstrate the potential of PLX cells for improvement of muscle function after trauma. The histological results indicate an effect of the PLX cells on myofiber regeneration and blood vessel formation. To conclude, allogeneic cell therapy seems to be promising for an intraoperative cell therapy of muscle trauma.

Time course of skeletal muscle regeneration after severe trauma. Winkler et al. 2010 (Acta Orthop. 2011 Feb;82(1):102-11. doi: 10.3109/17453674.2010.539498. Epub 2010 Dec 13.)

Abstract No. P134

Expression of a Distinct BMP Receptor Profile Determines Responsiveness of Primary Human Mesenchymal Stem Cells towards BMP2

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Objective
The aim of the project is to determine the expression and function of BMP signalling molecules quantitatively, especially BMP receptors, in primary human mesenchymal stem cells (hMSCs) to correlate their role in BMP2 signalling outcomes during bone regeneration.

Materials and Methods
Primary hMSCs of different donors isolated from bone marrow were stimulated with BMP2 for distinct timepoints. Phosphorylation levels of Smad1/5/8, p38 and AKT (Ser473) were analysed by western blot. Expression levels of BMP receptors, target genes and marker genes for osteoblastic differentiation were investigated by qRT-PCR. Furthermore, alkaline phosphatase enzyme activity was measured after BMP2 stimulation. Overexpression and siRNA knockdown approaches were used for validation of the results.

Results
Comparing the primary hMSCs from 9 donors we found significant differences in their responsiveness towards BMP2. 4 of the 9 hMSC donors did not show an increased ALP expression/activity upon BMP2 stimulation, the other 5 donors had diverse expression/activity levels. The measured levels of all of the 9 hMSC donors could be correlated to the expression level of one BMP receptor. siRNA mediated knockdown of the receptor in mouse mesenchymal precursor cells (C2C12) and human MSCs of one of the ALP positive donors confirmed the important role of this receptor for ALP activity. Ongoing experiments include the verification of the results by overexpression.

Conclusion
In bone regeneration both BMP2 and primary hMSCs are crucial players. Our finding suggests that variations in bone healing seen in individual patients might rely on distinct expression of individual BMP receptors. This knowledge about the role of individual BMP receptors for the osteogenic potential of hMSCs is relevant to understand and to improve bone fracture healing of non-unions in the future.

Abstract No. P135

Evaluation of Different Fungal Extract Influence on Expression of Extracellular Matrix Components in MSC Cultures

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The aim of the work was to evaluate the effects of different fungal extract influence on expression of extracellular matrix components coding genes in human skin mesenchymal stem cell cultures.
Four human skin-derived dermal mesenchymal stem cell (sMSC) cultures from early-passage (p3) and late-passage (p10) were used for in vitro extract testing. The cells were cultured in media with different fungal extract additives (L.edodes with polysaccharide concentration 1,56 mg/ml, L.edodes with polysaccharide concentration 40 mg/ml, and G.lucidum with polysaccharide concentration 1 mg/ml) in different concentrations (0,25%, 0,5%, and 1%). After assessment of morphology RNA was isolated and RT-qPCR was done to evaluate the expression of extracellular matrix components coding genes has1, col1A, col3, and eln. Expression data was interpreted by Livak's relative quantification method where housekeeping gene β-actin was used as reference.
Various extract additives in different concentrations showed no morphologically observed effect on sMSC cultures in both passages. All three extracts have a positive effect on has1 gene expression, but both L.edodes extracts showed the most positive effect on col3 gene expression. L.edodes extract with polysaccharide concentration 1,56 mg/ml shows most beneficial effect on extracellular matrix components expression.
The study data suggest the beneficial effects of fungal extracts and their prospect of use in development of skin anti-aging agents, in order to substitute synthetic ingredients.

Acknowledgements
This work was financially supported by The European Social Fund (ESF) project "Capacity building for interdisciplinary biosafety research" (Contacts Nr.2009/0224/1DP/1.1.1.2.0/09/APIA/VIAA/055) and by LU SP ZPK project "Evaluation of different fungal extract influence on expression of extracellular matrix components in MSC cultures".

Keywords: MSC, extracellular matrix components, RT-qPCR, Lentinula edodes, Ganoderma lucidum

Abstract No. P136

Xenogenic-free Culture Supplements for GMP-compliant Expansion of Mesenchymal Stromal Cells

Karen Bieback [1,*], *Andrea Hecker* [1], *Sven Kinzebach* [1], *Susanne Elvers Hornung* [1], *Stefanie Uhlig* [1], and *Harald Klüter* [1]

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The idea of stem cell therapy sounds simple: obtain sufficient numbers of cells from human tissues, isolate and expand the stem cells and then transplant the cells at the correct location. However, the translation into routine therapies is a complex, multistep process, necessary to comply with regulatory guidelines. Mesenchymal stromal cells (MSC) are interesting examples as these cells have been therapeutically applied in a very early phase of research. Scarcity of MSC often requires ex vivo expansion. Still expansion protocols rely on the use of fetal bovine serum (FBS), albeit critically rated by the regulatory authorities. “Humanized” culture conditions appear highly encouraging to replace FBS for clinical-scale manufacturing.

We compared the effects of pooled human serum (HS) as well as platelet lysate (pHPL) on MSC from bone marrow (MSC) and adipose tissue (ASC). Interestingly whereas expansion of MSC was strongly accelerated by pHPL, ASC proliferated stronger in HS. A differential proteomic approach comparing the human supplements combined with functional tests identified proteins with differential effects on MSC and ASC proliferation. Comparability assays ensured that basic therapeutic features of MSC such as senescence, differentiation potential, immunosuppression and secretion of bioactive factors were maintained. However, differences in cell size and adhesion force correlated to differences in gene and protein expression. These affected adhesion, migration and homing, important therapeutic features of MSC. Albeit the effect of changing the cell culture supplement is not ultimately clarified yet, clinical trials already utilize MSCs expanded in “humanized” supplements indicating feasibility, safety and efficacy. Nevertheless differences in the clinical outcome, e.g. in studies for treatment of graft versus host disease have been observed using either BM-MSCs in FBS or PL. To provide success of MSC-based therapies, the establishment of standardized manufacturing protocols and quality control parameters and assays is of utmost importance.

Abstract No. P137

Alginate encapsulation enhances neural differentiation of mouse embryonic stem cells

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The Central Nervous System is limited in its capacity for self-repair after damage. Thus, cell replacement therapies envisaging the use of pluripotent cells (suche as embryonic stem cells, ESCs) or stimulation of endogenous stem cells are currently the most promising strategies to cure an injured brain. Many published neural differentiation protocols for ESCs are based on monolayer cultures. However, it is also known that stimulation from the surrounding environment is crucial for the differentiation of cells towards the desired lineage. Biomaterials could be utilized to recapitulate the surrounding 3D physiological environment that cells encounter during in vivo differentiation.
In order to develop this strategy, we tested whether encapsulation of mouse ESCs within alginate beads could increase neural differentiation with respect to 2D cultures. Alginate was supplemented with the adhesion protein fibronectin (fn), the fn adhesion peptide RGD, and hyaluronic acid (HA, one of the major components of the neural extracellular matrix during development). Cells were cultured following established neural differentiation protocols. In few days, results showed that the cells were both viable and formed clusters. qRT-PCR and immunocytochemistry analyses demonstrate that cells grown in alginate and alginate-HA show increased differentiation toward neural lineages with respect to the 2D control and to fn and RGD modifications, with higher expression levels of the neural markers BIII-tubulin and NCAM. Immunocytochemistry also reveals that mESCs are able to make connections among themselves inside a cluster but not among clusters. Our data show that alginate and alginate-HA seem to be the best candidates in order to support and enhance mESCs neural differentiation.

Abstract No. P138

Human induced pluripotent stem cells for tissue-engineered cardiac repair

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Introduction
Myocardial infarction causes unrecoverable loss of cardiomyocytes. Engineered heart tissue (EHT) is an in vitro model of three-dimensional, force generating cardiomyocyte network with morphological and functional similarity to native heart tissue. In this study we transplanted EHTs from human induced pluripotent stem (hiPS) cell-derived cardiomyocytes (CM) on guinea pigs after cryo-injury mediated myocardial infarction and investigated whether hiPS-CM-EHTs support left ventricular function.
Methods
Human iPS-cells were generated by retroviral reprogramming of dermal fibroblasts. Cardiac differentiation of hiPS-cells was performed using an embryoid body-based three-stage differentiation protocol. EHTs were created from hiPS-CM (5*106 cardiomyocytes and 2*106 HUVECs per EHT) and cultivated for 3 weeks under auxotonic stretch between flexible silicone posts. Development of contractile force was monitored microscopically and macroscopically prior to transplantation. Left ventricular myocardial cryo-injury was induced in adult guinea pigs (n=9). 7 days after injury EHTs (2 per animal, n=4) or no-cell constructs (n=5) were implanted. Animals received immunosuppressant. Functional parameters were examined by echocardiography and histology at baseline, before and 28 days after transplantation.
Results
The cardiac differentiation protocol resulted in a cell population with 50% cardiomyocytes, which was further enriched by lactate-based selection to > 90% purity and directly used for EHT generation. HiPS-CM-EHTs developed contractile force and displayed morphological properties of native heart tissue. Cryo-injury resulted in large transmural scars (~30% of ventricular wall) which were verified histologically. Animals receiving cell-free constructs showed left ventricular dilatation 28 days after transplantation. The EHT-group showed less dilatation and significantly better ejection fraction. Survival of hiPS-cell derived cardiomyocytes was verified by immunohistochemical staining for dystrophin and MLC2v, the human origin of cardiomyocytes in the transplant by fluorescent-in-situ-hybridization.
Conclusion
Transplantation of hiPS cell EHTs in a guinea pig cryo-injury model provides early evidence that hiPS-CM-EHTs survive after transplantation and support cardiac function.

Abstract No. P139

Cardiomyocyte derived from induced pluripotent stem cells are more sensitive to ischemia than naïve cardiomyocytes

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OBJECTIVES: Cardiomyocytes derived from induced pluripotent stem cells (iPS-CM) may be suitable for myocardial repair. While their phenotype and function in standard culture conditions are comparable to that of naïve cardiomyocytes, the iPS-CM response to ischemia is not yet fully understood.

METHODS: The simulated in vitro ischemia model consisted of 3h hypoxia (1%O2) and glucose/serum deprivation. Cell damage was quantified by caspase-3/7, -8 and -9 activation, TUNEL and 7-Aminoactinomycin D presentation (7-AAD), visualization of the mitochondrial potential (JC-1), metabolic activity (MTS test) and ATP measurement. Activation of intracellular signaling cascades was evaluated by qRT-PCR and Western blotting. Finally, the protective effect of multipotent stromal cell-conditioned medium on iPS-CM and NMC in simulated ischemia was assessed.

RESULTS: In response to simulated ischemia, 48±5% murine iPS-CM and 23±3% neonatal murine cardiomyocytes (NMC) displayed poly-caspase activation ($p < 0.01$), with a similar pattern for individual activities of caspase -3/7, -8 and -9. 10±3% iPS-CM and 2.7±0.1% NMC were 7-AAD positive ($p=0.02$), while the proportion of TUNEL-positive cells was similar. The average mitochondrial membrane potential was reduced in iPS-CM but remained unchanged in NMC. The MTS conversion rate was significantly more reduced in “ischemic” iPS-CM than in NMC ($p=0.001$), as was the average cellular ATP content ($p=0.01$). Transcriptional upregulation of heat shock protein 70 was significant in iPS-CM ($p < 0.05$) as was the phosphorylation of STAT3 and total PKCε. MSC-conditioned medium restored metabolic activity in iPS-CM and NMC to similar extent ($p < 0.05$), but only iPS-CM reacted also with a significant increase in ATP synthesis ($p < 0.05$).

CONCLUSIONS: The response of iPS-CM to simulated ischemia is not identical to that of naïve cardiomyocytes. It remains to be determined whether this is a reprogramming-induced phenomenon or a reflection of cellular maturity. Concomitant treatment with MSC-conditioned medium may help improve the iPS-CM resistance to ischemia.

Abstract No. P140

Application of peripheral blood derived progenitor cells rescue biologically impaired bone fracture healing

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Delayed healing and pseudathrosis formation after fractures are clinical orthopaedic problems affecting 5-10% of all patients. With an aging population, skeletal fractures are increasing in incidence. There is an unmet need to induce predictable bone formation as well as improve implant fixation in the elderly. Sufficient angiogenesis is essential in fracture repair treatment. Peripheral blood derived CD133+ stem and CD31+ progenitor cells are reported to play a role in revascularisation. Thus, we supposed that a local administration of these cells to a fracture gap is a feasible option to treat biologically impaired fracture healing.

We analyzed availability, angiogenic and osteogenic potential of these cells derived from young and elderly, male and female probands in vitro. Besides, we performed first in vivo analysis to reveal the effect of aged cells on fracture healing after local transplantation. As evident from flow cytometric measurements, circulation of CD133+ cells in the peripheral blood increases with age, whereas the number of CD31+ cells remains unaltered. Tube formation assays and analysis of the osteogenic differentiation proved that CD31+ and CD31+/CD14- cells have a positive impact on osteogenic differentiation of MSCs beside their pro-angiogenic properties.

The regenerative potential of CD31+ and CD133+ cells could be proven in vivo by an increased callus formation and higher bone mineral density of callus tissue within the animals treated with the cells, compared to a control group treated identically but without additional cells or all peripheral blood mononuclear cells, respectively. Increased mineralization in the osteotomy gap was visible in histological analysis 42 days after surgery in the cell-treated animals.

Summarizing, we could prove that CD133+ and CD31+ stem and progenitor cells from peripheral blood feature bone regenerative capacities. Thus, an application of these cells to a fracture site is a promising approach for the treatment of impaired healing situations.

Abstract No. P141

Platelet-derived factors preserve SSEA-4 expression and maintain bone/marrow niche-forming potential of human MSPCs in vivo via GPCR/PDGFR signalling

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Availability of human stem cells (SC) in sufficient quantity and appropriate functionality is a prerequisite for regenerative SC therapy. We and others have previously shown that human platelet lysate (HPL) can efficiently replace FBS for preclinical mesenchymal stem/progenitor cell (MSPC) propagation. To understand the mechanism of HPL-regulated bone formation we propagated 11 pairs of human bone marrow-derived MSPCs either completely xeno-free (HPL-MSPC) or in FBS-supplemented conditions (FBS-MSPC). Proliferation/differentiation capacity and phenotype were tested in vitro and in vivo using a subcutaneous ectopic human bone and marrow niche (HuNiche) formation model in immune-compromised NSG mice (Blood 119:4971) including re-transplantation questioning the existence of skeletal SC.

Despite superior proliferation of HPL-MSPCs both lines were phenotypically virtually indistinguishable fulfilling basic MSPC criteria including osteo-, adipo- and chondrogenic differentiation in vitro. Interestingly, HPL-MSPC produced 4x heavier cartilage fragments during 3D chondrogenesis compared to FBS-MSPC in vitro. In the HuNiche model HPL-MSPC from all 11 donors spontaneously initiated endochondral bone formation in vivo (7/11 also forming functional niches attracting complete murine marrow). Only 2/11 corresponding FBS-MSPC-lines formed bone and 1/11 established a marrow niche.

Phenotypic profiling revealed higher SSEA-4 expression on HPL-MSPCs vs. FBS-MSPCs correlating with hematopoietic marrow niche formation in vivo. In a downstream target screen, cholera toxin but not pertussis toxin inhibited osteogenesis implicating GPCR-alpha-S involvement. Imatinib treatment inhibiting PDGF-R-beta or FBS exposure of HPL-MSPCs equally resulted in loss of SSEA-4 and impaired in vivo osteogenesis. Specific PDGF-R phosphorylation was found in HPL-MSPCs but not FBS-MSPCs despite equal PDGF-R-beta surface expression. Re-isolation of human MSPC and serial re-transplantation was only successful with HPL-MSPCs forming bone in secondary recipients and indicating preserved stemness.

We conclude that platelet-derived factors and PDGF-R signalling are crucial for maintaining MSPC potency mediated by HPL. This favours HPL-MSPCs for skeletal regeneration and for experimental studies addressing HuNiche biology.

http://www.jove.com/video/1523
Blood 119:4971

Abstract No. P142

Transplantation of expanded foetal intestinal progenitors contributes to colon regeneration after injury

Robert Fordham [1,*], *Shiro Yui* [2], *Nicholas Hannan* [1], *Christoffer Soendergaard* [2], *Alison Madgwick* [1], *Pawel Schweiger* [2], *Ole Nielsen* [2], *Ludovic Vallier* [1], *Roger Pedersen* [1], *Tetsuya Nakamura* [3], *Mamoru Watanabe* [3], and *Kim Jensen* [2]

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The mature intestinal epithelium is the most rapidly self-renewing tissue in adult mammals, consisting of differentiated villi and proliferative crypt compartments, where Intestinal Stem Cells (ISCs) reside. In mice at birth, the intestine is developmentally immature, lacking crypts and Paneth cells, the supportive cells of the adult ISC niche. Mature intestinal epithelium can be propagated from ISCs in vitro as three-dimensional 'organoids'. Here I describe the establishment and utilisation of long-term three-dimensional cultures from immature intestine, which exist as cystic epithelial spheroids, 'Foetal Enterospheres' (FEnS). FEnS represent a transient state in tissue maturation that can also be captured developmentally from human foetal tissue and pluripotent cells differentiated into posterior definitive endoderm.

FEnS are of primitive cellular composition, with distinct signalling requirements to adult organoids. Notably, they do not require exogenous stimulation of the Wnt pathway for their maintenance and do not contain secretory cells. However, canonical Wnt signalling is sufficient to induce maturation of foetal enteric progenitors to adult-like organoids. To assess their in vivo potential, FEnS were transplanted into a mouse model of ulcerative colitis. Foetal-derived cells were able to adhere to the denuded lamina propria within three hours after transplantation and engraft into the regenerating colonic tissue by three days. By one week after transplantation, the foetal cells formed adult crypt-like structures, morphologically indistinguishable from host crypts and with evidence of goblet cell differentiation. These FEnS-derived crypts were maintained for over one month, with localisation of proliferative cells to the nascent crypt base and expression of colonic epithelial markers. Altogether, FEnS demonstrated an ability to engraft into the injured adult colon and contribute to de novo cryptogenesis during colonic epithelial regeneration. These findings demonstrate that easily-expandable immature progenitors have the potential to mature in vivo and contribute to tissue repair.

Abstract No. P143

Hypoxic preconditioned Mesenchymal stem cells induces elevated expression of Cardioprotective Cytokines and Growth Factors

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Mesenchymal Stem Cells (MSCs) have ability to differentiate into various types of lineages which make them attractive candidates in the field of regenerative medicine. Transdifferentiation process of MSCs is mainly influenced by hypoxic microenvironment. Hypoxic preconditioning play a very important role in myocardial regeneration as it enhances the differentiation potential of stem cells and also upregulate the expression of different cardioprotective cytokines and growth factors. The present study main obejective was to see the effect of hypoxic insult on the expression of growth factors and thereby on the overall stimulation of MSCs. Rat bone marrow derived MSCs were cultured and assessed for their responsiveness to anoxia by an optimized dose of 0.25mM 2, 4, dinitrophenol (DNP) and then allowed to propagate under normal condition for 2 and 24 hours. Analysis of various cytokines and growth factors were done by RT-PCR and Determination of myogenic and angiogenic potential of these stem cells co-cultured with hypoxic cardiomyocytes were analyzed by flow cytometry after co-culturing them with hypoxic cardiomyocytes. The morphological examination of MSCs has indicated that the cells were slightly shrunken immediately after the anoxic insults. Analysis of various cytokines through RT-PCR has shown higher expression of SCF, TGFß, VEGF, IGF, HIF and HGF. The expression increased exponentially with the duration of anoxic insult. The coculture studies of hypoxic cardiomyocytes with MSCs showed higher fusion in conditioned medium as compared to normal medium. The results indicate that during hypoxia and ischemia, MSCs secrete cell survival factors that may operate via various signaling pathways enhancing their regenerating potential.

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Abstract No. P144

In vitro nephrogenesis of human induced pluripotent stem cells.

Krithika Hariharan [1,*], *Harald Stachelscheid* [1], *Manfred Gossen* [2], *Petra Reinke* [1], and *Andreas Kurtz* [1]

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Chronic Kidney disorders affect the functionality of the kidney at a slow pace ultimately leading to End Stage Renal Disorder (ESRD). Dialysis and kidney transplantation are currently the only available treatments for ESRDs. However, these have limitations like shortage of donor organs for transplantation and the requirement for lifelong immune suppression in the transplanted patient. These limitations have led to a quest for alternative therapies relying on regenerative mechanisms of the adult kidney and stem cells both in vivo and ex-vivo. Strategies like stimulation of resident adult kidney stem cells, mobilization of stem cells from bone marrow to migrate into the injured kidney or injection of mesenchymal stem cells have been proposed or are currently studied. We aim at generating renal progenitors from human induced Pluripotent Stem Cells (hiPSCs).
In an effort to develop a protocol to differentiate hiPSCs to the renal lineage, the process was segmented into several stages like mesoderm induction, Intermediate mesoderm specification, metanephric mesenchyme induction etc. to mimic renal organogenesis. Growth factors including activin A, BMP4 and 7, bFGF, BIOS and GDNF were screened for their morphogenic effect on hiPSCs. Mesoderm induction was indicated by the expression of transcription factors like brachyury and disappearance of pluripotency factors. Potential intermediate mesoderm specification due to Pax2 and Osr1 expression was also observed. The presence of a set of transcription factors – Six2, Lhx1, Osr1, Pax2, Eya1; either all or most, was used as a criterion to choose the best growth factor combinations capable of inducing renal morphogenesis. Prior studies highlight the potency of such a population in generating maximal cell types of the nephron. Demonstrating such a phenomenon in vitro would pave the way for potential cell therapy and drug testing applications; leading to several in vitro and in vivo applications of the protocol and the cells derived.

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Abstract No. P145

Cytokine-directed differentiation of hepatic hPSC-derivatives

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Human pluripotent stem cells (hPSCs) hold great promise in regenerative medicine. Hepatic derivatives of hPSCs might eventually serve as transplants for metabolic or acute liver diseases and are a valuable tool for research on disease models and for drug screening. So far, the generation of functional active hepatic derivatives in a sufficiently homogenous population for cell transplantation purposes is not yet well established and needs further attention.

In our study, we aimed for an efficient protocol that is applicable to specify hPSCs into an immature endodermal progenitor lineage prior to further terminal differentiation into hepatic cells. We evaluated a cytokine– and small molecule–based protocol stimulating the WNT pathway for direct conversion of hPSCs into a definitive endoderm progenitor population and investigated the GSK3beta inhibitor CHIR99021-driven activation of the WNT pathway by quantitative Western Blots of the active (non-phosphorylated) beta-catenin levels. The endodermal cells' differentiation status was determined by immunocytochemistry and qRT-PCR for the endodermal markers SOX17 and FOXA2. To enhance the hepatic differentiation of the endodermal cells, the WNT pathway was subsequently blocked with sFRP-5. Detection of phosphorylated GSK3beta and active beta-catenin via Western Blot were performed to analyse the sFRP-5 mediated inhibition of WNT signalling. The expression of early hepatic markers (GATA4, AFP) was determined by qRT-PCR.

In conclusion, our modified protocol allowed the specification of hPSCs into a homogenous endodermal progenitor cell population. Activation of the WNT pathway by the small molecule CHIR99021 supported a cell population expressing endodermal marker genes. Subsequent inhibition of the WNT pathway led to an improved hepatic differentiation of the endodermal cells showing an increased expression of early hepatic markers. Further experiments need to reveal if the cells differentiated under these conditions were suitable for cell transplantation approaches in vivo.

Abstract No. P146

Efficient derivation of cardiomyocytes from human iPS cell lines critically depends on precise modulation of Wnt signaling pathway

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Invention of iPS technology enables various new developments in the field of regenerative medicine. It can provide unlimited source of cardiac cells for cell replacement therapy as well as for disease modeling. Various protocols have been published showing targeted derivation of cardiomyocytes from human iPS cell lines. However line-to-line variability compromises the application of a particular protocol to robustly obtain cardiomyocytes from multiple iPS lines. Here we show the improvements of cardiac differentiation using small molecules modulating Wnt signaling. Moreover, we could further improve the purity of cardiac differentiation with addition of cardiac enrichment via lactate. Using our protocol we show efficient derivation of cardiomyocytes from multiple human iPS lines. In particular we demonstrate cardiomoycyte differentiation within 15 days with an efficiency of up tp 90 % as judged by flow cytometry staining against cardiac troponin T. Cardiomyocytes derived were functionally validated by immunohistochemical stainings as well as electrophysiological analysis. We expect our protocol to provides a robust basis for scale-up production of functional iPS cell-derived cardiomyocytes that can be used for cell replacement therapy and disease modeling.

Abstract No. P147

Urokinase receptor regulates differentiation of mesenchymal stem cells to osteoblasts and osteoblast-mediated osteoclast formation

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Multipotent mesenchymal stem cells (MSC), which are adult stem cells retaining self-renewal capability and unique multilineage potential, have emerged as the most promising candidate for osteoblastic differentiation related disorders, such as bone repair and vascular calcification. Molecular mechanisms underlying MSC osteogenic potential and differentiation remain, however, sparsely explored. Whether and how MSC-derived bone forming osteoblasts contribute to the communication with bone resorbing osteoclasts and to their function is poorly understood as well. We investigated a role for the multifunctional urokinase receptor uPAR in these processes. We observed that osteogenic differentiation of human MSC was accompanied by upregulated uPAR expression. uPAR silencing or overexpression in MSC resulted in inhibition or stimulation of osteogenic markers expression correspondingly. We elucidated the underlying molecular mechanisms and show that uPAR specifically regulates expression of the complement receptor C5aR in MSC upon osteogenic differentiation. We identified the NFκB pathway as the mechanism utilized by the uPAR-C5aR axis. We show involvement of the Erk1/2 kinase in this cascade. uPAR targeting in MSC resulted in decreased expression of M-CSF upon differentiation to osteoblasts that lead to impaired osteoclast formation and bone resorbing function in a co-culture of MSC-derived osteoblasts and human monocyte-derived osteoclasts. In vivo studies in a uPAR/LDLR double knockout mouse model of vascular calcification revealed impaired C5aR expression and calcification in aortic sinus plaques in uPAR-/-/LDLR-/- versus uPAR+/+/LDLR-/- control animals. These results suggest that uPAR-C5aR axis via the underlying NFκB transcriptional program controls osteogenic differentiation. They further point to uPAR requirement to maintain coordinated activity of osteoblasts and osteoclasts. This mechanism has consequences for vascular calcification in vivo

Abstract No. P148

Modification of neural stem cells to enhance their survival, neuronal differentiation and integration after brain transplantation

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Cell therapy could be a potential alternative treatment for neurodegenerative brain diseases. However, it is usually only a small proportion of pre-differentiated naïve transplanted cells (less than 5%) that survive post-transplantation period and the majority progressively die during the following weeks/months. Furthermore, transplanted cells usually have only a limited ability to influence the endogenous cell survival and regeneration. We used mouse precursor cells that were infected by a set of lentiviruses expressing genes that could, based on the published literature, affect the following processes: increase transplanted cell survival, enhance neuronal differentiation and stimulate the survival of endogenous cells in the injured area. Three months post-transplantation, the survival of transplanted cells was increased 10-fold over controls in all three regions examined (cortex, striatum and substantia nigra) and their differentiation was reversed compared to the neuronal phenotype. To confirm that the effects shown for mouse precursor cells could be transferred to human cells, we modified human neural stem cells and transplanted them in the striatum of the mouse brain. While in the control hemisphere there were only traces of surviving cells 1 month post-transplantation, in the genetically-modified hemisphere ~25% of the cells survived. The majority of genetically-modified cells exhibited a neuronal phenotype. The amount of transplanted cells that survives was stable over 3 months. Genetically-modified transplanted cells exhibited extensive cell process growth and branching, and their cell processes were found not only throughout the whole striatum, but also in the cortex and other brain regions.

Abstract No. P149

Use of complex transplant on a basis of mesenchymal stem cells of adipose tissue and hydrogel in cutaneus wound healing in experiment and clinic

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In the present study we have investigated the wound-healing effects of adipose tissue derived mesenchymal stem cells in combination with hydrogel when grafted into full-thickness skin defects. Twenty-five rats were divided into two equal groups randomly: treatment (hydrogel loaded with autologous AD-MSCs) and control. The size of the wounds was calculated for each group at 1, 10 and 20 days after grafting. Full thickness skin samples were taken from the wound sites for the morphological study on the 20th day after transplantation. Treatment group showed the most pronounced effect on wound closure, with statistically significant improvement in wound healing being seen on post-operative days 10 and 20. Thus, the graft of hydrogel loaded with AD-MSCs played an effective role during the healing of skin eatment was useful for tissue regeneration in patients with nonhealing wounds. Autologus AD-MSCs in combination with hydrogel was shown to be therapeutically effective. AD-MSCs represent a new cell source for therapeutic dermal wound healing.

Abstract No. P150

A dominant-negative acting Mpl as a tool to study Mpl pathways in the adult mouse

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Thrombopoietin signals via its receptor Mpl and regulates megakaryopoiesis, HSC maintenance and post-transplant expansion. MPL deficiency in patients results in thrombocytopenia and aplastic anemia. In the Mpl-/- mouse model the HSC defect and thrombocytopenia could be corrected by lentiviral overexpression of Mpl (Heckl et al., Blood 2011).
Expression of an intracellular-truncated and therefore signaling-deficient Mpl receptor in wildtype mice exerts dominant-negative effects. Mice transplanted with BM cells expressing the dominant-negative (dn)Mpl develop thrombocytopenia and HSC defects. dnMpl mice had 4-fold reduced lineage negative, Sca-1 and c-Kit positive (LSK) cell numbers and bone marrow (BM) cells did not engraft in secondary recipients. Furthermore, LSK cells of dnMpl mice were more often found in G1/S/G2 phase of the cell cycle indicating a loss of HSC quiescence. Due to the induced HSC defects in the hosts, a second wildtype BM transplant could engraft in dnMpl mice without further conditioning and led to longterm reconstitution of the BM (~41±12% chimerism, 4.8±5% in controls). To better understand the molecular changes in Mpl-signaling defective HSC, we performed transcriptome analysis. The expression profile of dnMpl LSK cells negatively correlated with known HSC stemness signatures which were positively enriched in Mpl corrected Mpl-/- LSK cells. Wnt, Jak/Stat and PI3K/Akt signaling were negatively enriched in dnMpl LSK cells while expression of genes involved in cell cycle progression were positively correlated. We confirmed distinct expression of typical markers of mouse HSC (Tie2, EPCR (CD201) and Esam1). Expression of all three markers in dnMpl mice was significantly reduced (**$p<0.005$) compared to control mice and similar to expression in Mpl-/- and Thpo-/- mice. In summary, we demonstrated the induction of HSC defects by dominant-negative inhibition of Mpl-signaling in vivo in the adult mouse. Further, we could identify genes and pathways that mediate the Mpl-deficient phenotype, which shed light on the molecular mechanism.

Heckl D. et al., Lentiviral gene transfer regenerates hematopoietic stem cells in a mouse model for Mpl-deficient aplastic anemia, Blood, 2011

Abstract No. P151

THE METAPROGEROL DRUG AND IR-LASER RADIATION SIGNIFICANTLY IMPROVE THERAPEUTIC POTENTIAL OF MSC TRANSPLANTATION IN THE TREATMENT OF CARDIOMYODYSTROPHY

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Systemic or local transplantation of mesenchymal stem cells (MSC) is currently the most frequently used cell therapy of various diseases. The reason is the MSC ability to the systemic homing in the regions of damage and production of paracrine agents activating regeneration. In this study, we tested the possibilities of enhancing the therapeutic efficiency of transplanted MSC by means of a number of physical and chemical agents applied to the recipient's organism. We demonstrated that administration of a cardiological drug metaprogerol or its analogues prior to the MSC transplantation significantly improved the therapeutic effect of MSCs administered intravenously to Wistar rats with doxorubicine-induced cardiomyodystrophy. Metaprogerol have been previously tested experimentally and in clinuical trials for the treatment of extensive acute myocardial infarctions. It was shown that administration of either metaprogerol alone or, especially, metaprogerol combined with MSC transplantation essentially increased the fraction of dividing cardiomyocytes and stromal cells (1.6-2 times by the PCNA index) in the heart muscle of rats 2 and 4 weeks post-administration. A similar effect was also revealed after an additional treatment of animals with low-intensity IR-laser radiation. The IR-treatment was applied several times before and after the MSC transplantation in the experiments on rats, as well as in the limited clinical trials with cardiological patients. In both cases of metaprogerol and IR-laser treatment, a more rapid regeneration of the normal level of metallothioneins in the damaged myocardium tissues was found (3-4 weeks for the MSC therapy combined with either agent vs. 1.5-2 months for the MSC therapy alone). The mechanisms of the stimulating action of metaprogerol and IR-laser radiation are probably related to the increase in the production of the key cytokines and growth factors, necessary for the normal functioning of so-called "niches" for adult stem cells which control their proliferation and differentiation.

Abstract No. P152

Perfusion feeding improves suspension culture of human pluripotent stem cells in controlled, stirred bioreactors

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Therapeutic and industrial applications of human pluripotent stem cells (hPSCs) and their derivatives require large cell quantities generated in defined conditions. Utilizing the defined culture medium mTeSR we have recently established single cell inoculated suspension cultures of hPSCs (Zweigerdt et al., Nature Prot. 2011), which form aggregates in stirred tank bioreactors (Olmer et al., Tissue Eng Part C Methods. 2012). Since they allow straight forward up-scaling and comprehensive monitoring and modulation of process parameters these systems are widely used in biotechnology for the mass culture of conventional mammalian cell lines aiming at the production of functional proteins. To ensure low medium consumption but the integration of all probes relevant for process monitoring including pO2 and pH, a “mini bioreactor platform” (DASGIP) was utilized. After establishing stirring-controlled aggregate formation up to 2 x 10^8 hiPSCs were generated per process run in 100 mL scale, whereby batch feeding was performed i.e. all culture medium was replaced once per day. Expression of pluripotency markers and cells ability to differentiate into derivates of all three germ layers was maintained underlining utility of this process. Yet, however, only linear growth rates were achieved and a relative low cell density of up to ~2 x 10^6 hiPSCs/ mL was obtained suggesting suboptimal conditions. Here we present novel data on how perfusion feeding can be technically established in mini bioreactors and that this feeding strategy results in more homogeneous process characteristics and substantially elevated cell yields.

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Olmer R, Lange A, Selzer S, Kasper C, Haverich A, Martin U, Zweigerdt R. Suspension culture of human pluripotent stem cells in controlled, stirred bioreactors. Tissue Eng Part C Methods. 2012;18(10):772-84.

Abstract No. P153

Functional correction and haematopoietic differentiation of human iPS cells from patients with X-linked chronic granulomatous disease

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Patients with X-linked chronic granulomatous disease (X-CGD) represent two thirds of CGD cases and carry mutations in the CYBB gene that codes for the NADPH-Oxidase. After phagocytosis of pathogens by neutrophils, the NADPH-oxidase in CGD patients cannot generate the reactive oxygen metabolites needed for killing opportunistic pathogens.
In clinical gene therapy studies for X-CGD patients, gene correction has never been persistent; therefore the transductions of stem cells and durability of transgene expression have to be improved. Additionally, the development of safer vector systems is needed to prevent insertional mutagenesis. To solve these problems we corrected CYBB mutations in iPS cells derived from X-CGD patients via homologous recombination and then differentiated them into haematopoietic stem cells and myeloid lineage cells.
In the context of the above, we isolated primary keratinocytes from plucked human hair of X-CGD patients and reprogrammed them by exogenous expression of human Oct4, Klf4, Sox2 and c-Myc genes using a Flp-recombinase excisable polycistronic lentiviral vector (kindly provided by Axel Schambach, Hannover). Pluripotency of the growing hiPS clones was confirmed demonstrating loss of exogenous reprogramming genes, expression of endogenous pluripotency markers and in their ability to form teratomas. CYBB mutation-specific correction was successfully achieved by homologous recombination. Subsequently, the reprogramming vector and the selection cassette in the targeting vector were excised by Flp-mediated recombination. SKY analysis of the corrected iPS clones demonstrated a normal caryotype. The corrected iPS cells were successfully differentiated into the myeloid hematopoietic lineage and the NADPH-oxidase activity was greatly restored, as demonstrated by chemiluminescence.
Overall, we established a strategy for correction and differentiation of X-CGD hiPS and proved their ability to produce superoxide in functional assays. The genetically corrected cells hold a great promise for future therapy of X-CGD.

Abstract No. P154

miR-181a promotes human neuronal differentiation and supports the generation of dopaminergic neurons

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MicroRNAs (miRNAs) control gene expression at the post-transcriptional level and are emerging as key regulators of neural cell proliferation, differentiation and fate choice. Recent studies have demonstrated that miRNA activity is required for the generation and maintenance of midbrain dopamine (DA) neurons, which are the prime target of Parkinson's disease. Gaining more insight into the role of miRNAs during human DA neuron specification may help to improve the generation of this neuronal subtype for regenerative purposes. So far, two miRNAs have been associated with this neuronal subtype, i.e. miR-133b and miR-132, which both inhibit the development of murine midbrain DA neurons. We used a population of long-term self-renewing neuroepithelial-like stem cells (lt-NES cells) derived from human pluripotent stem cells to identify novel miRNAs associated with human neuronal differentiation and fate specification. Based on gain- and loss-of-function analyses we show that miR-181a as well as other candidate miRNAs are required for the generation of neurons from lt-NES cells and contribute to the transition from self-renewal to differentiation. We further demonstrate that miR-181a positively affects the generation of neurons with dopaminergic fate, in part by activating WNT signaling in lt-NES cells. Thus, modulation of miRNAs might provide a tool for further enhancing the derivation of medically relevant neuronal subtypes from human pluripotent stem cells.

Stappert, L., Borghese, L., Roese-Koerner, B., Weinhold, S., Koch, P., Terstegge, S., Uhrberg, M., Wernet, P., Brüstle, O. (2013). MicroRNA-based promotion of human neuronal differentiation and subtype specification PLoS One 8(3):e59011
Roese-Koerner, B., Stappert, L., Koch, P., Brüstle, O., Borghese, L. (2013) Pluripotent stem cell-derived somatic stem cells as tool to study the role of microRNAs in early human neural development Curr Mol Med 13(5):707-722

Abstract No. P155

Efficient designer nuclease-based homologous recombination enables direct PCR screening for footprintless targeted human iPS cell clones

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The ability to genetically modify iPS cells, including the correction of gene defects by means of homologous recombination (HR) is of great interest regarding their potential for ex vivo gene therapy. Genetic engineering of human induced pluripotent stem cells (hiPSCs) via customized designer nucleases has been shown to be significantly more efficient than conventional gene targeting, but still typically depends on the introduction of additional genetic selection elements. In our study, we developed a protocol for the establishment of efficient non-viral and selection-independent gene targeting in hESCs and hiPSCs. For optimisation purposes, we first used an iPSC eGFP reporter system where ZFNs were applied to disrupt the eGFP open reading frame by NHEJ with an overall efficiency of up to 4% and to integrate a red fluorescent protein into the eGFP locus by HR. Without any antibiotic selection, we achieved HR-targeting efficiencies of up to 1.2 % and could show that the ZFN-treated pluripotent stem cells preserved their pluripotency and chromosomal integrity. Subsequently the developed protocol was applied to target the endogenous safe harbour locus AAVS1. Here, by using ZFNs and TALENs, targeting efficiencies comparable to our eGFP/RedStar reporter system were demonstrated for one hESC and two hiPSC lines, and stable transgenic PSC lines were generated by FACSorting. Moreover, the high targeting efficiencies obtained allowed for the direct PCR screening of correctly targeted clones by applying TALENs together with short single stranded oligonucleotide donors without any pre-selection. This will enable footprintless gene correction and transgene-independent isolation of mutation-corrected disease-specific iPSC clones and may ultimately lead to novel concepts for iPSC-based ex vivo gene therapies.

Abstract No. P156

Is there a way to convince MSCs to express functional surface receptors for improved homing?

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Mesenchymal stem/progenitor cells (MSC) hold a great promise for the development of alternative (cell based) therapies for numerous diseases. Despite their advantageous characteristics and their potential to improve functional outcome also after stroke, MSC therapy is still not optimal. Particularly the homing capacity of the cells towards ischemic regions seems barely sufficient to improve functional recovery. Thus, this study focuses on genetic cell engineering aiming to (transiently) overexpress relevant migratory and adhesional surface receptors on MSCs.
Vector constructs were designed for expression of C-X-C Motif Chemokine Receptor 4 (CXCR4; receptor for stromal derived factor 1 [SDF-1]), C-C-Motif Chemokine Receptor 2 (CCR2; receptor for monocyte chemotactic protein-1 [MCP-1]) or integrin alpha 4 (part of very late antigen 4 [VLA4]; receptor for vascular cellular adhesion molecule 1 [VCAM-1]). In addition to “closed” constructs (with stop codon) also constructs with c-terminal V5- or GFP-tag were designed. All vectors contain a T7/CMV promoter for in vitro transcription as well for eukaryotic expression. Finally, plasmids for production of lentiviral particles were constructed for stable transfection.
After testing the functionality of the constructs in HEK293T cells, MSCs from either human, ovine or rat origin were transfected with in vitro transcribed mRNA, pDNA or lentiviral particles. Surprisingly, the efficiency of the occurrence of target overexpressing cells was very low. This was all the more surprising, since control experiments with mRNA transfection (GFP) or lentiviral infection (GFP, dsRed, luc2) resulted in very good efficiencies (> 90%). It may be speculated that the ineffective target-expression is due to incorrect processing and/or transportation of the molecule. Therefore, more specialized and target cell specific strategies for molecular engineering need to be developed.

Abstract No. P157

The ADIPOA clinical trial - Adipose derived stromal cells for the treatment of osteoarthritis

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Within its 7th Framework Programme the European Community is funding a multinational research project aiming for the development of innovative treatment strategies for knee osteoarthritis (OA) by applying adipose-derived stem cells (ASCs). The ADIPOA consortium consists of 12 european partners focusing the expertise of more than 200 scientists to design and execute a "first-in-man-study" in accordance to the EMA guidelines, concerning the safety and clinical efficacy of an intraarticular injection of autologous ASCs into severely arthritic knee joints. The pivotal concept of intraarticular delivery is driven by the hypothesis that autologous ASCs cells exhibit anti-inflammatory/-fibrotic and chondroprotective/-inductive effects in OA and might potentially slow down or arrest the progression of cartilage degeneration.
A bi-centric clinical trial (Montpellier/France, Würzburg/Germany) was launched in 2012 after receiving approval of the national competent authorities in Germany and France. A total of 18 patients with severe OA of the knee joint will receive a single, escalating intraarticular dose of 2, 10 or 50 x 106 ASCs. The primary objective of the clinical trial is safety by recording adverse events and adverse reactions during a follow up-period of twelve months. Secondary endpoints include functional clinical parameters, joint function, pain measurement, and treatment satisfaction.
Up to date, thirteen patients received an intraarticular ASCs injection. Preliminary results on safety parameter confirmed the high tolerability of the ADSC application. Interim analysis of OA specific questionnaires (WOMAC, KOOS) as well as pain scores indicated an improvement in knee function and significant reduction in knee pain, that was independent from the admitted cell dose.
A critical monitoring over a longer time period has to validate these promising results. As these processes will expedite the testing of novel therapies, and are totally in keeping with EU legislation designed not only to ensure safety of ATMPs but also to facilitate their development.

Abstract No. P158

Skeletal Muscle Regeneration with Mesenchymal Stem Cells, Growth Factors and Multifunctional Scaffolds

Matthias Pumberger [1,*] , *Taimoor Qazi* [1], *Monika-Christine Ehrentraut* [1], *Philipp von Roth* [1], *Tobias Winkler* [1], *Carsten Perka* [1], *David Mooney* [2], and *Georg Norbert Duda* [1]

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Skeletal muscle has a limited potential to regenerate itself following injury and often results in functional deficiency. Transplantation of autologous mesenchymal stem cells (MSCs) after trauma showed a positive effect on the functional outcome (1). Furthermore, growth factors (GFs) delivered by a multifunctional scaffold increased the functional outcome following muscle trauma (2). The goal of this study was to investigate the potential effect of the MSCs and GFs (IGF-1 and VEGF) transplantation by a scaffold.

Each study group (n=10) (group1: alginate, group 2:alginate+GFs, group : alginate+MSCs, group 4: alginate+GFs +MSCs) was transplanted immediately after a standardized blunt open crush trauma of the soleus muscle of Sprague Dawley rats. The alginate hydrogel was placed directly onto to injuried muscle and functional muscle testing was performed after 7 and 28 days of the trauma bilaterally (total n=80). (fast twitch (FT) and tetanic contraction (TC) by sciatic nerve stimulation). None of the study groups showed significantly different muscle contraction force after 7 days (group 1-4: 53±8%; 56±12%; 62±13%; 57±10%). However, a significantly increase in muscle contraction force (FT) was observed after 28 days, when group 4 (83±10%) was compared to group 1 (62±9%) ($p<0.05$). Furthermore, it was the only study group which significantly increased its contraction force (FT) between day 7 (57±10%) and 28(83±10%) ($p<0.05$). The tetanic contraction failed statistical significance, however a similar trend could be observed. Interestingly, similar to better results were achieved with less cells when transplanted within a scaffold (1 Mio MSCs), compared to the prior established injection technique (2.5 Mio MSCs).

In conclusion, autologous MSC and GF transplantation showed a significantly increase in muscle contraction force. Futher research efforts should be directed towards skeletal muscle regeneration, in order to balance the dramatic clinical need in orthopedic and trauma surgery.

Abstract No. P159

Human induced pluripotent stem cells for cell-replacement therapy: Exploring their immunogenicity and immunomodulatory properties

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Human induced pluripotent stem cells (hiPSCs) are reprogrammed adult cells. They are characterized by their high proliferative capacity and their potential to give rise to all derivatives of the three germ layers thus holding a promising approach for cell-based therapies. The generation of human iPSCs i particularly laborious, hence, a human leukocyte antigen (HLA)-typed cell bank of allogeneic human iPSCs could present an alternative to autologous application. However, there are controversies about the immune effects that iPS derived cells may induce post transplantation.
The aim of this study was to define the potential risks of transplanted autologous and allogeneic, HLA-matched human iPSCs and their differentiated progenies by investigating their immunogenicity and immunomodulatory properties.
Undifferentiated and differentiated human iPSCs show low levels of HLA class I expression but no HLA class II and co-stimulatory molecule expression. Allogeneic human iPSCs elicit only a low proliferation of CD4+ or CD8+ T cells compared with primary allogeneic cells. And intriguingly, the proliferation of third party activated T cells could be suppressed by the addition of allogeneic undifferentiated and differentiated hiPSCs. The mechanisms underlying the immunogenic and immunomodulatory effects of human iPSCs are further elucidated within the ongoing studies.
This study possesses important implications to enable a safe transplantation of autologous and allogeneic human iPSCs along HLA barriers for cell-replacement therapy in regenerative medicine.

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Gutha P, Morgan JW, Mostoslavsky G, Rodrigues NP and Boyd AS (2013): Lack of immune response to differentiated cells derived from syngeneic induced pluripotent stem cells. Cell Stem Cell 12(4):407-12

Abstract No. P160

Inhibition of cell death signaling blocks retina regeneration in the murine retina

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Background: Retinal regeneration is evident in non-mammalian vertebrates after injury but the regenerative capacity is restricted in mammals. Recent studies suggested that Müller glia (MG) could be the potential source for neuronal regeneration. Here, we began to study the mechanism that link neuronal cell death and the induction of a potential regenerative program in mouse MG.

Methods: Murine retinas (p10) were explanted, mitogens and EdU were administered to stimulate and monitor the MG proliferation respectively. Two different cell death inhibitor were applied to block the neuronal cell death pathways. Various differentiation factors were used for induction of neuronal differentiation. Retinal cryosection were analyzed using immunostaining and confocal microscopy.

Results: We examined the temporal response of cell proliferation and cell death in retina explant ex vivo. Caspase3+ cells peaked at 48h (82±7 SEM/100µm; N=4) and the majority of Ki67+ cells are also Sox2+ (expressed by all retinal progenitors and MG). Intriguingly, at day 3, the combined application of two cell death inhibitors significantly diminished MG proliferation (Ki67+Sox2+) about 10-fold (0.4 ±0.1 SEM/100µm; N=4) compared to control (4 ±1 SEM/100µm; N=4; $p < 0.03$). Further, by investigating potential MG-derived neuronal regeneration, we observed Otx2+EdU+ (4±0.4 SEM/mm, n=3) cell progeny upon induction of differentiation by various approaches. Further, we confirmed that the potential new Otx2+ progeny is MG-derived using transgenic MG lineage tracing (hGFAP-Cre::RosaRFP mice). Otx2 is expressed by retinal progenitors, which may generate Otx2 expressing bipolar and photoreceptors neurons.

Conclusion: HB-EGF and EGF stimulate MG proliferation ex vivo. Application of defined neuronal cell death inhibitors blocks MG proliferation and reprogramming. A limited number of Otx2 progeny is MG-derived suggesting reprogramming to a progenitor or neuronal phenotype. Thus, we currently investigate (1) the molecular cues that regulate and restrict MG proliferation and stem cell competence and (2) the fate of MG-derived progeny.

Abstract No. P161

In vivo, single cell analysis of progenitor cells' response to injury in the zebrafish telencephalon

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The adult zebrafish has become a widely studied model for neurogenesis and regeneration. Unlike the adult mammalian brain, the adult zebrafish brain contains numerous neuronal progenitors in the neurogenic zones distributed throughout all brain subdivisions. Importantly, these progenitors, radial glia (RG) and their progeny, also become engaged in the regeneration of the injured brain, resulting in the tremendous regenerative capacity of the adult fish brain. As the activation of RG is crucial for the regeneration, we set out to analyze their reaction to stab-wound injury using clonal analysis and continuous live imaging.
We established for the first time in vivo imaging of RG cells in the adult brain that allowed us to follow a single RG cell up to 1 month. The live imaging revealed the increased activation of the quiescent RG cells in response to injury. However, we did not observe an increase in number of divisions within the single RG clone, suggesting the activation of quiescent RG as a mode to increase proliferation. Moreover, we observed migration of the RG progeny towards the injured brain parenchyma, probably engaging in the repair process. Finally, the clonal analysis revealed an increased number of mixed clones containing RG after injury, suggesting a change in the mode of the division of RG and increase of their asymmetric divisions.
Taken together, our data provide for the first time evidence of the single cell behavior of neural stem cells in vivo, suggesting their selective activation and engagement in the repair process.

Abstract No. P162

Adult stem cells in the small intestine are intrinsically programmed with their location-specific differentiation fate

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In mammals, the small intestinal epithelium is highly specialized along the cephalocaudal axis with different absorptive and digestive functions in duodenum, jejunum and ileum that is controlled by several transcription factors such as GATA4. However, so far it is unknown whether location-specific functional properties are intrinsically programmed within stem cells or if continuous signalling from the intestinal environment, such as the mesenchyme or the microbiota is necessary to maintain the location-specific functional properties of epithelial cells.
By using an intestinal organoid culturing technique, we cultured pure epithelial cells derived from location-specific crypts of mice and human to exclude the effect of extrinsic factors. We determined region-specific gene expression profiles after long-term organoid culture. We show that both mouse and human organoids maintain their functional identity corresponding with their original location.
We thus show that location-specific function is intrinsically programmed in the adult stem cells of the small intestine and that their differentiation fate is independent of extracellular signals. In light of the potential future clinical application of small intestine-derived organoids, our data imply that it is important to generate location-specific cultures to regenerate all essential functions of the small intestine.

Abstract No. P163

Development of a human cardiac muscle patch using a three-dimensional scaffold and custom bioreactor technologies

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After myocardial infarction, pathological remodeling processes in the damaged heart can lead to cardiac insufficiency. To date, main shortcomings of innovative therapeutic concepts are the lack of an ideal autologous cell source and robust cellular integration in the host tissue. Promising cell sources for future regenerative applications are adult cardiac tissue-derived cells (hCDCs).
hCDC-containing cardiospheres were isolated according to established protocols from autologous human heart biopsies. hCDCs were either seeded on three-dimensional (3D) decellularized biological matrices or on synthetic electrospun scaffolds that were cultured under static conditions and within bioreactors, exposing the cell-matrix constructs to defined biophysical signals. We further examined the influence of soluble factors produced by mesenchymal stem cells to the CDCs by culturing these two cell types under co-culture. In order to monitor cell phenotypic changes of the cells, cardiovascular-related protein profiles specific cardiac marrkers were screened by immunohistochemistry (IHC).
Our preliminary data highlight the possibility to establish a 3D myocardial tissue patch that can potentially serve as vital implant or in vitro test system for various applications. Exposure to defined biophysical signals allowed the maintenance of functional hCDCs. In future studies, we will focus on further exploring the impact of various culture conditions on cellular integrity and cell phenotype changes employing gene and protein expression analyses. Patch clamp technologies will be utilized to test the functionality of the engineered tissue.

Abstract No. P164

CellFinder: A Cell Data Repository

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CellFinder (http://www.cellfinder.org) is a comprehensive web-based knowledge base and data repository for characterizing different cells, cell types and tissues. Cells constitute natural units of information integration and processing. Cell type- and tissue-specific characteristics in these fundamental cellular processes, including gene and protein expression, metabolism and signalling are of paramount importance for various disciplines, ranging from basic cell biology to systems biology and medicine. CellFinder aims to provide a one-stop platform for the study of the characteristics of single cells by integrating data on gene- and protein expression, both in the spatial context (considering anatomical and structural properties, tissues, organs and organisms) and in the temporal context (considering cell differentiation, and development). CellFinder encompasses original experimental data, like expression values or images, and data extracted from literature by manual curation and text mining.

To date, CellFinder describes 3,394 cell types and 50,951 cell lines. The database currently contains 3,069 microscopic and anatomical images, 205 whole genome expression profiles of 194 cell/tissue types from RNAseq and microarrays and 553,905 protein expressions for 535 cells/tissues. Text mining of a corpus of more than 2,000 publications followed by manual curation added confirmed expression information on approximately 900 proteins and genes. CellFinder's data model is capable to seamlessly represent entities from single cells to the organ level, to incorporate mappings between homologous entities in different species, and to describe processes of cell development and differentiation. Its ontological backbone currently consists of 196,777 ontology terms incorporated from 10 different ontologies unified under the novel CELDA ontology (1).

CellFinder's web portal allows searching, browsing and comparing the stored data, interactive construction of developmental trees and navigating the partonomic hierarchy of cells and tissues through a unique body browser designed for bench scientists, biologists and clinicians.

CELDA - an ontology for the comprehensive representation of cells in complex systems. Seltmann S, Stachelscheid H, Damaschun A, Jansen L, Lekschas F, Fontaine JF, Nguyen-Dobinsky TN, Leser U, Kurtz A. BMC Bioinformatics. 2013 Jul 17;14:228.

Abstract No. P165

Treatment of lung diseases based on pluripotent stem cells

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Lung diseases are among the ten leading causes of death worldwide with still growing incidence. So far, lung transplantation is the only valid therapeutic treatment, which is however limited by the availability of donor organs and immunological complications. Pluripotent stem cells (PSCs), like embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), offer promising therapeutic alternatives to treat respiratory diseases, e.g. cystic fibrosis (CF) by cellular or tissue replacement therapy as well as disease modeling and drug screening.

On that account, we are aiming at an efficient protocol for the differentiation of human (h)PSCs into functional lung epithelial (progenitor) cells. To evaluate the earliest respiratory differentiation steps, we make use of the human (h)ESC (hES3) NKX2.1-GFP reporter cell line (kindly provided by the lab of A. Elefanty) expressing eGFP under the endogenous promoter of NK2 homeobox 1 transcription factor (NKX2.1), known as the earliest marker in lung development. With our current serum-free monolayer-based differentiation strategy we receive around 15 % NKX2.1-eGFP+ cells from hESCs. Preliminary data proof the endodermal origin and the respiratory phenotype of these NKX2.1-eGFP+ progenitors since the thyroidal or neuronal phenotype seem not to be favored. Moreover, first experiments addressing further maturation of NKX2.1-eGFP+ cells demonstrate their potential to differentiate into mature lung epithelial cells.

Additionally, we were able to generate CFTR+ (Cystic Fibrosis Transmembrane Conductance Regulator) cells from hESCs, a cell type affected in CF patients. Characterization of those CFTR+ cells is ongoing as well as the differentiation of mutant and gene-corrected CF-iPSCs into CFTR+ cells.

In summary, first steps have been made towards the efficient generation of NKX2.1+ lung epithelial progenitors and their further maturation as well as the generation of CFTR+ cells. These results provide the basis for cellular PSC-based therapies of respiratory diseases like CF and surfactant deficiencies.

Abstract No. P166

Studying adult neurogenesis using a simplistic niche model

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In recent years the natural phenomenon of adult neurogenesis (i.e. generation of new neurons in adult brain) gained increasing importance as a therapeutic approach to treat neurodegenerative diseases. However, current research efforts are confronted with a complex multivariate system regulating the activity of neural stem cells inside the hippocampal stem cell niche. Thus, ongoing studies are focused to unravel the complex interplay of the different exogenous signals involved in adult neurogenesis.
We utilize a powerful hydrogel platform based on star-shaped poly(ethylene glycol) and heparin that is capable of controlling a wide range of niche parameters independently to allow for screening of the individual exogenous cues. As such, the material allows to vary the stiffness, degradability and ligand density independently. This powerful matrix variability is combined with advanced life cell microscopy and fluorescence expressing neural cell lines. Specifically, we use bActin-GFP for morphology monitoring in three dimensions over time.
By correlating the observed cell behavior with the provided material-based exogenous cues, we hope to gain further insights into the complex regulatory network existent in the neurogenic stem cell niche.

Tsurkan et al. (2013), Defined Polymer–Peptide Conjugates to Form Cell-Instructive starPEG–Heparin Matrices In Situ. Adv. Mater., 25: 2606–2610
Freudenberg et al. (2009), A star-PEG-heparin hydrogel platform to aid cell replacement therapies for neurodegenerative diseases. Biomat., 30 (28): 5049–5060

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Abstract No. P167

DIRECT DIFFERENTIATION OF PATIENT IPS CELLS INTO SELF-RENEWING NEURAL PROGENITORS BY SMALL MOLECULES TO MODEL MITOCHONDRIAL DISEASES

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We report a rapid and feasible method to derive self-renewing neural progenitor cells (NPCs) from human pluripotent stem cells (PSCs). With an approach adapted from Li et al. (PNAS, 2011), NPCs could be obtained for induced pluripotent stem cells (iPSCs) generated from fibroblasts of three patients and control individuals. All patients carry a mitochondrial DNA (mtDNA) mutation in MT-ATP6. Mutations in this gene -encoding a mitochondrial ATP synthase subunit- are associated with neurodegenerative disorders ranging from NARP to Leigh syndrome (LS). Importantly, we were able to generate multiple iPSC clones employing episomal plasmid-based reprogramming and thus avoiding traditional viral-mediated transgene delivery. All patient iPSCs still displayed the same mutational load in mtDNA as their parental fibroblasts, even upon prolonged cultivation.
NPC identity and differentiation potential were confirmed by corresponding marker gene expression. Additionally, patient-derived NPCs were subjected to metabolic analysis, e.g. ATP quantification and Seahorse-based bioenergetic profiling.
In these assessments, we could observe responsiveness of NPCs to dihydrolipoic acid (DHLA), an oxygen radical scavenger currently being tested for the treatment of NARP patients (Couplan, PNAS, 2011). DHLA was capable to improve respiratory activity in patient NPCs to nearly 2-fold and to restore mitochondrial functionality upon conditions mimicking glucose shortage, which represents a potential cause of decompensation in NARP/LS patients. Thereby, a novel method of determining the metabolic state of neural tissue could be established, potentially enabling the study of mitochondrial encephalopathies and unravel their underlying molecular mechanisms.
Overall, NPCs from patient iPSCs represent an inexhaustible source of neurogenic tissue. We previously found that retroviral-mediated reprogramming may result into mtDNA sequence rearrangement (Prigione et al, Stem Cells 2011). Hence, non-viral generation of iPSCs, followed by small molecule-based derivation of NPCs may represent an advantageous strategy to establish faithful neuronal disease models with positional effect-free phenotypes, thus guarantying reproducibility and genomic stability.

Abstract No. P168

Adapting human pluripotent stem cells to high-content and high-throughput screening: challenges and applications in drug discovery

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Human pluripotent stem cells (hPSCs) hold great promise as model systems for studying human development and disease. They also provide specialised cells for regenerative medicine and for 'bioassays' used in early toxicity and efficacy assessment during drug development. These applications imply the optimisation of cost-effective, large-scale cultures of good-quality stem cells and the development of robust, cell-based assays. This includes automated fluid dispensing and miniaturised detection protocols that can be used in high-throughput and high-content screening (HTS/HCS) strategies. We are the pioneers in the adaptation of human pluripotent stem cells to HTS/HCS and will present the challenges and applications of such a technology, with an example of hPSC-assay development for regulators of self-renewal and early differentiation.

High-throughput screening assay for the identification of compounds regulating self-renewal and differentiation in human embryonic stem cells. Desbordes SC et al. Cell Stem Cell. 2008 Jun 5;2(6):602-12
Adapting human pluripotent stem cells to high-throughput and high-content screening. Desbordes SC, Studer L. Nat Protoc. 2013 Jan;8(1):111-30.

Abstract No. P169

Generation of human spastin-deficient neurons to model hereditary spastic paraplegia in vitro

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Hereditary spastic paraplegia (HSP) is a rare, heterogeneous group of genetic disorders with progressive spasticity in the lower limbs caused primarily by axonal degeneration of corticospinal motor neurons. The most frequent type of autosomal dominant paraplegia, spastic paraplegia 4 (SPG4), is found in 40% of all HSP cases. SPG4 is caused by mutations in the SPAST gene, which codes for the protein spastin. Research on HSP is complicated by limited access to patient-derived primary neurons. This obstacle may be overcome by the use of induced pluripotent stem cells (iPSCs). Here we report the generation and phenotypical assessment of iPSC-derived cortical cultures from SPG4 patients. To this end, fibroblasts of family members carrying a specific SPAST nonsense mutation were reprogrammed employing retroviruses or non-integrating Sendai viruses to obtain fully validated SPG4 iPSC lines. In order to enable HSP-specific phenotype analysis, iPSCs were further differentiated into neural cells with cortical phenotypes, which were enriched in glutamatergic neurons expressing the layer V and VI markers CTIP2 and TBR1, respectively. Both the short and the long isoform of spastin were found expressed in these cortical cultures. While spastin levels in SPG4 fibroblasts and iPSCs were comparable to those of control cultures, SPG4-derived neural cells failed to recapitulate the up-regulation of spastin observed during neuronal differentiation of non SPG4-derived cells. At the cellular level, SPG4 cortical neurons displayed impaired neurite outgrowth and formation of prominent axonal swellings, which may indicate impairment of axonal transport. IPSC-derived neurons from SPG4 patients may thus serve as valuable model system for studying pathomechanisms underlying neuronal degeneration in HSP.

Abstract No. P170

Evaluation of dynamic aggregate-based suspension cultures for human pluripotent stem cells

Andreas Elanzew [1], *Annette Pusch* [1], *Annika Sommer* [1], *Daniel Langendörfer* [1], *Simone Haupt* [1], and *Oliver Brüstle* [1]

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Human pluripotent stem cells (hPSCs) provide a fascinating tool for conducting compound screening and pharmaceutical drug development in an authentic cellular system. HPSC-derived somatic cells also represent a valuable cell source for regenerative medicine. These biomedical prospects have created an urgent need for generating large quantities of stem cells under standardized conditions in scalable systems. In our study we developed a new bioreactor mediated (BioLevitatorTM, Hamilton) protocol for the dynamic aggregate-based cultivation of hPSCs utilizing mTeSRTM medium. Our data show that human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSC) can be extensively expanded, yielding a maximum total fold increase of 4,79E+6 across 10 passages. Irrespective of the final cultivation volume (up to 45 ml), a mean of 2,28E+5 cells / ml was reached. In contrast, standard adherent control conditions yielded only an average cell yield of 1,20E+5 cells / ml. HPSCs maintained their proliferation capacity, pluripotency-associated marker-expression and differentiation potential after expansion in the BioLevitatorTM. Recently we further established a bioreactor-based protocol for the expansion of hPSCs utilizing a commercially available GMP-compliant medium. This system yielded a total fold increase of 8,95E+7 after 10 passages, emphasizing the suitability of such a process for the expansion of clinical grade hPSCs. We expect this technology to facilitate the standardized and automated scale-up of hPSCs and their derivatives for further downstream applications including potential cell replacement therapies.

Abstract No. P171

Human iPS cell-based in vitro modelling of metachromatic leukodystrophy

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Metachromatic leukodystrophy (MLD) is caused by a dysfunction of the enzyme arylsulfatase A (ASA). Under physiological conditions ASA acts as a key metabolic enzyme for the lysosomal degradation of the sphingolipid cerebroside sulfate (sulfatide). In patients suffering from MLD, sulfatide is not degraded and accumulates mainly in the myelin-forming cells of the nervous system, i.e. oligodendrocytes and Schwann cells, leading to demyelination and severe neurological symptoms. However, the precise pathomechanisms of MLD are still largely unknown. A human in vitro model based on disease-specific oligodendrocytes could provide a tool for modelling the pathogenesis of the disease on a cellular level. In this study, fibroblasts from four MLD-patients with distinct mutations in the ASA gene were successfully reprogrammed into induced pluripotent stem cells (iPSC) using a Sendai virus system. Subsequently, fully validated MLD-iPSC clones as well as healthy control iPSC clones were converted into radial glia-like neural stem cells (RGL-NSC), which exhibit a robust tripotential differentiation capacity upon growth factor withdrawal. Next, we investigated the protein and mRNA expression of ASA in fibroblasts and in RGL-NSCs and found an abundant expression of the enzyme in both cell types. However, enzyme activity assays clearly confirmed the loss of ASA activity in MLD cells. Since oligodendrocytes represent the key cell type in the course of MLD, RGL-NSCs were specifically differentiated along the oligodendrocyte lineage. In this context sulfatide storage in oligodendroglial cells was investigated using a specific antibody to sulfatide. Interestingly, major sulfatide depositions were specifically associated with O4-positive immature oligodendrocytes. In this context, we are currently analysing control and patient cells in a comparative fashion. Eventually, we expect this MLD model system to provide novel insight into the molecular pathogenesis of MLD and to serve as a tool to assess potential therapeutic strategies.

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Abstract No. P172

Efficient transduction of adipose tissue-derived stem cells with noncytpathic alphaviral vectors

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Mesenchymal Stem Cells (MSCs) are promising candidates for cell-based therapy to treat several diseases. The efficient and safe therapeutic transgene delivery into MSCs is one of the major problems due to the low efficacy of pDNA transfection and possible genotoxicity of non-regulated transduction with commonly used recombinant retroviruses. Here we suggest using alphaviral vectors as efficient and safe gene transfer system for MSCs. Alphaviruses are enveloped viruses containing a positive-strand RNA genome replicating in cell cytoplasm independently on nuclear function. In this study adipose tissue derived stem cells (ADSC) were isolated from human (healthy donor) and dog (English Beagle) adipose tissue aspirates. The cell stemness properties were confirmed by positive CD29, CD44, CD73, CD90, CD105 and negative CD19, CD34, CD45 and HLA-DRA markers, as well as by cell differentiation towards the osteogenic, hondrogenic and adipogenic pathways. Then cells were used to show the transduction efficiency by different alphaviral vectors based on Semliki Forest virus (SFV) and Sindbis virus (SIN) replicons. The ADSCs were infected with cytopathic SFV/DS-Red, Sin-Rep5-GFP vectors and noncytopathic SFV-PD-GFP and Sin-nspSer-LacZ vectors. The transgene expression was analyzed by FACS and microscopy analysis during 6 days post infection. The strong cytopathic effect was observed in both human and canine ADSCs types after infection with SFV/DS-Red and Sin-Rep5-GFP; moreover, cells were not able to restore the proliferation. In the case of noncytopathic alphaviral vectors, the mild cytostatic effect and high transgene expression level during up to 6 days was observed with transduction efficiency 30-40%. Therefore, noncytopathic alphaviruses are effective tools for gene delivery and prolonged transgene expression in ADSCs.

Abstract No. P173

THE IMPACT OF INDIVIDUAL'S IMMUNE REACTIVITY ON HUMAN MSCs AND ENDOGENOUS BONE REGENRATION

Sven Geissler [1], *Simon Reinke* [1,*], *Kathrina Schmidt-Bleek* [1], *Kerstin Juelke* [1], *Tony Hartwig* [1], *Hans-Dieter Volk* [1], and *Georg Duda* [1]

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Mesenchymal stromal cells (MSCs) are crucial for the regeneration of mesenchymal tissues. Furthermore, there is growing evidence that adaptive immunity contributes to endogenous regeneration processes. For example, endogenous bone fracture repair is modulated by T-cells even in the absence of infection. Because delayed or incomplete fracture healing is associated with poor long-term outcomes and high socioeconomic costs, we investigated the relationship between an individual's immune reactivity and healing outcome.

Our study revealed that delayed fracture healing significantly correlated with enhanced levels of terminally differentiated CD8+T-cells (CD8+TEMRA) in the peripheral patient's blood. This difference was long-lasting and reflects rather the individual's immune profile than a post-fracture reaction. Moreover, we could show that CD8+TEMRA migrate and accumulate in the human fracture hematoma and are locally the major producers of IFN-γ/TNF-α. We found that this high cytokine production of the CD8+TEMRA cells can not be modulated by the immunoregulatory activity of MSCs. In contrast, conditioned media of sorted and ex vivo stimulated CD8+TEMRA cells inhibit osteogenic differentiation and survival of human MSCs. The addition of neutralizing antibodies either against IFN-γ or TNF-α only slightly increased matrix mineralization, while the combination of both almost completely restored MSC survival and differentiation ability. The potential causal relationship between the enrichment of memory CD8+TEMRA and the pathogenesis of poor bone fracture healing, was further investigated in a mouse model. Depletion of CD8+TEMRA resulted in enhanced endogenous fracture regeneration and bone quality, whereas a transfer of CD8+TEMRA cells impaired the healing process.

Our data demonstrates the high impact of chronically activated adaptive immunity on adult stem/progenitor cell function and endogenous regeneration processes even in the absence of any infections. These results might open new opportunities for early and targeted intervention strategies to restore or enhance endogenous regeneration capacity.

Abstract No. P174

Investigating the translational landscape of mTOR signaling in hESC-derived models of autism

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Autism spectrum disorders (ASDs) comprise a range of complex developmental syndromes with significant unmet medical needs. Evidence indicates that molecular defects in autism interfere with the mechanisms of synaptic protein synthesis due to hyperfunction of the mechanistic target of rapamycin (mTOR) kinase pathway. Multiple syndromes on the autism spectrum are in fact caused by dominant mutations in negative regulators of mTOR, yet it remains elusive what subsets of mRNAs are translationally controlled and how mTOR hyperfunction underlies neuronal cellular and network dysregulation in autism.

In order to unravel autism-relevant gene expression networks controlled by mTOR at the post-transcriptional level we use a new powerful technique termed ribosome profiling, which enables global analysis of actively translated mRNAs and generates genome-wide maps of protein synthesis. Based on deep sequencing of ribosome-protected mRNA fragments, “ribo-seq” provides quantitative data on the translational state on a transcriptome-wide scale.

We employ ribosome profiling to uncover specific translational networks of genes in human pluripotent stem cell (hPSC) models of ASDs. These models include neurons differentiated from patient-derived induced PSCs (hiPSCs) and genome edited hPSCs with mutations in the TSC2 gene, a key negative regulator of mTOR signaling. In addition, we use known pharmacological inhibitors of the mTOR pathway to test whether pathophysiological gene translation signatures in these models can be reverted to normal.

In conclusion, by combining state-of-the-art protocols for cellular modeling of autism with cutting-edge deep sequencing technology we have the possibility to address the translational dysfunction underlying autism, which has the potential to open new avenues for pharmacological intervention in ASDs.

Abstract No. P175
Mesenchymal Stem Cells – Mediators of Bone Metastasis?

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Some cancers, such as prostate or breast cancer, show a strong tendency to metastasize to bone, a tissue of mesenchymal origin and a prominent site of mesenchymal stem cells (MSC) in an adult. Recent reports have suggested that bone-metastasizing cancers may mimic the process of homing of hematopoietic stem cells to their bone niche, in which MSC play a crucial role. In light of the growing awareness that MSC play a potentially important role in cancer, we aim to dissect the interaction and the dynamics between tumor cells and MSC in metastasis formation.
We found that prostate cancer cell lines, which are published to form bone metastasis in mouse models, show a characteristic migration pattern towards MSC, displaying dose dependency along a protein-based chemotactic gradient. This effect was stronger towards naïve MSC compared to MSC undergoing differentiation, fibroblasts and other cell types tested. This indicates that specific molecules secreted by naïve MSC are responsible for stimulating migration.
We have established a cell-based assay to measure migration of cancer cell lines towards signals emitted by primary bone marrow derived human MSC. It is based on trans-well migration chambers combined with impedance measurements for quantitative measurements of cell migration in a time-resolved fashion and high-throughput format. In order to investigate the secretome of MSC in an unbiased fashion we have generated a cell culture regime to culture MSC under protein-free and chemically defined conditions, after which the cell culture supernatant is subjected to fractionation by ion exchange as well as size exclusion chromatography. Chromatography fractions harboring chemotactic activity towards the cell lines investigated are currently analyzed by mass spectrometry to reveal the high molecular protein complex responsible for the observed migratory interaction between MSC and cancer cell lines harboring the potential to metastasize to bone.

Abstract No. P176

Modulation of sodium channel expression in human Dravet syndrome-specific neurons

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Dravet syndrome (DS) is a severe neurological disorder characterized by congenital epilepsy, which is mostly caused by haploinsufficiency of Nav1.1, a pore-forming α-subunit of voltage-gated sodium channels. To model the disease in patient-specific neurons, we generated DS-specific human induced pluripotent stem cells (hiPSCs) and derived long-term self-renewing neuroepithelial stem (lt-NES) cells to establish a stable, expandable neural precursor population. Upon growth factor withdrawal, DS lt-NES cells differentiate into neurons that show reduced Nav1.1 expression in comparison to healthy controls. Recent work suggests that the intracellular domain of an accessory channel subunit (β2) acts as an endogenous regulator of Nav1.1. We set out to explore whether ectopic β2-ICD expression is able to elevate Nav1.1 levels in human DS-specific neurons. To this end, a patient-derived lt-NES cell line was transduced with a lentiviral doxycycline-inducible construct coding for β2-ICD fused to GFP via a 2A peptide and sorted for GFP+ cells to near purity. Autocatalytic construct cleavage could be confirmed by Western blot analysis, and nuclear co-localization of transgenic β2-ICD could be verified by 3D microscopy. The β2-ICD transgenic cell line maintained expression of typical neural stem cell markers such as Dach1, Nestin, PAX6, PLZF, SOX1, SOX2 and ZO-1 and was able to differentiate into GABAergic neurons, the most relevant neuronal subtype in DS. After doxycycline-induced transgene expression a significant increase in Nav1.1 protein levels was detected in 6-week-old neuronal cultures by Western blot analysis compared to non-induced cultures. These results indicate that β2-ICD acts as regulator of cellular sodium channel homeostasis in human neurons and that its ectopic expression may counteract Nav1.1 deficiency in DS-specific neurons.

Abstract No. P177

Mesenchymal Stem Cell Conditioning Promotes Rat Oligodendroglial Cell Maturation

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In demyelinating diseases such as multiple sclerosis myelin repair activities based on recruitment, activation and differentiation of resident progenitor and stem cells can be observed. However, the overall degree of successful remyelination is limited. It is therefore of considerable interest to understand oligodendroglial precursor cell (OPC) homeostasis and maturation processes in order to develop remyelination therapies. Mesenchymal stem cells (MSC) were shown to exert positive immunomodulatory effects, to reduce demyelination, to increase neuroprotection and to promote adult neural stem cell differentiation towards the oligodendroglial lineage. We here addressed whether MSC secreted factors can influence primary OPCs in a myelin non-permissive environment. To this end we analyzed cellular morphologies, expression and regulation of key genes/proteins involved in oligodendroglial cell fate and maturation upon incubation with mesenchymal stem cell conditioned medium. This demonstrated that MSC derived soluble factors promote and accelerate oligodendroglial differentiation, even under astrocytic endorsing conditions. Accelerated maturation featured elevated levels of 2', 3'-cyclic nucleotide 3'-phosphodiesterase and myelin basic protein expression, reduced glial fibrillary acidic protein expression and was accompanied by downregulation of prominent inhibitory differentiation factors such as ID2 and ID4. We thus conclude that besides the previously established immunomodulatory and neuroprotective roles of MSCs these cells can also positively influence oligodendrogenesis in the adult central nervous system.

Jadasz JJ, Kremer D, Göttle P, Tzekova N, Domke J, et al. (2013) Mesenchymal Stem Cell Conditioning Promotes Rat Oligodendroglial Cell Maturation. PLoS ONE 8(8): e71814. doi:10.1371/journal.pone.0071814

Abstract No. P178

Derivation and maintenance of murine trophoblast stem cells under defined conditions

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Trophoblast stem (TS) cells are in vitro equivalents to the precursor cells of the placenta. TS cells are cultured in serum-rich medium with fibroblast growth factor 4, heparin and embryonic fibroblast conditioned medium. Here, we developed a simple medium consisting of ten chemically defined ingredients for culture of TS cells named TX medium. Gene expression and DNA methylation profiling demonstrated the faithful propagation of expression profiles and epigenomic characteristics of TS cells cultured in TX. Further, TX medium supported the de-novo derivation of TS cell lines. Finally, TS cells cultured in TX differentiate into all derivatives of the trophectodermal lineage in vitro, give rise to hemorrhagic lesions in nude mice, and chimerize the placenta indicating that they retained all hallmarks of TS cells. The fact that TX media formulation no longer requires fetal bovine serum and conditioned medium facilitates and standardizes the culture of this extraembryonic lineage in vitro. Using such a system will help in drug testing and modeling of placental disorders such as preeclampsia and HELLP syndrome.

Abstract No. P179

Embryonic stem cells carrying a transgenic BMP-reporter construct: A useful tool for the identification and analysis of teratogenic compounds in vitro

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Embryonic stem cells (ESC) are used as a tool for the identification of teratogenic activities in the analysis of chemicals or pharmaceuticals. In particular, the embryonic stem cell test (EST) has been scientifically validated some time ago and is now used for screening purposes. The differentiation of embryonic stem cells in various tissues is regulated by a set of essential signalling pathways, including the TGFß, Wnt, and Shh pathway as well as tyrosine kinase receptors mediating FGF or EGF signalling. For some of these essential signalling pathways, transgenic reporter mice have been developed that allow the in vivo analysis of pathway activity during embryonic development.
We have isolated and characterized embryonic stem cells from transgenic mice that carry a GFP transgene under the control of a BMP response element that has been shown to nicely recapitulate in vivo BMP activity (Monteiro et al, 2008). Our results show that the activity of the reporter gene can be used for the detection of teratogenic activities in vitro. In addition these cells provide a useful tool to characterize the molecular mechanism underlying the activity of chemicals or pharmaceuticals on the differentiation process during early embryonic development.

Monteiro, R.M., S.M.de Sousa Lopes, M.Bialecka, B.S.de, A.Zwijsen, and C.L.Mummery. 2008. Real time monitoring of BMP Smads transcriptional activity during mouse development. Genesis. 46:335-346.

Abstract No. P180

Highly efficient generation of neural stem cells from human pluripotent stem cells

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Human pluripotent stem cells (hPSCs) are an excellent resource for studies of cell fate specification, disease modeling and drug screening. In order to produce various neural cells from hPSCs, the induction to neural stem cells (NSCs) is the first important step. Conventional methods of NSC derivation involve embryoid body (EB) formation or co-cultures with stromal cell lines, which has several disadvantages such as long duration, difficulty to control the quality of derived NSCs and contamination with non-neural cells. To address the need for an easy and scalable process to generate NSCs, we have developed a neural induction medium, which can convert hPSCs into NSCs in one week with 80-90% efficiency but without the time consuming laborious processes of embryoid body formation and mechanical NSC isolation. To confirm the phenotype of derived cells, cells were stained with antibodies against the pluripotent marker Oct4 and neural markers including Sox1, Sox2 and Nestin.

Abstract No. P181

Epigenetic switch from transient drug-induced transcriptome responses to disturbed neurodevelopment from human embryonic stem cells

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Pluripotent stem cells are used increasingly as model system for early human development and to study potential disturbances by chemicals or other external factors. Transcriptomics and other systems-wide approaches have been used to gain information from such model systems. However, the evolution of changes in a cell differentiation system under the influence of chemicals or other stressors has never been investigated. Therfore, the significance of toxicogenomics data has remained unclear in the field of developmental toxicology. We addressed this question here in a comprehensive way. Using histone deacetylase inhibitors as model drugs, we examined neural differentiation of human embryonic stem cells to define the information provided by transcriptomics and to uncover the potential role of epigenetics in persistent drug effects. Normal differentiation proceeded in waves of gene expression changes, and pulsed exposure to trichostatin A or valproic acid triggered a distinct, but reversible change of histone acetylation and transcriptome alteration. Continuous drug exposure for 6 days lead to disturbed neurodevelopment associated with a characteristic pattern of transcriptome changes. Data from drug washout and pulse-chase experiments suggest that the final transcriptome changes after continuous drug treatment describe the altered cellular phenotype, but not chemical-induced signals. Analysis of histone acetylation confirmed that late transcriptome changes did not correlate with primary drug action. Moreover, short increases of histone acetylation were not sufficient to trigger disturbances of development. Instead, secondary histone methylation was identified as a potential persistence detector deciding on reversibility or adversity of drug exposure of different duration.

Abstract No. P182

Modeling Familial Motor-Neuron Disease (SPG11) Using Human Induced Pluripotent Stem Cells

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Hereditary spastic paraplegias (HSPs) are a heterogeneous group of monogenetic neurodegenerative disorders characterized by progressive lower limb spasticity. They are classified genetically by autosomal dominant, autosomal recessive and X-linked HSP. Partly due to lack of a good disease model, the underlying molecular mechanisms has not been studied in detail. So the overall aim of our research work is to generate a cellular human model for HSP using human induced pluripotent stem cells (hiPSCs) and to investigate neuronal pathology associated with this disease. Fibroblasts samples from clinically and genetically well characterized patients suffering from the most abundant autosomal recessive form of HSP (affecting the SPG11 gene) and age and sex matched controls are reprogrammed to hiPSCs. The reprogrammed cells or induced pluripotent stem (iPS) cells are then differentiated into different types of functional neurons, among them corticospinal motor neurons. The main focus is to elucidate the molecular mechanisms that leads to the development of disease in the patients.

Stevanin, G., et al., 2007. Mutations in SPG11, encoding spatacsin, are a major cause of spastic paraplegia with thin corpus callosum. Nat. Genet. 39, 366–372
Arlotta P, Molyneaux BJ, Chen J; et al. Neuronal subtype-specific genes that control corticospinal motor neuron development in vivo. Neuron 2005;45: 207-221.

Abstract No. P183

IPSC-based Modeling of Nijmegen Breakage Syndrome

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Nijmegen breakage syndrome (NBS) is a rare, autosomal, recessive genetic disorder first described in 1981 in patients living in Nijmegen, Holland. NBS patients display a characteristic facial appearance, microcephaly and a range of symptoms including elevated sensitivity to ionizing radiation, chromosome instability, a high frequency of malignancies, accelerated shortening of telomeres, abnormal cell cycle checkpoints, growth retardation and immunodeficiency. The clinical features overlap with those of Ataxia-telangiectasia, Ligase IV syndrome, Non-homologous end-joining factor 1 (NHEJ1) syndrome and Fanconi anemia.

We hypothesized that inducing pluripotency in NBS fibroblasts might by-pass ROS-mediated genome instability. To test this, we performed the following, a) Reprogrammed fibroblasts, derived from NBS patients, into iPS-cells (NBS-iPSC) as an in vitro model of the disease. b) Compared the transcriptomes of four NBS patient derived dermal fibroblasts to healthy foreskin fibroblasts (HFF1) in order to uncover molecular features and etiology of the disease. c) Compared the transcriptomes and stress responses of undifferentiated ES cells (H1 and H9), NBS-iPSCs and HFF-iPSCs.

Our findings are: All iPSCs expressed pluripotency associated proteins (Alkaline phosphatase, OCT4, NANOG, TRA1-81, TRA1-60, SSEA4) and pluripotency was further confirmed both in vitro (EB assays) and in vivo (teratoma formation). Comparative transcriptome and associated pathway analyses revealed (a) that NBS fibroblasts compared to healthy HFF1 seem to have a high impact on cell cycle regulation, apoptosis, p53 signalling and the Fanconi Anemia pathway. (b) The comparisons between ES cells, HFF-iPSCs and NBS-iPSCs revealed regulated genes and pathways associated with DNA replication, glycolysis, pyrimidine, fructose and mannose metabolism as well as DNA repair related pathways. Interestingly all of these pathways are known to be associated with ROS homeostasis.

(c)NBS-iPSCs retained a set of genes related to B cell receptor signaling pathway, ion transport, cell adhesion and others, not seen in ESCs and HFF-iPSCs.

Abstract No. P184

Modeling PMM2-CDG via generation of induced pluripotent stem cells (iPSCs) from PMM2-CDG patient´s fibroblasts

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PMM2-CDG, former known as Congenital Disorder of Glycosylation type Ia (CDG-Ia), is the most abundant form of the genetically and phenotypically heterogeneous CDGs which mainly impact the N-glycosylation machinery of the cell. N-glycans are indispensable for correct folding and stability of proteins, implicating great importance for both inter- and intracellular signaling. A total loss of N-glycans is lethal. Therefore type I CDGs genetically manifest as hypomorphic alleles not complete knockouts which are mostly associated with a reduction of the respective enzymatic activity resulting in hypoglycosylation of proteins. Clinical symptoms are broad and involve many organ systems especially the brain, liver, immune system and heart leading to psychomotor retardation and cardiomyopathy, for example. Currently, no options for treatment exist [1].

To study the impact of a reduced PMM2-activity on early developmental processes, induced pluripotent stem cells (iPSCs) from patients fibroblasts, carrying 10 % residual PMM2-activity were generated by reprogramming [2].

Morphologically, the generated patient specific PMM2-iPSCs displayed round and even colonies. Real-time PCR analysis and fluorescence microscopy revealed similar expression levels of the classical pluripotency markers OCT-3/4, NANOG and SSEA-4 as well as early differentiation markers as PAX6 (ectoderm), T (mesoderm) and SOX17 (endoderm) compared to healthy controls. Global gene expression profiling by deep-sequencing revealed close similarity to wild-type iPSCs and human embryonic stem cells.

Significant chromosomal abnormalities of the generated iPSCs were excluded after comparative genomic hybridization analysis (Array CGH). Vector copy number analysis revealed only two integration sites suggesting minimal perturbances of gene expression profile.

Initial lectin-blots of whole cell lysates of PMM2-iPSCs showed hypoglycosylation compared to wild-type iPSCs and human embryonic stem cells implicating a disease manifestation already on the embryonic level.

This model will create an important platform to dissect the effects of glycosylation in pluripotent stem cells and development.

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Abstract No. P185

Expansion and terminal hepatic differentiation of hESC-derived defenitive endoderm cells population

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Most applications of pluripotent stem cell-derived hepatic cells in disease modeling or drug screening harbour the need of high amounts of differentiated cells. Therefore, the generation of expandable multipotent progenitor cells derived from patient-specific pluripotent cells is of high interest for various applications in disease modelling aiming for new therapeutic strategies.

In our study we compared in a first step human ESC-derived definitive endodermal cells with cells that were expanded for one or two passages. In our expansion experiments, we analyzed a bulk population after a cytokine directed differentiation protocol for definitive endoderm and a sorted cell population (CD117+ / CD189+). The cells were analysed prior to and after the expansion phase for the endodermal markers Sox17 and FoxA2 as well as for the foregut endodermal and early hepatic markers GATA4, AFP and Hhex. In a second step these cells were further differentiated into hepatic cells. The maturation status of hepatic cells generated without, after one, or after two passages was determined by mRNA analyses of the hepatic marker genes Albumin, TTR, AFP, HNF4. Furthermore CyP1A2 activity (EROD assay) and Albumin secretion (ELISA) as well as subcellular localization of hepatic drug transporters was analyzed to asses the functional level of the respective hepatic cells.

Our results suggest that the expansion of endodermal progenitors in vitro is feasible, but reduces the differentiation capacity of these cells. Cells expanded for only one passage show a higher differentiation capacity than those expanded for two passages. Therefore, further improvements of the expansion protocol need to by studied, which aim for a more robust generation of an expandable endodermal cell population.

Abstract No. P186

Novel Neural Induction Method For Efficient Generation Of Neural Stem Cells Derived From Parkinson's Disease Patient-Derived Sample iPSC Lines

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Parkinson's disease (PD) is one of the most common neurodegenerative disorders affecting a million people in the U.S. alone, with 50,000 Americans being diagnosed with PD each year. The absence of physiologically relevant cellular models for PD represents a major bottleneck for PD research. Novel models are urgently needed to accelerate the discovery of disease mechanisms and drug targets and for screening purposes which could rapidly translate into a wide range of clinical and therapeutic applications. Patient-specific iPSC-derived cell types have become an attractive tool for disease modeling in vitro.

For neuronal differentiation, one commonly used approach is embryoid body (EB) formation followed by neural rosette isolation and expansion. This approach can generate neural stem cells (NSCs) which can differentiate into different neuronal cell types and glia and can be cryopreserved for further maturation. The current limitation is that the process is laborious, inefficient, and the cells usually need to be further purified. To overcome these limitations, we developed a novel neural induction method that allows for the generation of NSCs from iPSCs within 7 days without the need for EB formation. In this study, we differentiated 4 PD iPSC lines and 2 age-matched control lines into neural stem cells using a novel neural induction/expansion media to iPSCs from an adherent monolayer on different matrices or feeder cells. We demonstrate that the generated NSCs are karyotypically normal, and express known NSC markers: Nestin, Sox1 and Sox2. Furthermore, gene expression analysis distinguishes these NSCs from their parental iPSCs and fibroblasts, and clusters them together with control NSCs derived from H9 ESCs.

In summary, the novel neural induction medium allows for efficient and robust generation of NSCs from PD patient derived sample iPSCs and has the potential for large scale NSC generation to be utilized for HT/high content screening and drug discovery.

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Abstract No. P187

Transgenic BRAFV600E induces serration and stem cell loss in the mouse intestine, which is rescued by β-Catenin activity

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Sessile serrated adenomas (SSAs) represent benign precursors of a sizable fraction of all colon cancers. They are defined by their morphology and by distinct patterns of genetic alterations, for instance BRAFV600E mutations which frequently occur early in SSA. Subsequently, mutations activating the Wnt/β-Catenin pathway appear. How these mutations interact functionally is largely unknown. We therefore investigated signalling pathways controlling cell fates during SSA initiation. To this end, we engineered a mouse with an inducible BRAFV600E transgene, and analysed its effects on the intestinal epithelium in vivo and in organotypic culture. Transgenic BRAFV600E expression resulted in high levels of mitogen-activated protein kinase (MAPK) signalling, activation of MAPK target genes and rapid development of generalized serrated dysplasia. Unexpectedly, BRAFV600E induction also resulted in the conversion of intestinal stem cells (ISCs) to short-lived progenitors. Loss of ISCs was prevented by the activation of the Wnt/β-Catenin pathway, using a small-molecule inhibitor of GSK3beta or transgenic expression of stabilized β-Catenin. Our results indicate that strong and generalized MAPK signalling directly establishes multiple key features of advanced SSA, but is not compatible with the maintenance of ISCs in the absence of elevated β-Catenin activity. This suggests that genetic and epigenetic alterations activating the MAPK and Wnt/β-Catenin pathways must be co-ordinately selected to assure stem cell maintenance during serrated tumour progression. BRAFV600E-induced loss of stem cells could represent a novel fail-safe mechanism protecting intestinal tissue from oncogene activity.

Abstract No. P188

Investigations of cell-cell-interactions between mammary tumor cells and mesenchymal stem cells in in vitro and in vivo co-culture systems.

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Mesenchymal stem cells (MSCs) are known to localize to breast carcinomas, where they integrate into tumor-associated stroma. In recent years, the importance of tumor stroma for cancer initiation, development, local invasion and metastases has become increasingly clear. In this study, we addressed the influence of MSCs on the in vitro and in vivo growth, engraftment, proliferation, differentiation and vascularisation of breast cancer cells.
The breast cancer cell line MDA-MB231 (mammary ductal adenocarcinoma) was cultured alone as well as along with human adipose tissue derived MSCs in vitro and changes in morphology were monitored. Subsequently, alterations of protein and gene expression were investigated by flow cytometry and RT-PCR. Furthermore, MDA-MB231 cells were injected alone and together with MSCs subcutaneously and orthotropically into immunodeficient mice. The tumor growth along with metastasis was observed, tumors were surgically remove and changes in protein and gene expression were determined by flow cytometry and RT-PCR.
In vitro, human MDA-MB231 mammary ductal adenocarcinoma cells interact with human adipose tissue derived MSCs whereas MSC lose their stem cell character and differentiate. RNA expression indicates the formation of human endothelial cells and cancer-associated fibroblasts. In vivo, tumor growth and cell dissociation (metastasis) of MDA-MB231 cells is moderately stimulated though the addition of MSCs. During in vivo co-culture, MSCs lose their stem cell character and differentiate. First results indicate a possible contribution of human MSCs in the formation of tumor stroma.

Abstract No. P189

Neuronal and glial cell culture models derived from iPS cells for studying pathological mechanisms of Parkinson's Disease associated LRRK2 mutations

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Mutations in Leucine-rich repeat kinase 2 (LRRK2) have been identified to be strongly associated with familial Parkinson's disease (PD).
Recently, a number of studies used neuronal differentiation of iPSCs, derived from PD patients carrying LRRK2 mutations to investigate possible disease mechanisms. Using this approach, changes in neurite length and architecture [1,2], susceptibility to oxidative stress [2–5] as well as autophagy and proteasomal lysis [1,6,7] were revealed. These results, though, are based on few different cell lines, lacking isogenic controls. We plan to study pathological mechanisms of PD using differentiated neuronal cells, derived from isogenic hiPS cell lines. For this purpose we will insert a G2019S point mutation into non-mutant iPS cell lines using zinc-finger nucleases.
The original and the genetically engineered cell lines will be differentiated into neuronal cells, preferably dopaminergic neurons, to create an in vitro PD model. It has been reported, that R1441C mutation in LRRK2 leads to impaired dopamine transmission in mice [8]. Based on that, the influence of the G2019S LRRK2 mutation will be studied by comparing the electrophysiological properties of the differentiated neuronal cells.
As PD is an age-related disease we are going to study aging of our hiPSC-derived neuronal cells. In order to keep the differentiated neurons long enough in culture, the cells will be supported with astrocyte-conditioned medium or co-cultured with astrocytes.
Various groups have shown an involvement of LRRK2 in the immune system, especially in microglial activation [6,9-11]. Thus, iPSCs will be differentiated into macrophages/microglia. After the characterization and comparison of the phenotypes, these cells, co-cultured with the iPSC-derived neurons could serve as a model system to study LRRK2-dependent neuron-intrinsic and microglia-mediated neurotoxicity, respectively.
Ideally, a combination of hiPSC-derived neurons, astrocytes and microglia could serve as a mini-brain in the dish and be as close to a valid PD model as possible.

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Abstract No. P190

Modeling tuberous sclerosis pathophysiology using human pluripotent stem cell derived neurons

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The mTOR pathway is implicated in several aspects of CNS development and physiology, including regulation of neuronal maturation and synaptic plasticity. Several neurodevelopmental disorders which are linked to intellectual disability, autism spectrum disorders (ASD) and epilepsy are caused by mutations in genes implicated in the mTOR pathway, leading to mTORC1 hyperfunction and excessive protein translation. TSC1 and TSC2 code for the negative mTOR regulators hamartin and tuberin, respectively and heterozygous loss-of-function mutations of either gene causes tuberous sclerosis, a multi-system disorder with high prevalence of ASD. Using zinc finger nuclease-mediated genome editing, we have generated human embryonic stem cell (hESC) lines with heterozygous and homozygous ablation of TSC2. In order to identify morphological and functional defects in TSC2-deficient neurons, we have developed a protocol to differentiate rosette-derived neuroepithelial precursor cells towards neuronal cultures with functional inhibitory and excitatory synapses. TSC2-/- NSCs show a highly proliferative phenotype and are less prone to differentiation, even though functional neurons can be derived. We are now establishing a high content screening assay to study early aspects of neurogenesis, namely proliferation and differentiation.
We envision that these human cellular models of neuronal networks will help to elucidate the role of mTOR signaling in neurogenesis.

Abstract No. P191

Stem cell transplantation in mice for the detection of engraftment and cell differentiation: useful system for embryotoxicity testing

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Objective:
Potential application of embryonic stem cells require methods for testing their engraftment, distribution and differentiation in vivo. In vivo imaging methods (e.g. bioluminescence) were developed as procedure to determine the biodynamic of transplanted stem cells, organ engraftment and to follow in vivo distribution. The validated transplantation method was used to establish a teratoma formation test as a basis for testing embryotoxicity of drugs.
Methods:
Transfected 7AC5/FF (7AC5/Luc) cells were injected subcutaneously, intrahepatically, intrasplenically and intravenously into immunodeficient NOD/SCID mice. Luciferase signal of these cells was determined by imaging of the animals at different points in time. For the establishment of the embryotoxicity system drugs with different embryotoxic potential (non - saccharose, weak – valproic acid, strong – 5-fluoruracil (5-FU)) were investigated in the teratoma model. 7AC5 stem cells and derived teratomas were analysed concerning their expression profile of pluripotency and germ layer markers by RT-PCR, immunofluorescence and FACS analysis. Additionally, teratomas were histopathologically assessed with a focus to the three germ layers.
Results:
After transplantation of 7AC5/Luc into immunodeficient mice increasing luciferase signals were measured over time. After subcutaneous, intrahepatic and intrasplenic transplantation a signal could only be observed at the application site. After intravenous application an enrichment of cells and teratoma growth was detected in the lung and in the bone. Histological analysis revealed teratoma growth and germ layer structures. Expression profile of teratomas showed markers of the three germ layers and lower levels of pluripotency markers. 5-FU treatment reduced significantly tumour growth over time. Derived tumours showed germ layer structures.
Conclusion:
Stable transfection of 7AC5/FF cells with luciferase was established. Using in situ by bioluminescence, the kinetics of cell growth of transplanted cells after different application routes can be followed in vivo. Cytotoxic activity of reference drugs could be determined in vivo with the established screening system.

Abstract No. P192

Genome Editing of α-Synuclein in iPSCs from a Donor with Multiple System Atrophy

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Multiple system atrophy (MSA) is a neurodegenerative disorder of primarily glialorigin. Clinically it is distinct from Parkinson's disease with predominantly autonomic failure and motor impairment. Pathologically, the principal cellular targets are oligodendrocytesthat show abundant glialcytoplasmicinclusion bodies consisting of -synuclein(SNCA) aggregates and nigrostriataldegeneration. Protein misfoldingand aggregation of alpha-synucleinis a common feature across synucleinopathiesand an attractive target for drug development.In order to study the contribution of -synucleinto the disease phenotype of MSA, we generated induced pluripotentstem cells (iPSCs) from a donor with MSA and then created isogeniclines with SNCA deletions using the GeneArt®Precision TAL-nuclease fusion technology. We designed two sets of TALEs to create deletions in exon2 of the SNCA gene in patient derived iPSCsvia non-homologous end-joining (NHEJ). After functional verification of the SNCA TALENs carried out in HEK and U-2 OS cells, and confirmed with the Surveyor assay and Sanger sequencing, iPSCsfrom the MSA donor were edited with SNCA TALENs. Colonies that showed positive cuts in the Surveyor assay were picked and expanded. These colonies were further characterized by next gen-sequencing on the Ion Torrent PGM instrument to identify clonalpopulations with SNCA deletionalmutations. The edited clones with SNCA heterozygous deletion mutation are karyotypicallynormal and express pluripotencymarkers. These clones are in the process of re-editing to generate homozygous deletions, after which they will be further differentiated into appropriate cell types for phenotypic studies. In conclusion, TAL technology can be applied to generate isogenicdisease iPSCsfor studying the contribution of SNCA in the disease phenotype of MSA.

Cell. 2011 Jul 22; 146(2):318-31.
Nat Rev Neurol. 2013 Jan; 9(1):13-24.

Abstract No. P193

IN VITRO DIFFERENTIATION OF MURINE EMBRYONIC STEM CELLS INTO INTESTINAL CELLS

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In our study, we aim to generate embryonic stem cell (ES)-derived intestinal epithelial cells to use them as in vitro model for various biomedical applications. These cells provide an alternative cell source, which can be used for studying aspects of (tumor) stem cell biology or to build up organotypic tissue cultures as preclinical tool e.g. in absorption or toxicity studies.
Murine ES cells (CGR8) were stimulated with activin, gsk-3 inhibitor and dorsomorphin to form definitive endoderm (DE). For hindgut induction we used FGF and GSK-3 inhibitor and subsequently formed organoids were cultured in matrigel. Final maturation was achieved by addition of EGF, Noggin and R-spondin. Cells were characterized on mRNA and protein level using qPCR, immunocytochemistry and flow cytometry. Markers were sox17 foxa2 (DE), cdx2, klf5, cxcr4 (hindgut specification) and villin, cdx1, Bmi1, Mucin2, chromogranin A, IAP, lysozcyme, E-cadherin, ZO-1 (intestinal differentiation).
Treatment with GSK-3 Inhibitor, Dorsomorphin and Activin led to a population expressing markers for DE (Sox17 and Foxa2) and loss of embryonic markers (Oct4, Sox2, nanog). FACS analysis revealed a Sox17+/Foxa2+ population of about 20%. Further specification to hindgut cells was confirmed by up-regulation of cdx2. Long-time culture of intestinal organoids showed cell types expressing markers for proliferative, secretory and resorptive cell entities, which are organized as polarized structure including crypts, villi and tight junctions.
In order to generate a reliable and easy to reproduce intestinal test system, ES cells could be a novel and elegant cell source. In line with recently published data for human ES cells, our findings indicate that our differentiation protocol is also feasible to generate murine intestinal cells in vitro. Next steps would be a functional characterization and the attempt to culture these cells on a biological scaffold in order to provide a tight barrier system suitable for biomedical applications mentioned above.

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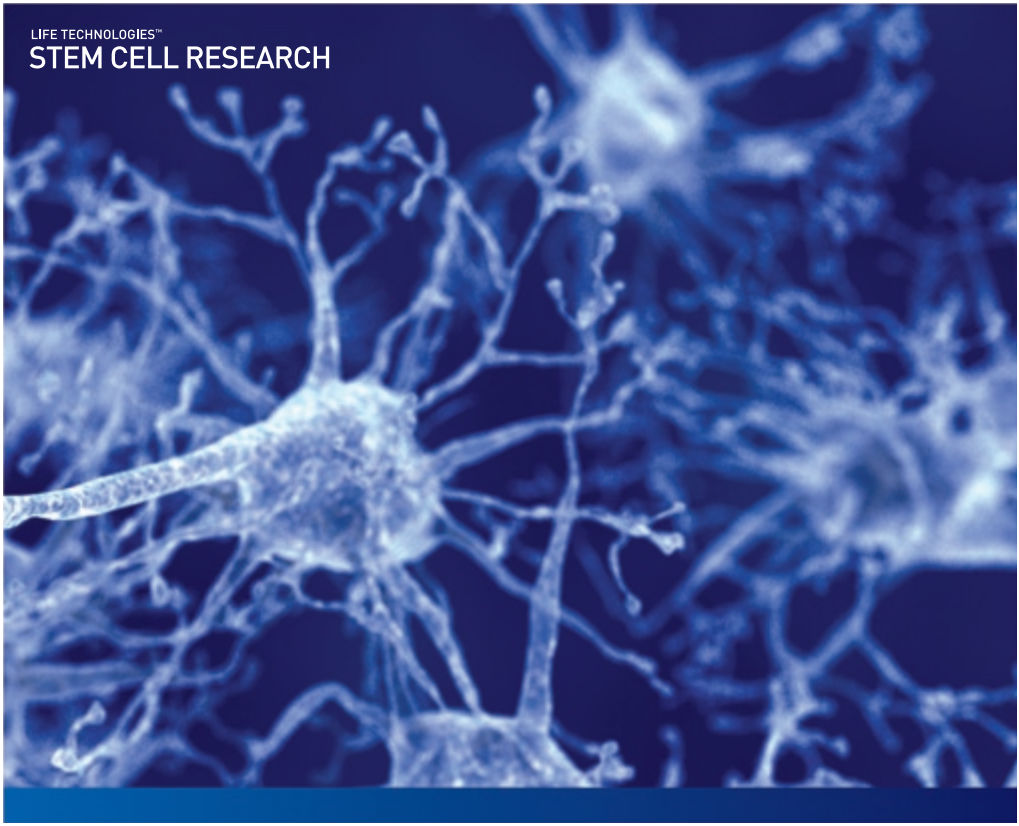

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