GermanStemCellNetwork

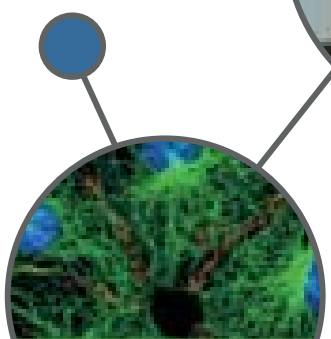
3rd International Annual Conference

of the German Stem Cell Network (GSCN)
9 – 11 September 2015

Campus Riedberg, Otto Stern Center
Goethe University
Frankfurt/Main

Program & Abstracts

www.gscn.org



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Organizer:
German Stem Cell Network (GSCN)
c/o Max Delbrück Center (MDC)
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web: www.gscn.org

Welcome address

Dear Friends and Colleagues,

It is a great pleasure to welcome you to the **3rd International Annual Conference of the German Stem Cell Network (GSCN)** at the Otto Stern Center in Frankfurt/Main.

The 3rd International Annual Conference of the GSCN will cover the latest findings from the field of stem cell biology. The interaction of scientists working on different types of stem cells (embryonic stem cells, stem cells in adult organs and cancer stem cells) has proven to be very fruitful at the GSCN Annual Conferences. Application of stem cells in regenerative therapies, disease modeling and drug development will be covered as well as strategic issues related to stem cell research, e.g. career development, public outreach, funding policies and clinical trials with stem cells.

Most speakers are selected from the best abstracts submitted leading up to the conference. The program will be completed by invited keynote lectures to present the international points of reference for this fast growing field of research. We are happy that Andras Nagy (Toronto), Paul Riley (Oxford), Hans-Willem Snoek (New York) and Lorenz Studer (New York) will present their most recent results.

Moreover, in 2015 the GSCN has established three awards for scientists in Germany: Young Investigator, Female Scientist and Publication of the Year. In the Presidential Symposium on Thursday, 10 September 2015, the awardees as well as the GSCN founding president Oliver Brüstle will give a presentation.

The final session of the conference will be a Joined Session with LOEWE Center for Cell and Gene Therapy Frankfurt (CGT) setting a spotlight on excellent science in the Frankfurt area.

I wish all participants a successful conference with new insights, new aquaintances and collaboration as well as reunion with colleagues.

Yours sincerely,

Thomas Braun (GSCN Acting president)

For the program committee

Daniel Besser (Berlin) • Thomas Braun (Bad Nauheim) • Ulrich Martin (Hannover) • Michael Rieger (Frankfurt) • Elly Tanaka (Dresden) • Andreas Trumpp (Heidelberg) • Harald von Melchner (Frankfurt)

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Conference information

Venue

Otto Stern Center
Campus Riedberg, Ruth-Moufang-Straße 2
60438 Frankfurt/Main, Germany

Date

Wednesday, 9 September to Friday, 11 September 2015

Registration

Regular fee:	*550 €*	Technical assistant fee:	*120 €*
Regular member fee:	*375 €*	Day ticket fee (onsite):	*200 €*
Student fee:	*300 €*	Day ticket member fee (onsite):	*160 €*
Student member fee:	*200 €*		

The registration fee includes attendance at all scientific sessions, poster and industry exhibition, lunch and coffee breaks, the Get-together, the Networking evening, free internet access and conference documents including badge, final program and abstract book.

Internet

Internet access via Wireless LAN is free of charge. Please pick up the login data at the registration.

Posters exhibition

Posters will be displayed during the conference in two sessions in the 1st floor of the Otto Stern Center. Authors are asked to be present at their poster during the poster session.
Even numbers will be presented 16:30 – 17:30 h and odd numbers 17:30 – 18:30 h.

You will find the number of your poster in this abstract volume. Posters in poster session I should be mounted on Wednesday, 12:00 – 16:30 h and removed latest on Thursday, 14:00 h. Posters in poster session II should be mounted on Thursday, 14:00 – 16:30 h and removed latest on Friday, 16:00 h.

Poster session I (P001 – P074)
Wednesday, 9 September 2015, 16:30 – 18:30 h

- Hematopoietic stem cells (P001 – P024)
- Stem cells in regenerative therapies (P025 – P038)
- Stem cells in regenerative therapies II: Mesenchymal stem/stroma cells (P039 – P048)
- Stem cells in disease modeling and drug development (P049 – P074)

Poster session II (P075 – P147)
Thursday, 10 September 2015, 16:30 – 18:30 h

- Pluripotency and embryonic stem cells (P075 – P093)
- Programing and Reprograming (P094 – P107)
- Somatic stem cells (P108 – P113)
- Stem cells in development (P114 – P130)
- Stem cells in disease: Cancer stem cells (P131 – P143)
- Computational stem cell biology (P144 – P147)

The poster session I is supported by Thermo Fisher Scientific – LSG.

The poster session II is supported by Fluidigm Europe B.V.

GSCN Awards

Travel awards

The following participants have been selected for the GSCN travel awards.

- *Onur Basak,* Hubrecht Institute, Utrecht, Netherlands
- *Debojyoti Chakraborty,* BIOTEC, Medical Faculty and University Hospital Carl Gustav Carus, Dresden
- *Christina Galonska,* Broad Institute of MIT and Harvard , Cambridge, MA, United States
- *Sarah Konze,* MH Hannover
- *Miha Modic,* Helmholtz Center Munich

The travel awards are supported by the GSCN member company Eppendorf AG.

eppendorf

Poster awards

There will be two poster awards for each poster session. Authors are asked to be present at the poster award ceremony, which will take place on Friday, 11 September 2015, 17:30 – 17:45 h in the lecture hall OSZ I.

The poster awards are supported by the member company Peprotech GmbH.

PEPROTECH
Manufacturer of Quality Cytokine Products

Scientific awards 2015

The German Stem Cell Network (GSCN) has presented its first awards to stem cell researchers in Germany. A top-class commission supported the GSCN in choosing the first awardees. The three awardees will give a presentation in the Presidential Symposium on Thursday, 10 September 2015, 14:00 – 16:00 h.

- **GSCN Young Investigator Award:** *Julia Ladewig,* Institute of Reconstructive Neurobiology of the University Hospital Bonn
- **GSCN Female Scientist Award:** *Magdalena Götz,* Institute of Stem Cell Research of the Helmholtz Zentrum München and chair of the Institute of Physiology – Department of Physiological Genomics of the Ludwig Maximilian University in Munich)
- The **GSCN Publication of the Year Award** (July 2014 to June 2015) goes to *Jichang Wang and Zsuzsanna Izsvák* of the Research Team „Mobile DNA“ at the Max-Delbrück-Centrum for Molecular Medicine (MDC) in Berlin-Buch. Their publication “Primate-specific endogenous retrovirus driven transcription defines naïve-like stem cells“ appeared in the journal *Nature* (Wang, J. et al., 2014, Nature, doi:10.1038/nature13804).

Social events

Get-together

On Wednesday, 9 September 2015, 18:30 – 21:00 h, all participants and exhibitors are invited to a **Get-together** with BBQ buffet in the Otto Stern Center.

Networking evening

The **Networking evening** with dinner buffet and DJ is open for all participants and exhibitors and will take place on Thursday, 10 September 2015, 20:00 – 01:00 h, in the Banquet hall I in the Casino at Campus Westend of the Goethe University Frankfurt/Main.

Address:
Festsaal I (Banquet hall I)
Campus Westend
Norbert-Wollheim-Platz 1
60323 Frankfurt am Main

Shuttle buses will leave at 19:30 h from the Otto Stern Center.

Pictures Otto Stern Center, Campus Westend and Banquet hall I © Goethe University

Public outreach event (in German)

Podiumsdiskussion zur aktuellen Forschung in Labor und Klinik: Moderne Zelltherapien – mit Stammzellen gegen Herzinfarkt und Blutkrebs

Bringen Stammzellen die Heilung, die von ihnen erwartet wird? Welche Fortschritte gibt es in der Forschung? Bei welchen Erkrankungen kommen zellenbasierte Therapien schon zur Anwendung? Und wie sieht der Weg von der Erforschung der Stammzellen über langjährige Studien bis hin zu ihrem klinischen Einsatz aus?

Patienten, ihre Angehörigen und die Öffentlichkeit haben heute ein gesteigertes Interesse daran, sich objektiv über Verlauf und Ursachen von Krankheiten als auch über die Chancen und Risiken von Therapien zu informieren. Der Hype um Stammzelltherapie der vergangenen Jahre ist einer realistischen Einschätzung bei stetigem Erkenntnisfortschritt gewichen.

In der Podiumsdiskussion kommen drei renommierte Wissenschaftler aus der Grundlagenforschung und Klinik zu Wort. Kurzfilme präsentieren ihre Arbeit. Anschließend skizzieren sie den neuesten Forschungsstand und Einsatzmöglichkeiten von Stammzellen bei Herzinfarkt und Blutkrebs und beantworten Fragen aus dem Publikum.

18:30	Einlass, Posterausstellung & Catering
19:00	**Podiumsdiskussion mit Kurzfilmen** • **Prof. Dr. Thomas Braun,** Direktor des Max-Planck-Instituts für Herz- und Lungenforschung, Bad Nauheim • **Prof Dr. Hubert Serve,** Universitätsklinikum Frankfurt, Direktor der Medizinischen Klinik II für Hämatologie und Onkologie • **Prof. Dr. Andreas Zeiher,** Universitätsklinikum Frankfurt, Direktor der Medizinischen Klinik III für Kardiologie • **Moderation: Dr. Stefanie Seltmann,** Deutsches Krebsforschungszentrum, Heidelberg
20:15 – 21:00	Ausklang mit Snacks, Getränken und Gesprächen

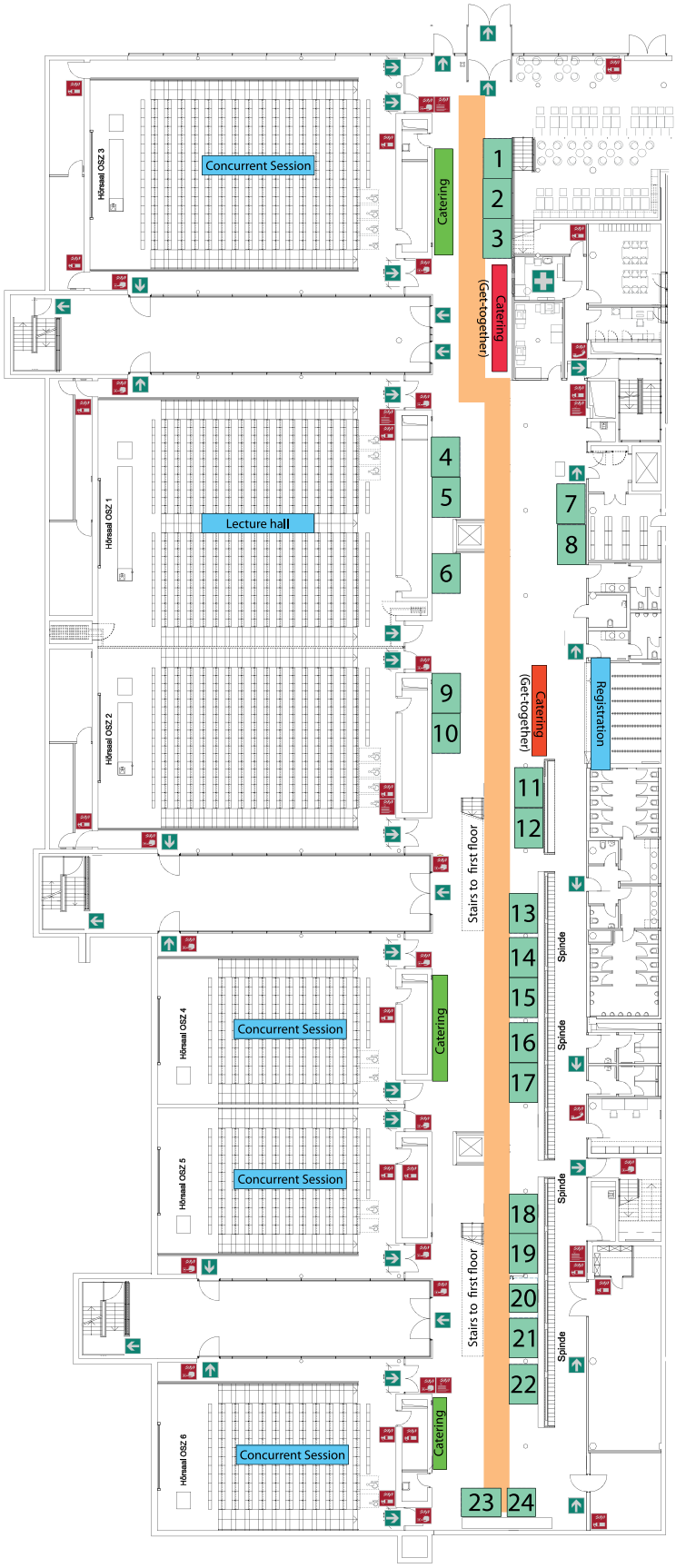
EINTRITT IST FREI!

Anfahrt:

Mit der U8 vom U-Bahnhof Uni Campus Riedberg (Richtung Südbahnhof) bis U-Bahnhof Holzhausenstraße. Von dort ca. 5 Minuten Fußweg bis zum Uni Campus Westend/Anbau Casino/Saal West.

Floor plan

Ground floor: Entrance II



First floor: Main Entrance (near U8/U9 Uni-Campus Riedberg)
Poster exhibition and seminar rooms

Legend

Company	booth
academics GmbH	23
AMS Biotechnology (Europe) Ltd	9
Becton Dickinson GmbH	2
BIOFROXX GmbH	15
BioLamina AB	14
Biological Industries	15
Cenibra GmbH	7
ChemoMetec A/S	19
Cygenia GmbH	24
Eppendorf AG	6
Fluidigm Europe B.V.	17
Gynemed GmbH & Co. KG	1
I&L Biosystems GmbH	18
Implen GmbH	20
Miltenyi Biotec GmbH	10
NEW ENGLAND BioLabs GmbH	22
OLS OMNI Life Science GmbH & Co. KG	21
PELOBIOTECH GmbH	12
PeproTech GmbH	3
PromoCell GmbH	16
Reprocell Inc.	12
STEMCELL Technologies SARL	13
Takara Clontech	11
Thermo Fisher Scientific – LSG	4
Thermo Scientific	5
UPM-Kymmene Corporation	8

Program Overview

WEDNESDAY, 9 September 2015

	OSZ 1	OSZ 3	OSZ 4	OSZ 6
11:00 – 12:00	Registration/Light lunch			
12:00 – 12:30	Opening			
12:30 – 13:15	**Keynote lecture I** *Paul Riley*			
13:15 – 14:00	**Keynote lecture II** *Hans-Willem Snoeck*			
14:00 – 14:30	Coffee break/Industry exhibition			
14:30 – 16:00		**Concurrent scientific working group session I**		
		Stem cells in regenera-tive therapies I	Pluripotency and embryonic stem cells	Computational stem cell biology
16:00 – 16:30	Coffee break/Industry exhibition			
16:30 – 18:30	**Poster Session I**			
16:30 – 17:30	posters with even numbers			
17:30 – 18:30	posters with uneven numbers			
18:30 – 21:00	**Informal GSCN Get-together** with dinner buffet in the foyer of the Otto Stern Center			

THURSDAY, 10 September 2015

	OSZ 1	OSZ 3	OSZ 4	OSZ 6
09:00 – 10:30		**Concurrent scientific working group session II**		
		Stem cells in disease modeling and drug development	Stem cells in disease: cancer stem cells	Stem cells in development
10:30 – 11:00	Coffee break/Industry exhibition			
11:00 – 12:30		**Industry session: "Technologies from GSCN industry partners"**		
		Eppendorf PeproTech Thermo Scientific	Takara Clontech STEMCELL Techn. I&L Biosystems	Biological Industries Fluidigm UPM-Kymmene Corp.
12:30 – 14:00	Lunch buffet/Industry exhibition/Poster viewing			
12:50 – 13:50	Showcase: Outreach + Patient Information (Seminar room 3)			
14:00 – 16:00	**Presidential Symposium**			
14:00 – 14:30	*Oliver Brüstle*			
14:30 – 15:00	Young Investigator *Julia Ladewig*			
15:00 – 15:30	Female Scientist Award *Magdalena Götz*			
15:30 – 16:00	Publication of the Year Award *Jichang Wang*			
16:00 – 16:30	Coffee break/Industry exhibition			
16:30 – 18:30	**Poster Session II**			
16:30 – 17:30	posters with even numbers			
17:30 – 18:30	posters with uneven numbers			

	OSZ 1	OSZ 3	OSZ 4	OSZ 6
18:30 – 19:30		**GSCN Members Meeting**		
19:30 – 20:00	Bus Shuttle to Networking evening			
20:00 – 01:00	**GSCN Networking evening** with dinner buffet and DJ at the Banquet Hall I, Casino, Campus Westend			

FRIDAY, 11 September 2015

	OSZ 1	OSZ 3	OSZ 4	OSZ 5	OSZ 6
09:00 – 10:30		**Concurrent scientific working group session III**			
		Hematopoietic stem cells	Somatic stem cells	SC in reg. therapies II: Mes-enchymal stem/ stroma cells	Programming and reprogramming
10:30 – 11:00	Coffee break/Industry exhibition				
11:00 – 12:30		**Concurrent strategic working group session**			
		Stem cell technologies	Career development		Clinical trials and regulatory affairs
12:30 – 14:00	Lunch buffet/Industry exhibition/Poster viewing				
12:45 – 13:45	Meet-the-expert-table: CRISPR/Cas Technology (Seminar room 3)				
12:45 – 13:45	Meet-the-expert-table: TALENs Technology (Seminar room 4)				
12:45 – 13:45	Drop-in booth on regulatory affairs and clinical trials (Registration desk)				
14:00 – 14:45	**Keynote lecture III** *Andras Nagy*				
14:45 – 15:30	**Keynote lecture IV** *Lorenz Studer*				
15:30 – 16:00	Coffee break/Industry exhibition				
16:00 – 17:30	**Joined Session with LOEWE Center for Sell and Gene Therapy Frankfurt (CGT)**				
17:30 – 17:45	**Poster award ceremony**				
17:45 – 18:00	**Outlook and closing remarks**				
18:00	End of conference				
19:00 – 20:00	**GSCN public event** at the Hall West, Campus Westend				
20:00 – 21:00	Reception public event				

Legend

- Opening/Evening events/Members meeting/Outlook
- Keynote Lectures/Presidential Symposium
- Concurrent scientific working group sessions
- Concurrent strategic working group sessions
- Industry session
- CGT Session
- Poster Sessions and Poster award ceremony
- Showcase/Meet-the-expert-tables/Drop-in booth

Program

WEDNESDAY, 9 September

11:00 – 12:00 Registration and light lunch

OSZ 1: Opening

12:00 – 12:15 Welcome to the Goethe University, Otto Stern Center
Enrico Schleiff, Vice President, Goethe University

12:15 – 12:30 Opening
Thomas Braun (Acting President, GSCN)

12:30 – 13:15 **Keynote lecture I**
K1 – Epigenetic reactivation of adult epicardium underpins endogenous heart repair
Paul Riley, University of Oxford, U.K. (Chair: *Thomas Braun*)

13:15 – 14:00 **Keynote lecture II**
K2 – Modeling human lung development and disease using pluripotent stem cells
Hans-Willem Snoeck, Columbia University, New York, U.S.A. (Chair: *Ulrich Martin*)

14:00 – 14:30 Coffee break/Industry exhibition

Concurrent scientific working group session I

OSZ 3: Stem cells in regenerative therapies I (Chairs: *Michael Cross/Ulrich Martin*)

14:30 – 14:45 Overview
Michael Cross, University of Leipzig

14:45 – 15:00 T01 – Atrial & ventricular stem cells from same heart show different functional competencies for cardiac repair but are superior to endothelial progenitors
Parul Dixit, University of Otago, Dunedin, New Zealand

15:00 – 15:15 T02 – Cardiomyogenesis of human pluripotent stem cells is associated with transformation of metabolism and expression of specific PDLIM5 splice variants
Sarah A. Konze, Hannover Medical School

15:15 – 15:30 T03 – Gene modification in stem cells targeting megakaryopoiesis
Lisette Johanna Latorre Rey, Paul Ehrlich Institute, Langen

15:30 – 15:45 T04 – A novel cell surface marker for the enrichment of transplantable dopaminergic precursor cells from differentiated human pluripotent stem cells
Daniela Lehnen, Miltenyi Biotec GmbH, Bergisch Gladbach

15:45 – 16:00 Working group discussion “Stem cells in regenerative therapies”

OSZ 4: Pluripotency and embryonic stem cells (Chairs: *Hans Schöler/Mathias Treier*)

14:30 – 14:45 Overview
Hans Schöler, Max Planck Institute for Molecular Biomedicine, Münster

14:45 – 15:00 T05 – Foxd3 promotes the exit from naïve pluripotency and prevents germline specification through enhancer decommissioning
Alvaro Rada-Iglesias, CMMC, University of Cologne

15:00 – 15:15 T06 – Ground state conditions induce rapid reorganization of core pluripotency factor binding before global epigenetic reprogramming
Christina Galonska, Havard University, Cambridge, U.S.A.

15:15 – 15:30 T07 – TDP-43 safeguards pluripotency by regulation of alternative polyadenylation
Miha Modic, Helmholtz Center Munich and MRC LMB Cambridge, U.K.

15:30 – 15:45 T08 – LncRNA Panct1 maintains mouse embryonic stem cell identity by recruiting TOBF1 to OCT-SOX sequences in early G1
Debojyoti Chakraborty, Dresden University of Technology

15:45 – 16:00 T09 – Decoding the heterogeneity of pluripotent stem cells at the single cell level
Simon Hastreiter, ETH Zürich BSSE, Basel, Switzerland

OSZ 6: Computational stem cell biology (Chairs: *Georg Füllen/Ingo Röder*)

14:30 – 14:45 Overview
Georg Füllen, IBIMA, University of Rostock

14:45 – 15:00 T10 – Morpheus: computational modeling for stem cell biologists
Walter de Back, Dresden University of Technology

15:00 – 15:15 T11 – Inference of spatiotemporal effects on cellular state transitions from time-lapse microscopy
Michael Strasser, Helmholtz Center Munich

15:15 – 15:30 T12 – Prospective identification of hematopoietic lineage choice
Carsten Marr, Helmholtz Center Munich

15:30 – 15:45 T13 – On the quantification of hematopoietic tissue remodeling in aging and disease: simulations, measures and predictions
Christoph Baldow, Dresden University of Technology

15:45 – 16:00 Working group discussion “Computational stem cell biology”

16:00 – 16:30 Coffee break/Industry exhibition

16:30 – 18:30 **Poster session I: P001 – P074** supported by Thermo Fisher Scientific – LSG
P001 – P024: Hematopoietic stem cells
P025 – P038: Stem cells in regenerative therapies
P039 – P048: Stem cells in regenerative therapies II: Mesenchymal stem/stroma cells
P049 – P074: Stem cells in disease modeling and drug development

Even numbers: please present you poster from 16:30 – 17:30
Odd numbers: 17:30 – 18:30

18:30 – 21:00 Get-together for all participants
Dinner buffet in the foyer and outside of the Otto Stern Center

THURSDAY, 10 September

Concurrent scientific working group session II

OSZ 3: Stem cells in disease modeling and drug development (Chairs: *Karl-Ludwig Laugwitz/Oliver Brüstle*)

09:00 – 09:15 Overview
Karl-Ludwig Laugwitz, Technische Universität München

09:15 – 09:30 T14 – Infection, innate immune signaling and cancer in the stomach – stem cell-derived organoids as a new model
Sina Bartfeld, Hubrecht Institute, Utrecht, Netherlands

09:30 – 09:45 T15 – Modeling reactive gliosis in mESC-derived retina organoids
Manuela Völkner, DZNE Dresden

09:45 – 10:00 T16 – Patient iPSC-derived NPCs as a model for mtDNA mutations reveal HCS/HTS-suitable phenotypes
Raul Bukowiecki, Max Delbrück Center, Berlin

10:00 – 10:15 T17 – Intracellular calcium-release and autophagy modulation trigger ataxin-3 positive nuclear inclusions in Machado-Joseph disease-specific neurons
Johannes Jungverdorben, Institute of Reconstructive Neurobiology, Bonn

10:15 – 10:30 Working group discussion "Stem cells in disease modeling and drug development"

OSZ 4: Stem cells in disease: cancer stem cells (Chairs: *Thomas Brabletz/Andreas Trumpp*)

09:00 – 09:15 Overview
Thomas Brabletz, FAU Erlangen

09:15 – 09:30 T18 – Epigenetic age-predictions in cancer: aging signatures are associated with malignant transformation and clinical prognosis
Wolfgang Wagner, RWTH Aachen Medical School

09:30 – 09:45 T19 – Triple-negative inflammatory breast cancer: syndecan-1 is a biomarker and regulator of cancer stem cell phenotype via IL-6/STAT-3 and Notch-3
Sherif A. Ibrahim, Faculty of Science, Cairo University, Egypt

09:45 – 10:00 T20 – Targeting tumor-initiating stem cells in colorectal carcinoma
Ilaria Lunger, Goethe University Hospital and LOEWE Center Frankfurt

10:00 – 10:15 T21 – Unraveling the subclonal architecture of adult B-ALL at a single cell level
Bartosch Wojcik, Goethe University Hospital and LOEWE Center Frankfurt

10:15 – 10:30 Working group discussion "Stem cells in disease: cancer stem cells"

OSZ 6: Stem cells in development (Chairs: *Jan Lohmann/Thomas Braun*)

09:00 – 09:15 Overview
Jan Lohmann, Centre for Organismal Studies (COS) Heidelberg

09:15 – 09:30 T22 – Understanding the curious case of trophoblast differentiation
Micha Drukker, Helmholtz Center Munich

09:30 – 09:45 T23 – The Ca^{2+}-regulated phosphatase calcineurin controls proportional growth of zebrafish regenerating fins
Christopher Antos, Max Planck Institute for Molecular Cell Biology and Genetics, Dresden

09:45 – 10:00 T24 – Antagonistic roles of miR-181a and orphan nuclear receptor GCNF in regulating human neural stem cell maintenance
Laura Stappert, Institute of Reconstructive Neurobiology, Bonn

10:00 – 10:15 T25 – Direct Nkx2-5 transcriptional repression of Isl1 controls cardiomyocyte subtype identity
Jessica Haas, Klinikum r.d.I. der TUM, Muinch

10:15 – 10:30 T26 – Hypoxia induced epigenetic mechanism underlying Isl1+ cardiac progenitor cell homeostasis control
Xuejun Yuan, Max Planck Institute for Heart and Lung Research, Bad Nauheim

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

Industry session: "Technologies from GSCN industry partners"

OSZ 3: Main supporter (chair: *Albrecht Müller*)

11:00 – 11:05 Introduction

11:05 – 11:35 C1 – Robust expansion of human pluripotent stem cells: integration of bioprocess design with transcriptomic and metabolomic characterization
Margarida Serra, Instituto de Tecnologia Química e Biológica (ITQB), Universidade Nova de Lisboa (UNL), Oeiras, Portugal, representing Eppendorf AG

11:35 – 12:05 C2 – A human iPS cell platform to study pancreatic disease
Alexander Kleger, University Hospital Ulm, representing PeproTech GmbH

12:05 – 12:35 C3 – Advances in pluripotent stem cell culture and differentiation
David Kuninger, Thermo Fisher Scientific – LSG

OSZ 4: Supporter (chair: *Hartmut Geiger*)

11:00 – 11:05 Introduction

11:05 – 11:35 C4 – Consistency of pluripotent stem cell culture systems and differentiation protocols for generation of functional human stem cell derived hepatocytes
Mia Emgård, Takara Clontech

11:35 – 12:05 C5 – Reprogramming of somatic cells to induced pluripotent stem cells using a novel synthetic self-replicating RNA vector in a feeder-free system
Alexandra Blak, STEMCELL Technologies SARL

12:05 – 12:35 C6 – High throughput electrospinning to generate scaffolds containing cells for regenerative medicine applications
Thomas Hayes, BioInicia S.L./I&L Biosystems GmbH

OSZ 6: Supporter (chair: *Ana Martin-Villalba*)

11:00 – 11:05 Introduction

11:05 – 11:35 C7 – An optimal serum-free, xeno-free culture system for the isolation, expansion and differentiation of clinical grade human mesenchymal stem cells
Orit Ecker-Cohen, Biological Industries / Biofroxx GmbH

11:35 – 12:05 C8 – Explore what makes every stem cell unique from inside out
Mark Lynch, Fluidigm Europe B.V.

12:05 – 12:35 C9 – GrowDex® hydrogel for 3D cell cultures
Lauri Paasonen, UPM-Kymmene Corporation

12:35 – 14:00 Lunch buffet/Industry exhibition/Poster viewing supported by STEMCELL Technologies SARL

Seminar room 3 (1st floor)

12:50 – 13:50 Showcase: Outreach + Patient Information (Chairs: *Ira Herrmann/Tobias Cantz*)

OSZ 1: Presidential Symposium

14:00 – 14:30 PS1 – Evolving stem cell-based strategies for targeting neurological disorders
Oliver Brüstle, Institute of Reconstructive Neurobiology, Bonn

14:30 – 15:00 Young Investigator Award
PS2 – *In vitro* cell culture models to decipher brain development and neuronal integration
Julia Ladewig, Institute of Reconstructive Neurobiology, Bonn

15:00 – 15:30 Female Scientist Award
PS3 – From neurogenesis to neural repair
Magdalena Götz, Helmholtz Center Munich

15:30 – 16:00 Publication of the Year 2015 Award (07/14 – 06/15)
PS4 – Primate-specific endogenous retrovirus-driven transcription defines naive-like stem cells
Jichang Wang (w. *Zsuzsanna Izsvak*), Max Delbrück Center, Berlin

16:00 – 16:30 Coffee break/Industry exhibition

16:30 – 18:30 **Poster session II: P074 – P147** supported by Fluidigm Europe B.V.
P075 – P093: Pluripotency and embryonic stem cells
P094 – P107: Programing and reprograming
P108 – P113: Somatic stem cells
P114 – P130: Stem cells in development
P131 – P143: Stem cells in disease: Cancer stem cells
P144 – P147: Computational stem cell biology

Even numbers: please present you poster from 16:30 – 17:30
Odd numbers: 17:30 – 18:30

18:30 – 19:30 **Membership meeting**

19:30 – 20:00 Bus transfer

20:00 – 01:00 GSCN Networking evening for all participants
with dinner buffet and DJ at the Banquet Hall I, Casino, Campus Westend

FRIDAY, 11 September

Concurrent scientific working group session III

OSZ 3: Hematopoietic stem cells (Chairs: *Timm Schröder/Claudia Waskow*)

09:00 – 09:15 Overview
Timm Schröder, ETH Zürich BSSE, Basel, Switzerland

09:15 – 09:30 T27 – CCND1/CDK4-mediated cell cycle progression provides a competitive advantage for human hematopoietic stem and progenitor cells *in vivo*
Nicole Mende, Dresden University of Technology

09:30 – 09:45 T28 – The H3K4 histone methyltransferase Setd1b is required for haematopoietic stem cell homeostasis in mice
Kerstin Schmidt, Biotechnology Center TU Dresden

09:45 – 10:00 T29 – Molecular and functional heterogeneity of dormant HSCs
Nina Cabezas-Wallscheid, German Cancer Research Center (DKFZ) Heidelberg

10:00 – 10:15 T30 – High efficiency gene correction in hematopoietic cells by template-free CRISPR/Cas9 genome editing
Frank Schnütgen, University Hospital Frankfurt

10:15 – 10:30 Working group discussion "Basic, translational and applied hematopoiesis"

OSZ 4: Somatic stem cells (Chairs: *Ana Martin-Villalba/Michael Rieger*)

09:00 – 09:15 Overview
Ana Martin-Villalba, German Cancer Research Center (DKFZ) Heidelberg

09:15 – 09:30 T31 – Visualization of the short-range Wnt gradient in the intestinal stem cell niche
Henner Farin, Georg-Speyer-Haus, Frankfurt

09:30 – 09:45 T32 – Clonal heterogeneity within the exocrine pancreas
Damian Wollny, German Cancer Research Center (DKFZ) Heidelberg

09:45 – 10:00 T33 – Single cell analysis of cellular mechanisms regulating adult neural stem cell fate
Onur Basak, Hubrecht Institute, Utrecht, Netherlands

10:00 – 10:15 T34 – *In vivo* Ribotag analysis reveals translational control during neural stem cell differentiation
Avni Baser, German Cancer Research Center (DKFZ) Heidelberg

10:15 – 10:30 Working group discussion "Somatic stem cells and development"

OSZ 5: Stem cells in reg. therapies II: Mesenchymal stem/stroma cells (Chairs: *Dirk Strunk/Karen Bieback*)

09:00 – 09:15 Overview
Dirk Strunk, Paracelsus Medical University, Salzburg, Austria

09:15 – 09:30 T35 – The transcription factor GATA2 controls mesenchymal stem cell fate
Alexander Tolkachov, Center for Cardiovascular Research, Charité, Berlin

09:30 – 09:45 T36 – Attenuation of alloimmunity to human endothelial colony forming cells (ECFC) by interaction with mesenchymal stem/progenitor cells (MSPC)
Naima Souidi, Berlin-Brandenburg Center for Regenerative Medicine and Institute of Medical Immunology, Charité, Berlin

09:45 – 10:00 T37 – Murine bone marrow derived mesenchymal stem cells as a putative therapy approach for influenza-virus induced pneumonia
Lina Jankauskaite, Universities of Giessen and Marburg Lung Center (UGMLC), Giessen

10:00 – 10:15 T38 – Regenerative potential of human pluripotent stem cell-derived MSCs in a Gunn rat liver injury model
Lucas-Sebastian Spitzhorn, University Hospital of Düsseldorf

10:15 – 10:30 Working group discussion "Stem cells in regenerative therapies" – future progress with MSCs

OSZ 6: Programing and reprograming (Chairs: *Micha Drukker/Frank Edenhofer*)

09:00 – 09:15 Overview
Micha Drukker, Helmholtz Center Munich

09:15 – 09:30 T39 – An inducible overexpression system for rapid and highly efficient induction of mature cell types from human pluripotent stem cells
Matthias Pawlowski, Cambridge Stem Cell Institute, U.K.

09:30 – 09:45 T40 – Reprogramming triggers endogenous LINE-1, Alu and SVA retrotransposition in human induced pluripotent stem cells affecting host gene expression
Gerald Schumann, Paul Ehrlich Institute, Langen

09:45 – 10:00 T41 – Efficient patterning of human pluripotent stem cells towards distinct mesendodermal subtypes simply by modulation of the bulk cell density
Henning Kempf, Hannover Medical School

10:00 – 10:15 T42 – Direct *in vivo* glia-to-neuron conversion in the postnatal mouse cerebral cortex
Sophie Péron, Institute of Physiological Chemistry, Johannes Gutenberg University Mainz

10:15 – 10:30 Working group discussion "Pluripotency and reprograming"

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

Concurrent strategic working group session

OSZ 3: **Stem cell technologies** (Chairs: *Frank Emmrich/Andreas Bosio*)

11:00 – 11:30 S1 – Technologies in genetic engineering of stem cells
Ulrich Martin, Hannover Medical School

11:30 – 12:00 S2 – Genetic engineering of human pluripotent stem cells using TALENs nucleases
Sebastian Diecke, Max Delbrück Center, Berlin

12:00 – 12:30 S3 – Mammalian genome engineering in the era of CRISPR/Cas
Ralf Kühn, Max Delbrück Center, Berlin

OSZ 4: **Career development** (Chairs: *Insa Schröder/Hartmut Geiger*)

11:00 – 11:15 Introduction
Insa Schröder, GSI Helmholtzzentrum für Schwerionenforschung GmbH, Darmstadt

11:15 – 12:00 S4 – How to apply in research and industry
Hanna Proner, academics/Die Zeit, Hamburg

12:00 – 12:30 Panel discussion

OSZ 6: Clinical trials and regulatory affairs (Chairs: *Andreas Kurtz/Halvard Bönig*)

11:00 – 11:05 Introduction
Andreas Kurtz, Berlin-Brandenburg Center for Regenerative Medicine, Charité, Berlin

11:05 – 11:25 S5 – Regulatory aspects and classifications of stem cell based medicinal products
Egbert Flory, Paul Ehrlich Institute, Langen

11:25 – 11:45 S6 – Accelerating the clinical development of cell based therapies
Natalie Mount, Cell Therapy Catapult, London, U.K.

11:45 – 12:00 S7 – The ADIPOA-1/-2 trial – Adipose tissue derived stromal cells for osteoarthritis therapy
Oliver Pullig, Fraunhofer IGB, Würzburg branch

12:00 – 12:15 S8 - Cell Therapy for bone defects: *in vitro*, *in vivo*, and first clinical results
Ingo Marzi, Goethe University Hospital, Frankfurt

12:30 – 14:00 Lunch buffet/Industry exhibition/Poster viewing supported by UPM-Kymmene Corporation

12:45 – 13:45 Meet the expert tables: Stem cell technologies supported by Schering Stiftung
Please register at the registration (limited to 10 participants each)
Seminar room 3: CRISPR/Cas Technology *(Ralf Kühn)*
Seminar room 4: TALENs Technology *(Sebastian Diecke)*

12:45 – 13:45 Drop-in booth on regulatory affairs and clinical trials with *Egbert Flory, Natalie Mount,* u.a. (Registration)

OSZ 1: Joined Session with LOEWE Center for Cell and Gene Therapy Frankfurt (CGT)

14:00 – 14:45 **Keynote lecture III**
K3 – Pluripotency in the artificial cell space
Andras Nagy, Lunenfeld-Tanenbaum Research Institute, Toronto, Canada
(Chair: *Harald von Melchner*)

14:45 – 15:30 **Keynote lecture IV**
K4 – Modeling and treating neural disease using human pluripotent stem cells
Lorenz Studer, Memorial Sloan Kettering Cancer Center, New York (Chair: *Lenhard Rudolph*)

15:30 – 16:00 Coffee break/Industry exhibition

CGT Session (Chair: *Lenhard Rudolph*)

16:00 – 16:20 CGT1 – Regulation of second heart field progenitor cells: discovering key molecular players
Gergana Dobreva, Max Planck Institute for Heart and Lung Research, Bad Nauheim

16:20 – 16:40 CGT2 – Functional systems analysis of the adult muscle stem cell identifies crucial regulators of muscle regeneration
Johnny Kim, Max Planck Institute for Heart and Lung Research, Bad Nauheim

16:40 – 17:00 CGT3 – Hematopoietic stem cell fate decision control at single cell resolution
Michael Rieger, Goethe University Hospital and LOEWE Center Frankfurt

17:00 – 17:20 CGT4 – The Sleeping Beauty transposon system for genetic engineering in stem cells
Zoltán Ivics, Paul Ehrlich Institute, Langen

Closing ceremony

17:30 – 17:45 Poster award ceremony and announcement of industry quiz winners (*Daniel Besser; GSCN*)

17:45 – 18:00 Outlook and closing remarks
Incoming president: *Ulrich Martin*

End of GSCN conference

Announcement (please inform the registration desk for transfer)

19:00 – 21:00 GSCN public event: Moderne Zelltherapien – mit Stammzellen gegen Herzinfarkt und Blutkrebs (in German, page 9)

Speaker abstracts

3rd International Annual Conference
German Stem Cell Network

9 – 11 September 2015

Keynote lectures: K1 – K4

K1 Epigenetic reactivation of adult epicardium underpins endogenous heart repair
Paul Riley

K2 Modeling human lung development and disease using pluripotent stem cells
Hans-Willem Snoeck

K3 Pluripotency in the artificial cell space
Andras Nagy

K4 Modeling and treating neural disease using human pluripotent stem cells
Lorenz Studer

Abstract No. K1

Epigenetic reactivation of adult epicardium underpins endogenous heart repair

Paul Riley

Oxford Stem Cell Institute, UK

We adopt the paradigm of understanding how the heart develops during pregnancy as a first principal to inform on adult heart repair and regeneration. Our focus is on the epicardium and epicardium-derived cells (EPDCs) which line the outside of the forming heart and contribute vascular endothelial and smooth muscle cells to the coronary vasculature, interstitial fibroblasts and cardiomyocytes. The epicardium can also act as a source of signals to condition the growth of the underlying embryonic heart muscle. In the adult heart, whilst the epicardium is retained it is effectively quiescent. We have sought to extrapolate the developmental potential of the epicardium to the adult heart by stimulating dormant epicardial cells using a protein called Thymosin β4 (Tβ4), to give rise to new muscle and vasculature at the site of injury. A hallmark of this reactivation is the re-expression of embryonic epicardial genes, of which Wilm's tumour 1 (Wt1) sits on top of the hierarchy as critical for stimulating epithelial-mesenchymal transition (EMT). The mechanism for Tβ4-mediated reactivation remains elusive. Here, we report findings arising from an in vivo chromatin immunoprecipitation screen to map epigenetic changes mediated by SWI/SNF (BAF/BRG) chromatin remodellers in complex with Tβ4 and histone post-translational modifications at the level of Wt1, both in the developing and adult (intact and injured) heart. We reveal an in vivo requirement for exogenous Tβ4 (priming) for the epigenetic reprogramming of the adult epicardium and the role of an antisense Wt1 lncRNA in stabilising the expression of nascent Wt1 transcripts within adult EPDCs. Further studies are on-going to dissect out the Tβ4-mediated epigenetic mechanism of Wt1 reactivation. Understanding how to restore embryonic potential to adult EPDCs, following injury, may underpin the development of resident cell-based therapies for heart repair.

This work was generously supported by the British Heart Foundation and Wellcome Trust.

Abstract No. K2

Modeling human lung development and disease using pluripotent stem cells

Hans-Willem Snoeck

Columbia Universtity College of Physicians and Surgeons, New York, USA

Generating lung and airway epithelial cells from human pluripotent stem cells (hPSCs) has applications in regenerative medicine for lung diseases, drug screening and disease modeling, and provides a model to study human lung development. We have developed a strategy to differentiate hPSCs into anterior foregut endoderm, and used this as a platform for studies aimed at achieving differentiation of these cells into lung and airway epithelial cells. Long-term differentiation yielded cultures where >90% of the cells were committed to a lung or airway epithelial fate and contained goblet, Clara, ciliated, type I and, after addition of maturation media containing dexamethasone, predominantly (> 50%) type II alveolar epithelial cells after 50 days of culture. Importantly, the type II alveolar epithelial cells generated were capable of surfactant protein-B uptake and release, providing evidence of specific function. Furthermore, we developed a flow cytometric approach to isolate type II cells from the cultures based on their function. Based on this model, we developed three-dimensional cultures where human branching morphogenesis and alveogenesis are recapitulated. These culture models allowed the dissection of novel molecular mechanisms involved in alveolar maturation.

Abstract No. K3

Pluripotency in the artificial cell space

Andras Nagy

Mount Sinai Hospital and University of Toronto, Canada

The discovery of a defined set of transcription factors that can induce reprogramming of somatic cells to pluripotent stem cells (iPSCs) has had an unprecedented impact on our view on future cell transplantation-based tissue repair and restoration of faulty physiological functions. Somatic cell reprogramming is a several weeks long process through which cells reach pluripotency, the developmental state similar to embryonic stem cells. This cascade of events and the driving forces behind the phenomenon are very poorly understood. It is, however, crucial to uncover the fine details of this process in order to comprehend the true properties of iPSCs and so better tailor their future therapeutic use.

We have recently developed a reprogramming method utilizing a transposon-mediated delivery of the reprogramming transgenes. This system has several advantages over the viral delivery-based alternative. Most notably, it allows for a seamless removal of the transgenes once pluripotent stem cells have been generated and they are no longer needed for stem cell self renewal. We also combined the doxycycline inducible transgene expression system with the transposon delivery-based reprogramming and found that these transgenes are very efficiently regulatable by adding or withdrawing doxycycline. *In vivo* differentiated somatic cells derived from iPSCs can be reprogrammed to “secondary” iPSCs (2ºiPSc) by simply adding doxycycline to the culture medium.

By analyzing the effect of reprogramming factor expression dynamics on the reprogramming process, we have discovered additional classes of pluripotent cells that might have a different spectrum of phenotypes than that of ES cell-like iPS cells. Here, we explore alternative outcomes of somatic reprogramming by fully characterizing reprogrammed cells independently of preconceived definitions of partial or fully reprogrammed iPSC states. These alternative pluripotent states may have different therapeutic values depending on the target disease.

Abstract No. K4

Modeling and treating neural disease using human pluripotent stem cells

Lorenz Studer

Memorial Sloan Kettering Cancer Center, New York, USA

Human pluripotent stem cell-based disease modeling is a powerful strategy for recreating disease phenotypes *in vitro*. Our group has demonstrated that human pluripotent cell-based disease models can yield novel insights into disease mechanisms and serve as platform for drug discovery. I will present recent progress in modeling early-onset genetic disorders such as familial dysautonomia (FD), primary Herpes Simplex encephalitis (HSE) and Hirschsprung's disease (HD) including data on modeling disease severity. I will also discuss some of the challenges and potential solutions involved in modeling late-onset disorders such as Parkinson's disease (PD) including questions about how to model time and age *in vitro*. Furthermore, I will present data on recreating human enteric neuron development, diversity and function from human pluripotent stem cells and demonstrate the use of those cells for a combined cell and drug based therapeutic intervention in models of HD.

Moving from preclinical towards potential clinical interventions, I will also discuss ongoing work aimed at using pluripotent stem cells as a clinical grade source of dopamine neurons. We have previously shown that differentiation protocols that transit through a floor plate intermediate yield large numbers of authentic dopamine neurons from hESCs or hiPSCs. Current work is geared towards the prospective isolation of selective subtypes of midbrain dopamine neurons at the developmental stage optimal for cell therapy and promoting graft integration *in vivo* by engineering the cell surface properties of the transplanted dopamine neurons. All those studies are performed under conditions suitable for translational application. An important open question for any transplantation paradigm to the central nervous system is the mechanism of graft action. Using *in vivo* optogenetic studies in Parkinsonian mice, we have started to probe - in real time - the role of human neuronal activity and graft integration in triggering behavioral recovery. In conclusion, my presentation explores the potential of human pluripotent stem cells both as a tool for drug discovery and as a vehicle for developing novel cell therapy approaches in PD and beyond.

Presidential symposium lectures: PS1 – PS4

PS1 Evolving stem cell-based strategies for targeting neurological disorders
Oliver Brüstle

PS2 In vitro cell culture models to decipher brain development and neuronal integration
Julia Ladewig

PS3 From neurogenesis to neural repair
Magdalena Götz

PS4 A primate-specific endogenous retrovirus HERVH: from genomic scrap to stem cell regulator
Jichang Wang

Abstract No. PS1

Evolving stem cell-based strategies for targeting neurological disorders

Oliver Brüstle

Institute of Reconstructive Neurobiology, University of Bonn

Abstract not available

Abstract No. PS2: Junior Investigator Award

In vitro cell culture models to decipher brain development and neuronal integration

Vira Iefremova[1], George Manikakis[1], Kevin Weynans[1], Laura Ostermann[1], Franz-Josef Müller[2], Oliver Brüstle[1], Philipp Koch[1], and Julia Ladewig[1,]*

[1]University of Bonn and Hertie Foundation, Bonn

[2]Centre for Integrative Psychiatry, Kiel

*Presenting author

The advent of cellular reprogramming and the direct conversion of somatic cells enable the generation of defined human neural cell types in virtually unlimited numbers allowing to study brain development and neuronal integration. We generated defined and developmental distinct neural stem cells from pluripotent stem cells recapitulating the developmental progression of neuroepithelial stem cells into radial glia cells of defined regional identity. We also developed lineage selection strategies to isolate pure populations of human neurons form heterogeneous cultures. We applied such neurons to investigate migration and integration of human neurons in the context of stem/progenitor containing grafts. Here we identified, that autoattraction of neurons by neural progenitors inhibits emigration of neurons from transplanted cells, a phenomenon which can be blocked by receptor tyrosine kinase inhibition. Using transcription factor-based direct neuronal conversion in combination with small molecules we efficiently generated neuronal cultures from postnatal human fibroblasts. We translated these protocols to individuals with defined cortical developmental defects to study cell-intrinsic neuronal developmental alterations. Starting from pluripotent cultures we derived standardized and highly reproducible cortical organoid cultures allowing to study early aspects of brain development in health and disease. Thus *in vitro* culture models combining the most recent technologies of reprogramming, differentiation and direct cell conversion are powerful tools to enhance neuronal integration and to decipher pathways associated with normal brain development and developmental malformations.

Abstract No. PS3: Female Scientist Award

From neurogenesis to neural repair

Magdalena Götz

Institute for Stem Cell Research, Helmholtz Center Munich and Biomedical Center, University of Munich

Much progress has been made in recent years to regenerate neurons in injury models either by transplantation, recruitment or direct reprogramming. An unresolved issue is to which extent these regenerated neurons indeed integrate into the circuitry in an adequate manner. While projection of the transplanted neurons to their adequate target sites has been demonstrated, nothing is yet known about the nature of the specificity of the synaptic input. This is, however, essential for adequate restoration of neural circuitry and an important issue for circuits, like the cerebral cortex, that can easily be driven into aberrant activity, e.g. epileptiform activity.

We addressed this issue by using a layer-specific lesion model of the mouse cerebral cortex that allows assessing the layer-specific integration of regenerated neurons. We used mono-synaptic Rabies virus to retrogradely trace the neurons forming synapses onto the regenerated neurons. These data show a remarkable specificity of input that is almost indistinguishable from the connectivity of neurons in the same layer generated during development. Multiphoton live imaging reveals the remarkable fast integration of regenerated neurons into the circuitry with little reshaping of the growing dendritic trees and final spine density reached within 6-8 weeks. Functional imaging revealed orientation-tuning of these newly integrated neurons, thus demonstrating for the first time the capacity for adequate integration of regenerated neurons – a major prerequisite for regenerating circuitry function.

While the regenerated neurons in the above selective lesion model with induced apoptotic death of neurons survive indeed for long-time, in other injury contexts new neurons often die even when recruited from the endogenous sites[1]. We therefore set out to identify major hurdles of neuronal survival and identified a novel key pathway overcoming this major hurdle in direct reprogramming.

Together, these approaches now allow overcoming two major roadblocks in repairing neuronal networks after brain injury.

1 *Thored, P. et al., 2007, Stroke, 38, 3032-3039*

Abstract No. PS4: Publication of the Year Award

A primate-specific endogenous retrovirus HERVH: from genomic scrap to stem cell regulator

Jichang Wang[1,], Gangcai Xie[2], Avazeh T Ghanbarian[3], Laurence D. Hurst[3], and Zsuzsanna Izsvák[1]*

[1]Max-Delbrück-Center for Molecular Medicine, Berlin
[2]CAS-MPG Partner Institute for Computational Biology, China
[3]University of Bath, UK
*Presenting author

Naive embryonic stem cells hold great promise for research and therapeutics as they have broad and robust developmental potential. While such cells are readily derived from mouse blastocysts it has not been possible to isolate human equivalents easily, although human naive-like cells have been artificially generated (rather than extracted) by coercion of human primed embryonic stem cells by modifying culture conditions or through transgenic modification. Here we show that a sub-population within cultures of human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) manifests key properties of naive state cells. These naive-like cells can be genetically tagged, and are associated with elevated transcription of HERVH, a primate-specific endogenous retrovirus. HERVH elements provide functional binding sites for a combination of naive pluripotency transcription factors, including LBP9, recently recognized as relevant to naivety in mice. LBP9–HERVH drives hESC-specific alternative and chimaeric transcripts, including pluripotency-modulating long non-coding RNAs. Disruption of LBP9, HERVH and HERVH-derived transcripts compromises self-renewal. These observations define HERVH expression as a hallmark of naive-like hESCs, and establish novel primate-specific transcriptional circuitry regulating pluripotency.

1 *Jichang Wang, Gangcai Xie, Manvendra Singh, Avazeh T. Ghanbarian, Tamás Raskó, Attila Szvetnik, Huiqiang Cai, Daniel Besser, Alessandro Prigione, Nina V. Fuchs, Gerald G. Schumann, Wei Chen, Matthew C. Lorinc, Zoltán Ivics, Laurence D. Hurst & Zsuzsanna Izsvák, (2014), Primate-specific endogenous retrovirus-driven transcription defines naive-like stem cells.* Nature*, 516, 405 - 409*

CGT lectures: CGT1 – CGT4

CGT1 Regulation of second heart field progenitor cells: discovering key molecular players
Gergana Dobreva

CGT2 Functional systems analysis of the adult muscle stem cell identifies crucial regulators of muscle regeneration
Johnny Kim

CGT3 Hematopoietic stem cell fate decision control at single cell resolution
Michael A. Rieger

CGT4 The Sleeping Beauty transposon system for genetic engineering in stem cells
Zoltán Ivics

Abstract No. CGT1

Regulation of second heart field progenitor cells: discovering key molecular players

Gergana Dobreva

Goethe University Frankfurt and Max Planck Institute, Bad Nauheim

Heart formation requires tight spatial and temporal control of cardiac progenitor cell specification, expansion, and differentiation. The earliest population of cardiac progenitors, referred to as the first heart field, fuses at the midline and differentiates into the myocardium of the heart tube. After the initial heart tube formation, the heart grows by addition of second heart field progenitor cells to its anterior and venous poles. Multiple transcription factors and signalling molecules, expressed differentially in these two progenitor populations are integrated in regulatory networks, which strictly control a transcriptional program that ensures proper heart development. The transcription factor Isl1 marks the second heart field cardiovascular progenitors and plays a key role in regulating the proliferation, survival, migration and differentiation of these cells, but the mechanisms underlying its function are poorly understood. We found that the Isl1/Ldb1 transcriptional complex plays a key function in orchestrating a network for transcriptional regulation and coordination in three-dimensional space during cardiac progenitor expansion and differentiation.

Abstract No. CGT2

Functional systems analysis of the adult muscle stem cell identifies crucial regulators of muscle regeneration

Johnny Kim, Ting Zhang, Stefan Günther, Carsten Künne, Mario Looso, Marcus Krüger, Yonggang Zhou,* and *Thomas Braun*

Max Planck Institute for Heart and Lung Research, Bad Nauheim
*Presenting author

Skeletal muscle stem cells (MuSC), also called satellite cells, are indispensable for maintenance and regeneration of adult skeletal muscles. Yet, a comprehensive picture of the regulatory events controlling the fate of MuSC is missing. We determined the proteome of purified adult MuSC from transgenic reporter mice against which we designed a high-throughput loss-of-function screen. This approach led to the systematic identification of 120 genes important for MuSC self-renewal and differentiation. In depth in vivo analysis of one candidate, the arginine methyltransferase Prmt5, revealed that MuSC-specific inactivation of Prmt5 in adult mice prevents expansion of MuSC, abolishes long-term MuSC maintenance and abrogates skeletal muscle regeneration by direct epigenetic silencing of the cell cycle inhibitor p21. We reason that Prmt5 generates a poised state, which keeps MuSC in a stand-by mode thus allowing rapid MuSC amplification under disease conditions. Strikingly, Prmt5 is dispensable for proliferation and differentiation of muscle progenitor cells during embryonic development demonstrating that epigenetic regulation via Prmt5 determines significant differences between embryonic myogenesis and adult muscle stem cell dependent regeneration.

Abstract No. CGT3

Hematopoietic stem cell fate decision control at single cell resolution

Michael A. Rieger

Goethe University Hospital Frankfurt

Hematopoietic stem cells (HSCs) serve for the life-long regeneration of blood cells. They have the unique ability of life-long self-renewal and of differentiation into all blood cell lineages. These delicate fate decisions need strict molecular control to either avoid HSC depletion and organ failure or excessive HSC expansion, clonal dominance and leukemia development. Despite the fact that a substantial knowledge of this balance has been gained over the last decade, further understanding of the molecular mechanisms that integrate extrinsic signals and intrinsic factors in HSC self-renewal and differentiation control are needed for therapeutic protocols for regenerative medicine.

We have unraveled several molecules including non-coding RNAs that are instrumental in the HSC fate decisions of self-renewal and differentiation during development and adult blood regeneration. The elucidation of the molecular mechanisms sheds light on how extrinsic factors integrate their signals to immediately alter the mode of HSC self-renewal programs into differentiation induction programs, and how excessive self-renewal leading to expansion of HSCs over time are kept in control by negative feed-back mechanisms. The absence of these mechanisms results in the emergence of pre-leukemic alterations and finally leads to leukemia induction. The continuous observation of HSCs by a unique video-microscopy-based cell tracking system allows us to follow individual HSCs and all their progeny for weeks during their development, without losing individual cell identities. This technology enables us to investigate functional changes caused by these molecular mechanisms in real-time at single cell resolution. Our findings are utilized in the development of rational approaches to manipulate these novel molecular mechanisms for advanced applications of HSCs in cell and gene therapy.

Abstract No. CGT4

The Sleeping Beauty transposon system for genetic engineering in stem cells

Zoltán Ivics[1,], Marta Holstein[1], Andreas Gogol-Döring[2], Csaba Miskey[1], Ismahen Ammar[3], Lisa Wiedemann[1], Franka Voigt[4], Attila Sebe[1], Carsten Rudolph[5], Martin Schleef[6], Orsolya Barabas[4], Wilfried Kues[7], and Zsuzsanna Izsvák[3]*

[1]Paul Ehrlich Institute, Langen
[2]German Centre for Integrative Biodiversity Research (iDiv), Leipzig
[3]Max Delbrück Center for Molecular Medicine, Berlin
[4]EMBL, Heidelberg
[5]ethris GmbH, Planegg
[6]Plasmid Factory, Bielefeld
[7]Friedrich Loeffler Institute, Greifswald
*Presenting author

The Sleeping Beauty (SB) transposon system yields efficient stable gene transfer following gene delivery into the germline of mammals as well as into stem cells that are relevant targets for regenerative medicine and gene- and cell-based therapies.

We compared distributions of SB and piggyBac (PB) transposon insertions as well as MLV retrovirus and HIV lentivirus insertions in human CD4+ T cells. The SB transposon displayed the least deviation from random with respect to genome-wide distribution. We identify remarkable parallels between integration site distributions of the PB transposon and the MLV retrovirus, and detect unequal biases across the four systems with respect to targeting genes whose deregulation has been previously linked to serious adverse events in gene therapy clinical trials.

We applied the SB system for non-viral gene delivery in both mouse and human hematopoietic stem cells. We optimized our protocols by applying minicircle vectors to deliver the transposon and synthetic mRNA to deliver the transposase into this hard-to-transfect cell type. With this optimized protocol in hand, we are in the process of setting up preclinical gene therapy for several inherited rare diseases.

We have begun to dissect the molecular determinants of target site selection by the SB transposase. We have solved the crystal structure of the catalytic domain of the SB100X hyperactive transposase. The structure can be used prospectively to predict amino acids whose mutagenesis could result in altered function of the transposase. One such position, implicated to be involved in making contacts with the target DNA during transposition, was subjected to saturation mutagenesis. Screening for excision and full transposition revealed two mutants proficient in excision but deficient in integration. Such mutants could be developed as useful tools for advanced, transposon-based genetic engineering in iPS cells.

Selected presentations

3rd International Annual Conference
German Stem Cell Network
9 – 11 September 2015

Selected presentations: T01 – T42

(the PXXX number indicates an accompanying poster presentation)

T01 Atrial & ventricular stem cells from same heart show different functional competencies for cardiac repair but are superior to endothelial progenitors
Parul Dixit

T02 Cardiomyogenesis of human pluripotent stem cells is associated with transformation of metabolism and expression of specific PDLIM5 splice variants
Sarah A. Konze

T03 Gene modification in stem cells targeting megakaryopoiesis
Lisette Johana Latorre Rey

T04 A novel cell surface marker for the enrichment of transplantable dopaminergic precursor cells from differentiated human pluripotent stem cells
Daniela Lehnen

T05 Foxd3 promotes the exit from naïve pluripotency and prevents germline specification through enhancer decommissioning
Alvaro Rada-Iglesias

T06/P080 Ground state conditions induce rapid reorganization of core pluripotency factor binding before global epigenetic reprogramming
Christina Galonska

T07/P086 TDP-43 safeguards pluripotency by regulation of alternative polyadenylation
Miha Modic

T08 LncRNA Panct1 maintains mouse embryonic stem cell identity by recruiting TOBF1 to OCT-SOX sequences in early G1
Debojyoti Chakraborty

T09/P082 Decoding the heterogeneity of pluripotent stem cells at the single cell level
Simon Hastreiter

T10 Morpheus: computational modeling for stem cell biologists
Walter de Back

T11 Inference of spatiotemporal effects on cellular state transitions from time-lapse microscopy
Michael Strasser

T12 Prospective identification of hematopoietic lineage choice
Carsten Marr

T13 On the quantification of hematopoietic tissue remodeling in aging and disease: simulations, measures and predictions
Christoph Baldow

T14 Infection, innate immune signalling and cancer in the stomach – stem cell-derived organoids as a new model
Sina Bartfeld

T15/P071 Modelling reactive gliosis in mESC-derived retina organoids
Manuela Völkner

T16 Patient iPSC-derived NPCs as a model for mtDNA mutations reveal HCS/HTS-suitable phenotypes
Raul Bukowiecki

T17 Intracellular calcium-release and autophagy modulation trigger ataxin-3 positive nuclear inclusions in Machado-Joseph disease-specific neurons
Johannes Jungverdorben

T18 Epigenetic age-predictions in cancer: Aging signatures are associated with malignant transformation and clinical prognosis
Wolfgang Wagner

T19/P133 Triple-negative inflammatory breast cancer: Syndecan-1 is a biomarker and regulator of cancer stem cell phenotype via IL-6/STAT-3 and Notch-3
Sherif A. Ibrahim

T20 Targeting tumor-initiating stem cells in colorectal carcinoma
Ilaria Lunger

T21 Unravelling the subclonal architecture of adult B-ALL at a single cell level
Bartosch Wojcik

T22 Understanding the curious case of trophoblast differentiation
Micha Drukker

T23 The Ca2+-regulated phosphatase calcineurin controls proportional growth of zebrafish regenerating fins
Christopher Antos

T24 Antagonistic roles of miR-181a and orphan nuclear receptor GCNF in regulating human neural stem cell maintenance
Laura Stappert

T25 Direct Nkx2-5 transcriptional repression of Isl1 controls cardiomyocyte subtype identity
Jessica Haas

T26 Hypoxia induced epigenetic mechanism underlying Isl1+ cardiac progenitor cell homeostasis control
Xuejun Yuan

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Abstract No. T01

Atrial & ventricular stem cells from same heart show different functional competencies for cardiac repair but are superior to endothelial progenitors

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Introduction:
Deciding the best cell type for cardiac regeneration still remains a challenge, however, no studies have directly compared the functional efficacy of cardiac stem cells (CSCs) with extra-cardiac SCs isolated from the same patient. Even within the heart, atrial CSCs and ventricular CSCs may have different functional properties relevant to cardiac repair. In this study the functional characteristics of endothelial progenitor cells (EPCs), right atrial (RAA) CSCs and left ventricular (LV) CSCs isolated from the same patients were compared.

Method & Results:
Flowcytometry analysis of CSCs (n=12 patients) revealed higher expression of CD90 (involved in cell adhesion and migration) in RAA CSCs compared to LV CSCs. However, scratch assay showed superior wound closure in LV CSCs suggesting that LV CSCs have a better migration potential. Moreover, the expansion properties of RAA CSCs were better than LV CSCs in terms of higher cumulative population doublings. To simulate *in vivo* ischemic environment, cells were subjected to hypoxia and serum starvation. RAA and LV CSCs exhibited similar pattern of resistance to apoptotic cell death (evaluated as percentage of annexin +ve cells and proliferation under hypoxia. Interestingly, EPCs exhibited highest resistance to apoptotic, but they also showed the lowest proliferation under hypoxia. Secretion of paracrine factors is an important mechanism behind cardiac regeneration. Therefore, to compare the paracrine angiogenic and wound healing potential, HUVECs were cultured in culture supernatant (CS) from all the 3 cell types. RAA-CS showed the highest increase in tube formation capacity, while the scratch assay showed comparable wound closure with both LV and RAA CS, but was lowest in EPC CS. The secretion of the angiogenic factor VEGF-A was lowest in EPCs and comparable between RAA and LV CSCs. The results suggest that the three cell types are different in functional properties even within the same patient.

Dixit P, Katare R: Challenges in identifying the best source of stem cells for cardiac regeneration therapy. Stem Cell Res Ther 2015, 6:26.
Gnecchi M, He H, Liang O, Melo L, Morello F, Mu H, Noiseux N, Zhang L, Pratt R, Ingwall J, Dzau V: Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. Nat Med 2005, 11:367 - 368

Abstract No. T02

Cardiomyogenesis of human pluripotent stem cells is associated with transformation of metabolism and expression of specific PDLIM5 splice variants

Sarah A. Konze[*], *Ruth Olmer*, *Henning Kempf*, *Sebastian Werneburg*, *Andreas Pich*, *Robert Zweigerdt*, and *Falk F. R. Buettner*

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Human pluripotent stem cells (hPSCs), both embryonic (hESCs) and induced (hiPSCs), can be differentiated into derivatives of all germ layers and are promising tools in regenerative medicine. Coronary heart diseases rank top in the list of death-causing diseases worldwide, particularly in industrialized countries. Cell replacement therapies using *in vitro* differentiated cardiomyocytes would provide a promising perspective to cure patients in the future. Understanding the molecular mechanisms during differentiation is a prerequisite for improvement of differentiation protocols and standardized cardiomyocyte production. By stable isotope labeling of amino acids in cell culture (SILAC), we quantitatively assessed differences in the proteomes of hPSCs and cardiomyocytes derived thereof (hPSC-CMs). Over 3500 proteins were identified in this approach, of which approximately 700 and 400 were significantly more than 1.5-fold up- and down-regulated, respectively, from hPSCs to hPSC-CMs. We observed a strong up-regulation of enzymes involved in glycolysis and pentose phosphate pathway in the differentiated cardiomyocytes. In contrast, enzymes involved in oxidative phosphorylation were down-regulated at the level of embryoid bodies but reconstituted in pure CMs, suggesting an unexpected switch from aerobic to (temporarily) anaerobic glucose metabolism during differentiation. Additionally, when looking at specific proteins that were up-regulated in cardiomyocytes, the PDZ and LIM domain protein 5 (PDLIM5), a scaffold protein known to be expressed in various cell type-specific isoforms, attracted our attention. By qPCR and western blot analysis we found that the shortened isoform g (corresponding to transcript variant 7 on the mRNA level) of PDLIM5 is up-regulated at the same time as expression of the early cardiac transcription factor NKX2.5 is first detectable, suggesting a specific role of this PDLIM5 isoform in the early differentiation steps towards cardiomyocytes.

Abstract No. T03

Gene modification in stem cells targeting megakaryopoiesis

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Platelets are essential for hemostasis and play important roles in inflammation and angiogenesis. Reduction in platelet numbers or defects in their function cause thrombocytopenias or thrombopathias. Gene correction or addition may cure platelet disorders and enables the development of models to study the underlying causes of disease.

As platelets are anuclear blood cells, the genetic modification has to take place in the nucleated precursor cells, the hematopoietic stem cells (HSC) or even the pluripotent stem cells (PSC). The transplantation of in-vitro transduced HSC allows the modification of megakaryopoiesis and platelets in-vivo. To avoid ectopic expression in other blood cells, expression should be restricted to MK. We developed third generation lentiviral vectors for MK-specific expression by the use of lineage-specific promoters of the platelet-specific human glycoprotein (GP) Iba, hGP6 and hGP9 and murine platelet factor 4 (mPf4) genes. Specificity of expression was investigated in-vitro in MK differentiation assays and *in vivo* after bone marrow transplantation in the mature blood lineages and the stem cell compartment. GFP expression in culture-derived MK was 3-6 fold increased from MK-specific compared to the PGK control vector and lower in non-megakaryocytic progenitors (less than 6 %). The mPf4 and hGPIba promoters showed unexpected high activity in HSC, while the hGP6 promoter expressed most MK-restricted. The specificity also remained in a shorter, 348bp hGP6 promoter fragment.

A potential source for transfusions are PSC-derived platelets, however, their *in vitro* generation is still inefficient. We established *in vitro* megakaryocyte/platelet differentiation from murine PSC with >30% platlelet/MK specific, CD41+/CD42d+ cells and ultrastructurally confirmed platelets. Megakaryopoiesis-specific transcription factors or modulators could be expressed to support differentiation, but expression has to be tightly controlled. We therefore explore the use of tet-responsive or lineage-specific vectors for the inducible or restricted expression during differentiation also in combination with the UCOE element to prevent vector silencing.

Abstract No. T04

A novel cell surface marker for the enrichment of transplantable dopaminergic precursor cells from differentiated human pluripotent stem cells

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Parkinson's Disease (PD) is the second most frequent neurodegenerative disorder, mainly caused by death of dopaminergic (DA) neurons in the ventral mesencephalon (VM). Cell replacement therapies with fetal VM DA neurons can be beneficial for PD patients. However, as fetal tissue is restricted, embryonic stem cells or induced pluripotent stem cells (hiPSC) are considered as an alternative source for the derivation of DA neurons for future cell therapy of PD. Current differentiation protocols result in 40 – 90 % DA neurons. Additional sorting could help to increase the purity of cells and standardize the cell manufacturing process.

We performed a flow cytometry screening to find sortable cell surface antigens correlating with a mesencephalic DA (mesDA) phenotype. DA neurons were generated from hiPSC using a combined embryoid-body / dual SMAD inhibition protocol. As mesDA neurons arise from the midbrain floor plate we screened antibodies for co-labelling of cell surface epitopes with FOXA2, a key transcription factor. Among the hits one marker was co-expressed with FOXA2 and suitable to isolate mesDA progenitors utilizing MACS® Technology.

Sorted cells were viable, turned into mature dopaminergic neurons *in vitro* and - as analyzed by flow cytometry, whole genome expression profiling and RT-PCR - were enriched for midbrain specific markers and devoid of pluripotency markers.

Immunomagnetic isolation of differentiated hiPSCs prior to intrastriatal transplantation in a rat model of PD did not affect graft survival. Grafts of sorted mesDA cells were less proliferative and gave rise to dopaminergic neurons with a more mature phenotype as compared to those of unsorted cells. After comparison of different sorting and transplantation regimens, longterm behavioral studies in 6-OHDA-PD rat model are currently ongoing.

In conclusion, we describe a novel cell surface marker which might be used as a tool to purify mesDA progenitor cells for regenerative therapy of PD patients.

Abstract No. T05

Foxd3 promotes the exit from naïve pluripotency and prevents germline specification through enhancer decommissioning

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Following implantation, mouse epiblast cells transit from a naïve to a primed state in which they are competent for both somatic and primordial germ cell (PGC) specification. Using mouse embryonic stem cells (mESC) as an *in vitro* model to study the transcriptional regulatory principles orchestrating peri-implantation development, here we show that the transcription factor Foxd3 is necessary for the exit from naïve pluripotency and the progression to a primed pluripotent state. During this transition, Foxd3 acts as a repressor that dismantles a significant fraction of the naïve pluripotency expression program through the decommissioning of active enhancers associated with key naïve pluripotency and early germline genes. Subsequently, Foxd3 needs to be silenced in primed pluripotent cells to allow the reactivation of relevant genes required for proper PGC specification. Our findings uncover a wave of activation-deactivation of Foxd3, crucial for the exit from naïve pluripotency and subsequent PGC specification.

Rada-Iglesias, A., Bajpai, R., Swigut, T., Brugmann, S.A., Flynn, R.A., and Wysocka, J. (2011). A unique chromatin signature uncovers early developmental enhancers in humans. Nature 470, 279–283.

Rada-Iglesias A, Bajpai R, Prescott R, Brugmann S, Swigut T and Wysocka J. Epigenomic mapping annotates human neural crest enhancers and predicts a new role for nuclear receptors NR2F1/2. Cell Stem Cell 2012 Nov 2;11(5):633-48.

Abstract No. T06/P080

Ground state conditions induce rapid reorganization of core pluripotency factor binding before global epigenetic reprogramming

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Mouse embryonic stem cells (mESCs) cultured under serum/LIF conditions exhibit heterogeneous expression of pluripotency-associated factors that can be overcome by two inhibitors (2i) of the MEK and GSK3 pathway, respectively. Several studies have shown that the "ground state" induced by 2i is characterized by global hypomethylation and specific transcriptional profiles, but little is known about the contributing effectors. Here we show that 2i conditions rapidly alter the global binding landscape of OCT4, SOX2 and NANOG. The dynamic binding influences enhancer activity and shows enrichment for regulators linked to Wnt and Erk signaling. Epigenomic characterization provided limited insights to the immediate transcriptional dynamics suggesting these are likely more secondary effects. Likewise, knock-out of the PRC2 component EED to prevent H3K27me3 deposition had minimal effect on the transcriptome, rendering it largely dispensable for continued repression of bivalent genes and de novo silencing in 2i.

Abstract No. T07/P086

TDP-43 safeguards pluripotency by regulation of alternative polyadenylation

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Alternative polyadenylation (APA) of RNAs is increasingly being recognized as important posttranscriptional mechanism governing gene dosage that is essential for pluripotent stem cell (PSC) differentiation. Over half of mammalian genes harbor multiple polyadenylation sites that regulate translation and RNA localization. APA-pattern is dynamically regulated during early PSC differentiation and reprogramming. However, the factors regulating APA are still poorly understood. We elucidated a novel TDP-43 function in global regulation of APA, role of APA in paraspeckle formation and its connection to the differentiation of PSCs.

We show that unique subnuclear structures termed paraspeckles are formed independent of lineage type during exit from the pluripotency and are important for stabilization of the early commitment state. The long nonpolyadenylated isoform of non-coding RNA NEAT1 serves as the scaffold for paraspeckles assembly produced only upon differentiation. Undifferentiated PSCs express only the short NEAT1 isoform that doesn't polymerize paraspeckles. Importantly, we present evidence that TDP-43 maintains production of nucleoplasmic NEAT1 short isoform by directly regulating APA of nascent NEAT1 transcripts in undifferentiated cells, and that TDP-43 itself is sequestered in paraspeckles upon differentiation leading to formation of NEAT1 long isoform and paraspeckle assembly. This indicates the existence of a feed-forward feedback mechanism underlying paraspeckle assembly during onset of differentiation. Furthermore, we analyzed how paraspeckle assembly regulates exit from pluripotency; we show that cells lacking NEAT1 and paraspeckle proteins exhibit severe developmental delay. Together, this work implicates developmental regulation of APA in RNA-mediated nuclear restructuring leading to stabilization of the early differentiation state and pluripotency breakdown.

Abstract No. T08

LncRNA Panct1 maintains mouse embryonic stem cell identity by recruiting TOBF1 to OCT-SOX sequences in early G1

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Long noncoding RNAs (lncRNAs) are implicated in diverse biological processes including regulation of pluripotency and several lncRNAs have been catalogued to be important for embryonic stem (ES) cell maintenance. However, the functions of lncRNAs remain largely undefined. Here we show that the lncRNA Panct1 is required for ES cell identity by transiently recruiting the putative X-chromosome encoded protein TOBF1 (Transient Octamer Binding Factor 1) to genomic sites that resemble the canonical Oct-Sox motif. Knockdown by RNAi or Cre/LoxP mediated conditional knockout of Panct1 prevents recruitment of TOBF1 to its DNA binding sites, which subsequently leads to downregulation of OCT4 expression and exit from pluripotency. Strikingly, an octamer-like motif present in Panct1 mRNA is requited for localizing TOBF1 to promoters of pluripotency genes during a short time window immediately following mitosis. Our data reveal a tightly coordinated spatial and temporal pattern of gene regulation guided by lncRNA-mediated recruitment of a protein to chromatin in a cell-cycle dependent manner and suggest that lncRNAs can function as barcodes for identifying genomic addresses to maintain cellular states.

Chakraborty D., Paszkowski-Rogacz M., Berger N., Ding L., Mircetic J. et al. Nature (under revision)
Chakraborty D. Kappei D., Theis M., Nitzsche A.,Ding L. et al. Nature Methods (2012)

Abstract No. T09/P082

Decoding the heterogeneity of pluripotent stem cells at the single cell level

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Pluripotent stem cells have the unique ability to both self-renew indefinitely and to differentiate into all cell lineages of the embryo. The maintenance of pluripotency is controlled by a network of transcription factors and epigenetic modifiers centered around Oct4, Sox2 and Nanog which in turn is regulated by extracellular signals. It has been shown that the expression levels of key pluripotency factors like Nanog are highly heterogeneous at the single cell level in mouse embryonic stem cell (ESCs) cultures in standard Serum+LIF conditions. In contrast, dual inhibition of GSK3 and MEK signaling (2i) converts the culture into a more homogeneous ground state.

So far, it is still elusive how pluripotency factors regulate each other in single cells and what the dynamics and significance of their heterogeneity are. In this study we found two functionally distinct subpopulations of low Nanog expressing cells and distinguish selective and inductive effects of the 2i condition on Nanog heterogeneity. We further aim to decipher the regulatory logic in single cells by manipulating the pluripotency network and linking cellular dynamics to future fate choices. Long-term continuous time-lapse imaging of knock-in reporter cell lines enables us to quantitatively measure endogenous protein levels of key pluripotency factors in single ESCs over many days and microfluidic devices will allow us to precisely stimulate single cells and observe their immediate responses.

With our approach the cellular dynamics of ESC self-renewal and differentiation can be analysed at an unprecedented resolution and the data provide a rich resource for the development of accurate computational models of network dynamics.

Abstract No. T10

Morpheus: computational modeling for stem cell biologists

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TU Dresden
*Presenting author

Computational modeling is already an important tool to understand and predict the stability and heterogeneity of stem cells. The increasing interest in spatial organization and interactions within the stem cell niche makes the need for modeling even more pronounced. However, the complexity of constructing suitable computer simulations hampers the accessibility of these tools.

Morpheus is a modeling environment for the simulation of multiscale and multicellular systems that has been developed with a focus on usability [1]. It supports the modeling of interactions between genes, signals and cells in terms of ordinary and stochastic differential equations, reaction-diffusion models and cell-based models. All these models can be coupled to explore feedbacks between the scales. Using the modeling environment does not require programming expertise, as models are constructed and simulated through an intuitive graphical user interface.

Morpheus has for instance been used to study cell fate decisions and reprogramming in pancreatic cells and spatial patterning in colonies of embryonic stem cells. It is also used in education, e.g. in a GSCN workshop teaching computational modeling to stem cell biologists.

Morpheus is freely available as a desktop application for Windows, Mac OSX and Linux from the Website [2].

[1] J. Starruß, W. de Back, L. Brusch and A. Deutsch. Morpheus: a user-friendly modeling environment for multiscale and multicellular systems biology. Bioinformatics, 30(9):1331- 1332, 2014.
[2] More information and download: http://imc.zih.tu-dresden.de/wiki/morpheus

Abstract No. T11

Inference of spatiotemporal effects on cellular state transitions from time-lapse microscopy

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Time-lapse microscopy allows to monitor cell state transitions, e.g. differentiation, in a spatiotemporal context. Combined with single cell tracking and appropriate cell state markers, transition events can be observed within the genealogical relationship of a proliferating population. However, to understand whether these cell fate choices are determined by cell-intrinsic processes (e.g. fluctuations in the gene regulatory network) or cell-extrinsic queues (e.g. cell-cell interactions), statistical analysis of these transition events observed in the experiments is required. We present a method to infer spatiotemporal features predictive of the state transition events observed in time-lapse microscopy data. We first formulate a generative model, simulate different scenarios, such as time-dependent or local cell density-dependent transitions, and illustrate how to estimate univariate transition rates. Second, we formulate the problem in a machine-learning language using regularized linear models. This allows for a multivariate analysis and to disentangle indirect dependencies via feature selection. Finally, we explicitly use the genealogical structure of the data to validate if the estimated model is sufficient to explain correlated transition events of sister cells. Our method can accurately recover the relevant features and reconstruct the underlying interaction kernels if a critical number of samples is available. As an outlook we discuss then necessary steps for the method's application to time-lapse experiments of differentiating hematopoietic stem cells.

Abstract No. T12

Prospective identification of hematopoietic lineage choice

Felix Buggenthin[1], *Florian Buettner*[1], *Manuel Kroiss*[1], *Michael Strasser*[1], *Michael Schwarzfischer*[1], *Philipp Hoppe*[2], *Timm Schroeder*[2], *Fabian Theis*[1], and *Carsten Marr*[1,*]

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Differentiation alters the molecular properties of a stem cell, but also its shape and movement characteristics. We present a deep neural network that prospectively predicts lineage choice in hematopoietic progenitor cells up to three generations before conventional molecular lineage markers, using only raw time-resolved brightfield image patches and cell speed. Our approach allows identifying groups of cells with differentially expressed lineage-specifying genes without molecular labeling.

Abstract No. T13

On the quantification of hematopoietic tissue remodeling in aging and disease: simulations, measures and predictions.

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Aging is most apparent on the tissue level as a progressive decline of functional ability. Although a wide range of molecular mechanisms has been identified that are closely connected to cellular aging, the translation on the phenotypic level of the overall tissue is rarely understood. The distinct age-related increase in the incidence of hematopoietic diseases, such as myeloproliferative neoplasms and leukemias, has long been associated with alterations of hematopoietic tissue structure with age. Novel methods for cell fate analysis, such as the use of genetic barcodes, allow assessing the clonal architecture of hematopoiesis even in in-vivo situations. However, it is still a challenging problem to quantify clonal contributions over time and, therefore, to estimate and predict future developments.

We developed a simple mathematical model of a self-stabilizing hematopoietic stem cell population to generate a wide range of possible clonal developments, reproducing typical, experimentally and clinically observed scenarios. We use the resulting model scenarios to suggest and test a set of statistical measures that should allow for an interpretation and classification of relevant clonal dynamics. We identified a measure of clonal suppression to sensitively detect the onset of exponential growth in a poly- clonal setting and report on our results to which extent this and other measures are suitable to prospectively estimate atypical developments.

Additionally to the insights into structural principles of age-related tissue remodeling, our effort to establish a reliable classification of pathological and non-pathological clonal dynamics has a direct potential for clinical applications. Leukemogenesis is a well-known and severe problem in gene therapy patients. Based on the tight post-therapy monitoring of clonal devel- opments in these patients our identified measures and the resulting categorisation can aggregate time course data and provide estimates for the risk of atypical clonal developments and predict the manifestation of leukemia.

Alice Gerrits et al.: Cellular barcoding tool for clonal analysis in the hematopoietic system. Blood 2010
Leonid V Bystrykh et al.: Counting stem cells: methodological constraints. Nature Methods 2012

Abstract No. T14

Infection, innate immune signalling and cancer in the stomach – stem cell-derived organoids as a new model

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Gastric cancer is the third leading cause of cancer deaths worldwide. Gastric cancer originates from stem cells and the development is causally related to chronic infection with the bacterium *Helicobacter pylori*. The infection drives inflammation via innate immune signaling.

Troy and Lgr5 are Wnt target genes specifically expressed at the bottom of gastric glands. While Lgr5 marks stem cells in the antrum, in the corpus Troy and Lgr5 together mark a subpopulation of differentiated chief cells that can regain stem cell capacity upon damage.

Mouse and human gastric stem cells can initiate ever-expanding 3 dimensional organoids *in vitro*. The organoids harbor stem cells, progenitor cells as well as four lineages of the stomach. Infection of organoids with *Helicobacter pylori* reveals cell-type specific inflammatory responses.

Organoids grown from tumors and matched healthy tissue allow evaluation of drug responses. Organoids may bridge the gap between cancer genetics and patient trials and allow personalized medicine.

Abstract No. T15/P071

Modelling reactive gliosis in mESC-derived retina organoids

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Pluripotent stem cell derived retinal organoids are a new experimental model system for studies on retinal degeneration and regeneration. Here we started to develop a retinal organoid-based model to study reactive gliosis, a glial response frequently associated with neuronal damage and disease.

We first characterized the developmental and molecular state of radial Müller glia (MG), the major type of glia in the retina, in the organoid system. We observed the first MG in developing retina organoids on day 18 and postmitotic MG by day 22. Next, we characterized cell death in the mESC-derived retina organoid system using TUNEL assay. Cell death increases with ongoing neurogenesis, and photoreceptors and bipolar cells (8% ± 5 SEM) are less affected compared to amacrine and ganglion cells (25% ± 1 SEM). Strikingly, MG in retina organoids do not express glial fibrillary acidic protein (GFAP), a hallmark of reactive gliosis, or other known gliosis associated markers in response to the organoid system immanent cell death.

Reactive gliosis does not occur due to ontogenetic cell death *in vivo*, but is induced upon retina explant culture. We hypothesized that the organoid system offers a unique opportunity to study reactive gliosis ex vivo. Thus, we asked if reactive gliosis can be induced in the retina organiod system. Strikingly, application of neuronal damage-related signaling factor TNF on day 20 induced GFAP expression in MG, increased cell proliferation, as well as a loss of retinal stratification within 5 days.

In sum, our data suggests that MG in the retinal organic system become quiescent and non-reactive at the end of retinogenesis. Stimulation of retinal organoids with TNF is sufficient to induce MG reactive gliosis and a retinal pathologic phenotype. We conclude, the organoid system offers a new model system and unique opportunity to study the mechanisms of reactive gliosis *ex vivo*.

Abstract No. T16

Patient iPSC-derived NPCs as a model for mtDNA mutations reveal HCS/HTS-suitable phenotypes.

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Mitochondria fulfill a central role in the cellular energy metabolism. Since the editing the mitochondrial DNA (mtDNA) by traditional gene targeting approaches is inefficient there is a considerable lack of suitable mitochondrial disease models analysing mtDNA mutations in the respective nuclear genome background. However, patient cells containing the mutated mtDNA as well as the nuclear genome can be reprogrammed to induced pluripotent stem cells (iPSCs) and further differentiated to neuronal cells debilitated by mitochondrial dysfunction (Bukowiecki, 2014).

We generated iPSC clones from control and patient individuals. Furthermore, we adapted a protocol to derive neural progenitor cells (NPCs) that is based on defined media supplemented with small molecules. Therefore, the neural induction is very stable, reproducible, and operator-independent. We extensively characterized our NPCs with electron microscopy and at the bioenergetic as well as transcriptomic level. Analysing also numerous other published NPC populations derived with various protocols, we established a comprehensive comparison that could be relevant for the whole field of stem cell-derived and ex vivo NPCs/NSCs.

All our three patients carry an mtDNA mutation in MT-ATP6. Mutations in this gene -encoding a mitochondrial ATP synthase subunit- are associated with severe infantile maternally inherited Leigh syndrome (MILS). When a certain genotype is predominant throughout the mtDNA copies per cell it can reach a grade of homoplasmy with disease penetrance. Importantly, all our iPSC lines still displayed the same homoplasmy in mtDNA as their parental fibroblasts, even upon prolonged cultivation and differentiation confirmed by mtDNA deep-sequencing. We established high-throughput-scalable screening methods to assess the mitochondrial disease phenotype, i.e. mitochondrial membrane hyperpolarization.

Overall, our NPCs as well as their functional neuronal derivatives could be utilized for high-content screening (HCS). This model could help to unravel molecular pathological mechanisms of disorders in the basal ganglia and to identify novel drug candidates for therapeutic intervention.

Bukowiecki et al., 2014, Gerontology
Prigione, ... , Bukowiecki et al., 2014, Stem Cells

Abstract No. T17

Intracellular calcium-release and autophagy modulation trigger ataxin-3 positive nuclear inclusions in Machado-Joseph disease-specific neurons

Johannes Jungverdorben[1,*], *Peter Breuer*[2], *Philipp Koch*[3], *Ullrich Wüllner*[2], *Michael Peitz*[1], and *Oliver Brüstle*[1]

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Machado-Joseph disease (MJD) or spinocerebellar ataxia type 3 is the most frequent form of inherited spinocerebellar ataxia worldwide. Expansion of a polyQ repeat increases the aggregation propensity of ataxin-3, leading to the formation of ataxin-3-positive inclusions, a hallmark of MJD. Our previous studies employing patient-specific MJD neurons showed that excitation by glutamate or NMDA and subsequent Ca2+ entry via voltage-gated calcium channels activates calpain-mediated cleavage of ataxin-3, which initiates the generation of SDS-insoluble ataxin-3 microaggregates (Koch et al., Nature 480:543-46, 2011). Here we set out to explore whether and to what extent intracellular calcium release contributes to this disease-initiating step. We investigated the early expressed purinergic receptor family for the induction of cytosolic calcium increase and found that ATP-stimulation of P2Y receptors triggered induction of microaggregates as early as 6 days after initiation of neuronal differentiation. Microscopically visible neuronal intranuclear inclusions (NIIs) positive for ataxin-3 and ubiquitin could be detected selectively in ATP-stimulated MJD cultures. Since both glutamate and ATP-mediated aggregation rely on receptor types with largely variable spatio-temporal distribution pattern, we also explored the potential contribution of ubiquitously expressed ryanodine receptors as well as sarco/endoplasmic reticulum calcium-ATPase (SERCA) for their capacity to modulate aggregate induction. Both activation of ryanodine receptors and SERCA inhibition resulted in ataxin-3 NIIs after only 8 days of differentiation with SERCA inhibition yielding >80% MJD neurons carrying ataxin-3-positive aggregates. However, these paradigms still showed high variability of NII induction between independent experiments. We reasoned that this variability might be due to fluctuations in proteasomal degradation and autophagy. Indeed, calibration of media conditions towards inhibition of autophagic flux resulted in robust and disease-specific NII formation in MJD neurons. Our data point to a complex pathogenentic system where dysregulation of intracellular calcium homeostasis and autophagy conspire to initiate the process of protein aggregation in MJD neurons.

Abstract No. T18

Epigenetic age-predictions in cancer: Aging signatures are associated with malignant transformation and clinical prognosis

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Aging is associated with highly reproducible DNA-methylation (DNAm) changes at specific sites in the genome. We have recently demonstrated that such modifications can be used as biomarker for biological aging and that age-associated DNAm patterns are entirely reversed by reprogramming into induced pluripotent stem cells. On the other hand, accumulation of age-associated DNAm may contribute to higher prevalence of malignant diseases in the elderly. In this study, we analyzed epigenetic aging signatures in 5,621 DNAm profiles of 25 cancer types from The Cancer Genome Atlas (TCGA). Overall, age-associated DNAm patterns hardly reflect chronological age of cancer patients, but they are coherently modified in a non-stochastic manner, especially at CpGs that become hypermethylated upon aging in non-malignant tissues. This coordinated regulation in epigenetic aging signatures can therefore be used for aberrant epigenetic age-predictions, which facilitate disease stratification. For example in acute myeloid leukemia (AML) higher epigenetic age-predictions are associated with increased incidence of mutations in RUNX1, WT1, and IDH2, whereas mutations in TET2, TP53, and PML-PARA translocation are more frequent in younger age-predictions. Furthermore, epigenetic aging signatures correlate with overall survival in several types of cancer (such as lower grade glioma, glioblastoma multiforme, esophageal carcinoma, chromophobe renal cell carcinoma, cutaneous melanoma, lung squamous cell carcinoma, and neuroendocrine neoplasms). In conclusion, age-associated DNAm patterns in cancer are not related to chronological age of the patient – they rather capture the epigenetic state of aging from the tumor initiating cell. CpGs that become hypermethylated in normal aging are coherently modified in cancer. Furthermore, the apparent epigenetic age-predictions correlate with clinical parameters and overall survival in several types of cancer, indicating that regulation of DNAm patterns in age-associated CpGs is relevant for cancer development.

Weidner CI, Lin Q, Koch CM, Eisele L, Beier F, Ziegler P, Bauerschlag DO, Jöckel KH, Erbel R, Mühleisen TW, Zenke M, Brümmendorf TH, Wagner W. Aging of blood can be tracked by DNA methylation changes at just three CpG sites. Genome Biol 2014;15:R24.

Wagner W, Weidner CI, Lin Q. Do age-associated DNA methylation changes increase the risk of malignant transformation? Bioessays 2015;37:20-24.

Abstract No. T19/P133

Triple-negative inflammatory breast cancer: Syndecan-1 is a biomarker and regulator of cancer stem cell phenotype via IL-6/STAT-3 and Notch-3

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Syndecan-1 (Sdc-1) is a heparan sulfate proteoglycan that is highly expressed on the surface of epithelial cells and a corecetor for growth factors and chemokines modulating tumor adhesion, invasion, and metastasis. Recently, we addressed the potential role of Syndecan-1 in regulating breast cancer stem cells via regulation of LRP-6 and IL-6-mediated STAT3 signaling. Inflammatory breast cancer (IBC) is a particularly aggressive form of breast cancer and posses a chemo- and radio-resistant stem cell phenotype. Herein, we characterized the expression level of Sdc-1 and cancer stem cell marker CD44 in triple negative IBC (n=15) and non-IBC (n=15) patients. Moreover, we deciphered the functional impact of Syndecan-1 knockdown *in vitro* using RNA interference on the breast cancer stem cell phenotype of the triple negative IBC Sum-149 cells and HER-overexpressing non-IBC SKBR-3 cells employing real time PCR, flow cytometry and western blot. Our data indicate upregulation of Sdc-1 and CD44 in triple negative IBC compared to those of non-IBC patients. Aldehyde dehydrogenase-1 activity revealed that Syndecan-1 knockdown Sum-149 and SkBR-3 cells exhibited significantly reduced putative cancer stem cell pools by 5% compared to controls. In Sum149 cells, the CD44(+)CD24(-/low) phenotype decreased significantly by 29% upon siRNA-mediated Syndecan-1 depletion. Intriguingly, qPCR results indicate downregulation of IL-6, CCL20, gp-130, Notch-1, -3 and -4 by 20-30% in Sdc-1-silenced Sum-149 cells compared to controls. Furthermore, activation of STAT-3 and NFkB transcription factors was reduced by >30% in Syndecan-1-depleted cells compared to controls. At the functional level, Syndecan-1-silenced Sum-149 and SKBR-3 cells exhibited reduced colony formation by 40-50% compared to controls. In conclusion, Syndecan-1 acts as a biomarker and a modulator of cancer stem cell phenotype for triple negative IBC via IL-6/STAT3 and Notch-3 signaling pathway, thus it emerges as a promising novel target for therapeutic approaches in IBC patients.

Abstract No. T20

Targeting tumor-initiating stem cells in colorectal carcinoma

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Colorectal cancer (CRC) is the second most common cause of tumor-related mortality in Germany. Despite advances in conventional treatment, a high proportion of patients develop a recurrence of the disease and the 5-year overall survival is only about 50%. Recent findings establish colon cancer as a stem cell-driven disease. Only certain cancer cells, so-called tumor-initiating stem cells (TICs), are responsible for the development, persistence and therapy resistance of the tumor.

The purpose of our study is to identify surface markers and target molecules for a TIC-directed diagnosis and therapy. We identified a very aggressive TIC population of colon cancer SW480 cells with a strong capacity to initiate tumor growth *in vivo*. SILAC-based proteomics detected distinct molecular differences between the aggressive TIC population and the bulk of tumor cells and revealed potential markers for TICs enrichment and targets for cellular and molecular therapies.

Screening small molecule inhibitors against these targets (e.g. AKT inhibition by MK-2206) determined their potency as potential therapeutics by decreasing CRC cell and TIC proliferation. More important, increased apoptosis was observed particularly in the TIC fraction, leading to a significant reduction of tumorsphere formation of CRC patient material. Furthermore, we showed that a pretreatment of tumor cells with inhibitors reduced significantly the stemness capacity to initiate tumors *in vivo*. The *in vivo* treatment of mice with established CRC tumors resulted in a severe reduction of tumor progression. Knockdown experiments confirmed the target selectivity and showed a reduction of tumor cell proliferation, an increased cell death *in vitro*, and a significant reduction of the TIC capacity to initiate tumor formation *in vivo*.

Taken together, our research strategy has revealed promising targets for specific elimination of TICs for advanced CSC treatment.

Abstract No. T21

Unravelling the subclonal architecture of adult B-ALL at a single cell level

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The existence of a leukemic hierarchy and a stable phenotype of leukemia initiating (stem) cells (LICs) in acute lymphoblastic leukemia (ALL) remain controversial. This is in contrast to the findings in acute myeloid leukemia, where the immune phenotype of the LIC-enriched fraction has been already described. Despite the proven subclonal composition of ALL, the investigation of the genealogy and molecular composition of functionally defined subclones, that may be unrelated to their surface marker phenotype, require stable maintenance of cellular identity in culture systems. Since cell lines do not represent the heterogeneity of the disease and there is a general difficulty to culture primary ALL patient material, solving questions regarding plasticity of marker expression, cancer stem cell hierarchy or clonal evolution theory remain hampered.

We established a patient-derived ALL culture system with a stable karyotype for 6 months to isolate distinct clonal subpopulations from the same patient. Within these patient-derived ALL cultures, we identified single cell clones, showing both stable and plastic surface marker profiles. Some subclones are highly leukemogenic in immunocompromised NSG mice, and others from the same patient with the same prospective phenotype, have low or no leukemogenic potential *in vivo*. Quantitative proteomics of these subclones with high and low leukemogenicity revealed distinct differentially-regulated pathways which might open new therapeutic windows for treatment of highly aggressive LICs in ALL. Further, new surface markers and molecular mechanisms which are directly correlated to the aggressive phenotype will help to prospectively identify and specifically target LICs to improve therapy and minimize therapy related side effects.

Abstract No. T22

Understanding the curious case of trophoblast differentiation

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The emergence of trophoblast-like cells during the course of human pluripotent stem cell (PSC) differentiation is puzzling. This is since trophoblasts are NOT among the known progeny of pluripotent cells in the human embryo. By performing genome scale transcriptional and epigenomic analysis of consecutive trophoblast progenitor stages derived and purified from human PSCs using surface markers CD249 (APA) and SSEA-5, we identified a putative gene activation cascade that establishes the trophoblast fate from human PSCs. The cascade consists of activating protein 2 (AP-2) and GATA transcription factors, which are induced directly by TGF-beta signaling in response to BMP4 treatment, and that subsequently regulate induction of a second layer set of novel and known trophoblast fate promoting genes. We have validated this cascade thorough shRNA analysis. Furthermore, we show that the promoters of the initial genes in the cascade are poised for rapid activation in PSCs (exhibiting H3K4me3 H3K27me3 marks). Conversely, the second layer set of genes, which likely finalizes the commitment of the cells to the trophoblast lineage, are marked by methylation in human PSCs, and the canonical histone repressive modification H3K27me3 is not present in their promoters. We speculate that the “dormancy” of trophoblast commitment genes in human PSCs represents an attempt to silence a pathway leading to placental development, but this can be overridden by TGF-beta signaling *in vitro*. This work provides a putative mechanistic explanation for trophoblast differentiation from human PSCs, and grounds for understanding epigenetic priming during PSC commitment.

Abstract No. T23

The Ca2+-regulated phosphatase calcineurin controls proportional growth of zebrafish regenerating fins

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Animals that regenerate organs and appendages control the growth of stem and progenitor cells to reform lost structures to the same dimensions as the original structures. This proportional regeneration involves coordinating rapid allometric (disproportional) growth with the restoration of isometric (proportional) cell proliferation once the correct tissue dimensions are reached. It is unknown what executes this coordinated control. We show that the calcium-dependent phosphatase calcineurin regulates this control. Calcineurin inhibition results in continued allometric outgrowth of regenerating fins beyond their original dimensions. Congruent with these results, calcineurin activity is low when the rate of progenitor cell proliferation is highest, and its activity increases as the regeneration rate decreases. Furthermore, inhibition of calcineurin in uninjured adult fins switches isometric growth into allometric growth, demonstrating that calcineurin regulates appendage allometry. Previous results show that the rate of regenerative outgrowth is controlled by position along the proximodistal axis, but it is unknown what this positional control is. Our growth rate measurements and morphometric analysis of proximodistal asymmetry indicate that calcineurin inhibition shifts fin regeneration from a distal isometric growth program to an allometric proximal program. This shift is associated with the promotion of retinoic acid signaling, a signal transduction mechanism that affects positional information along the proximodistal axis. Furthermore, we provide evidence that calcineurin regulates potassium conductance by interacting and regulating a potassium leak channel that has been shown to promote allometric growth. In summary, we identified a calcineurin-mediated mechanism that operates as a molecular switch between distal isometric growth and proximal allometric growth.

Abstract No. T24

Antagonistic roles of miR-181a and orphan nuclear receptor GCNF in regulating human neural stem cell maintenance

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Based on experiments in human embryonic stem cell-derived neuroepithelial-like stem (lt-NES) cells, we have previously demonstrated that miR-181a promotes human neuronal differentiation (Stappert et al. PloS One, 2013). Here, we describe that this effect is partially caused by miR-181a-mediated targeting of GCNF/NR6A1. GCNF is an orphan nuclear receptor known for its function as transcriptional repressor of pluripotency genes. The data presented here, however, point to a yet unrecognized role of GCNF in regulating neural stem cell maintenance. Knockdown of GCNF in lt-NES cells promoted neuronal differentiation, similar to the effect of miR-181 gain-of-function, and was sufficient to counteract the effects of miR-181a inhibition. In turn, sustained expression of GCNF interfered with neuronal differentiation, while preserving the neural rosette morphology and other characteristic features of undifferentiated lt-NES cells. To further assess the potency of GCNF in repressing neuronal differentiation, we pushed the cells towards differentiation by interfering with Notch signaling. Treatment with the Notch inhibitor DAPT was not able to compensate for the impaired neuronal differentiation induced by ectopic GCNF. Down-stream analysis revealed that GCNF might, in fact, act in parallel to Notch signaling to maintain lt-NES cells in an undifferentiated state by preventing premature expression of neurogenic genes. Taken together, this study uncovers a mechanistic role of miR-181a and GCNF in regulating human neural stem cell fate, thus providing new prospects for maintaining and expanding human neural stem cells *in vitro*.

Abstract No. T25

Direct Nkx2-5 transcriptional repression of Isl1 controls cardiomyocyte subtype identity

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During cardiogenesis, most myocytes arise from cardiac progenitors expressing the transcription factors Isl1 and Nkx2-5. Here, we show that a direct repression of *Isl1* by Nkx2-5 is necessary for proper development of the ventricular myocardial lineage. Overexpression of Nkx2-5 in mouse embryonic stem cells (ESCs) delayed specification of cardiac progenitors and inhibited expression of *Isl1* and its downstream targets in Isl1+ precursors. Embryos deficient for Nkx2-5 in the Isl1+ lineage failed to downregulate Isl1 protein in cardiomyocytes of the heart tube. We demonstrated that Nkx2-5 directly binds to an *Isl1* enhancer and represses *Isl1* transcriptional activity. Furthermore, we showed that overexpression of Isl1 does not prevent cardiac differentiation of ESCs and in *Xenopus laevis* embryos. Instead, it leads to enhanced specification of cardiac progenitors, earlier cardiac differentiation, and increased cardiomyocyte number. Functional and molecular characterization of Isl1-overexpressing cardiomyocytes revealed higher beating frequencies in both ESC-derived contracting areas and *Xenopus* Isl1-gain-of-function hearts, which associated with upregulation of nodal-specific genes and downregulation of transcripts of working myocardium. Immunocytochemistry of cardiomyocyte lineage-specific markers demonstrated a reduction of ventricular cells and an increase of cells expressing the pacemaker channel Hcn4. Finally, optical action potential imaging of single cardiomyocytes combined with pharmacological approaches proved that Isl1 overexpression in ESCs resulted in normally electrophysiologically functional cells, highly enriched in the nodal subtype at the expense of the ventricular lineage. Our findings provide an Isl1/Nkx2-5-mediated mechanism that coordinately regulates the specification of cardiac progenitors toward the different myocardial lineages and ensures proper acquisition of myocyte subtype identity.

Dorn T, et al. STEM CELLS. Apr;33(4):1113-29 (2015)

Abstract No. T26

Hypoxia induced epigenetic mechanism underlying Isl1+ cardiac progenitor cell homeostasis control

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Congenital heart defects (CHDs) are the most frequent birth defects, affecting high numbers of live births in the world. Genetic studies have shown that the majority of de novo mutations in CHDs are mutations in epigenetic modifiers, which could function to integrate the environmental signals or cues into gene expression pattern during heart development. Temporal and spatial regulation of oxygen availability is one essential environmental cue for cardiogenesis during embrynonic development, deregulation of which often leads to heart malformation. However the epigenetic mechanism underlying cellular response to oxygen availability regulation and downstream events in CPC during heart development is still elusive. Here we show that while Isl1+ CPCs are physiologically non-hypoxic, earlier physiological and acute non-physiological hypoxia induction inhibits cell proliferation and promotes lineage specification to cardiomyocytes due to inversely regulating the expression of cardiac transcription factor Isl1 and Nkx2.5. Mechanistically hypoxia induction activates class III histone deacetylase (HDAC) Sirt1, silences Isl1 gene expression in a Hes1 dependent manner and relieves class II HDAC mediated Nkx2.5 suppression during Isl1+ CPC lineage specification. Consistently ablation of Sirt1 in Isl1+ CPCs restores reduced cell expansion capacity upon hypoxia induction and rescues acute hypoxia induced heart malformation during heart development. Thus, our findings link hypoxia, co-regulation of Isl1 and Nkx2.5 gene expression and Isl1+ CPC lineage specification during embryonic heart development by an epigenetic relay of class II and Class III histone deacetylases, which might contribute to a better understanding of the pathophysiology of CHDs.

Abstract No. T27/P013

CCND1/CDK4-mediated cell cycle progression provides a competitive advantage for human hematopoietic stem and progenitor cells *in vivo*

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Hematopoietic stem cell (HSC) function is well maintained over time and it is thought that one main reason is a tight control on cell cycle entry and, inversely, HSC activation is measured by a G0 to G1 phase shift. Still, it remains unknown whether the transit time through distinct cell cycle phases itself influences HSC activity. Therefore, we tested the effect of a 'shortened' G1 cell cycle phase on fate choices of human HSCs, reasoning that a 'short' G1 phase may provide less opportunity for the delivery of differentiation-inducing signals compared to a 'long' G1 phase. To shorten G1 experimentally, we overexpressed the G1 phase regulator Cyclin D1 and its catalytic partner cyclin-dependent kinase 4 (CCND1/CDK4, termed 4D) in human $CD34^+$ HSPCs. As a result, the G1 phase of HSPCs was shortened while overall cell cycle length was unaffected. Consequently, there was no difference in HSPC proliferation and colony formation *in vitro*. In contrast, serial transplantation experiments revealed an increased HSPC engraftment in secondary recipients compared to controls, suggesting that an accelerated transit through G1 improves the maintenance and function of hematopoietic stem cells *in vivo*. Consistently, 4D transduced HSPCs were protected from differentiation-inducing signals evidenced by an increased preservation of HSC numbers upon stimulation with LPS or growth factors. These results suggest that a shortened G1 phase transit is a mechanism providing resistance for human HSPCs to hematopoietic stress signals and favors their long-term maintenance. Interestingly, overexpression of the second G1 phase regulatory complex CCNE1/CDK2 promoted a more accelerated transit through G1, but resulted in the rapid loss of functional HSPCs *in vivo*. Thus, our data suggests that a balanced transit time through early and late G1 cell cycle phase is a key regulator for human HSPC function and provides a new regulatory platform to ensure lifelong hematopoiesis.

Abstract No. T28

The H3K4 histone methyltransferase Setd1b is required for hematopoietic stem cell homeostasis in mice

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Lineage- and cell-specific transcription programs are established and maintained by a tightly controlled network of epigenetic modifications. All expressed genes are trimethylated at histone 3 lysine 4 (H3K4me3) on promoter nucleosomes. The responsible enzymatic machinery belongs to the trithorax group of proteins and contains 6 different enzymes: MLL1-4, SETD1A and SETD1B. While the founding member MLL1 has been identified due to frequent involvement in human 'mixed-lineage leukemias', much remains elusive about the specific roles of the other H3K4 histone methyltransferases.

The present study addresses the function of Setd1b in the adult mouse by applying conditional mutagenesis based on the tamoxifen-inducible Rosa26-CreER(T2) system. Upon deletion of Setd1b mice progressively weaken, display dysplastic blood counts and splenomegaly and do not survive beyond 30 weeks. Subsequent flow cytometric analysis of hematopoietic stem cells (HSCs) in the bone marrow revealed abnormal ratios between individual stem cell types; especially short-term HSCs (Lin- Sca-1+ c-Kit+ CD34+ Flt3-) were significantly enriched. In the spleen we further detected a high degree of myeloid-biased hematopoiesis resulting in diffuse infiltration of splenic tissue with granulocytes and megakaryocytes. From these findings we were able to classify the Setd1b-deficient phenotype as myeloid neoplasia with features of human 'chronic myelomonocytic leukemia' (CMML). To rule out putative effects originating from the hematopoietic stem cell niche, we employed bone marrow reconstitution experiments as well as hematopoietic-specific deletion of Setd1b using Vav-Cre. In either case a comparable neoplastic phenotype was induced confirming the intrinsic requirement of Setd1b in the HSC compartment.

In conclusion, we characterized Setd1b as a crucial factor for haematopoietic stem cell homeostasis in mice. Future studies will shed light on the molecular pathways that depend on Setd1b function. Given the known role of Mll1 in the maintenance of HSCs, this study highlights the importance of epigenetic regulation for both steady-state and malignant hematopoiesis.

Abstract No. T29

Molecular and functional heterogeneity of dormant HSCs

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Hematopoietic stem cells (HSC) harbor the highest self-renewal capacity and generate a series of multipotent progenitors (MPP) that differentiate into lineage-committed progenitors and subsequently mature cells. To explore essential HSC features, we present integrated quantitative proteome, transcriptome, and methylome analyses of five FACS-sorted HSC and MPP populations (MPP1-4) as previously described in our laboratory (Wilson et al., Cell, 2008). From the characterization of more than 6,000 proteins, 27,000 transcripts, and 15,000 differentially methylated regions (DMRs), we identified coordinated changes associated with early differentiation steps. DMRs show continuous gain or loss of methylation during differentiation, and the overall change in DNA methylation correlates inversely with gene expression at key loci. Our data reveal the differential expression landscape of 493 TFs and 682 lncRNAs and highlight specific expression clusters including Wnt and Lin28-Hmga signaling, the imprinted-gene-network, Hox genes, retinoic acid metabolism. We also found an unexpectedly dynamic pattern of transcript isoform regulation, suggesting a critical regulatory role during HSC differentiation, and a cell cycle/DNA repair signature associated with multipotency in MPP2 cells. To address differentiation potential of MPP2-4 we linked our OMICs data with functional reconstitution experiments (Cabezas-Wallscheid et al. 2014). We have now expanded this analysis to dormant HSCs identified by label-retaining assays (Wilson et al., 2008). Using single-cell RNA-seq analysis we addressed the heterogeneity within this population and provide first markers and molecular pathways specifically operational in dormant HSCs.

Abstract No. T30

High efficiency gene correction in hematopoietic cells by template-free CRISPR/Cas9 genome editing

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A significant fraction of inherited monogenic disorders are caused by patient-specific mutations dispersed over the entire locus of the affected gene. Correcting these mutations by introducing healthy gene copies into the genome of the diseased cells proved successful in several clinical trials. In most of these trials the healthy genes were inserted randomly throughout the genome by retroviral vectors and were thus deprived of their endogenous control elements resulting in complications including gene silencing and activation of cancer genes. The development of genome editing tools capable of modifying any prespecified genomic sequence with unprecedented accuracy opened up a wide range of new possibilities in gene manipulation including targeted gene repair. In particular, CRISPR/Cas9, a prokaryotic adaptive immune system, and its swift repurposing for genome editing was widely adopted as the thus far simplest genome editing tool. In this study, we exploited the nonhomologous end joining repair mechanism (NHEJ) triggered by RNA guided CRISPR/Cas9 endonuclease (RGN) induced DNA double strand breaks to repair point mutated Cytochrome b-245 heavy chain (CYBB) genes causing chronic granulomatous disease - a life threatening immunodeficiency disorder.

We used an integrase defective lentiviral (IDLV) strategy to deliver template free RGNs into human hematopoietic cells harboring mutationally inactivated EGFP reporter- or bona fide CYBB genes. We could efficiently repair an EGFP frameshift mutation in both PLB leukemia and primary CD34 hematopoietic progenitor cells. Likewise, RGNs effectively corrected patient specific frameshift mutations in XCGD (CYBB null) PLB cells but not equivalent nonsense or missense mutations.

Frameshift mutations can be effectively repaired by NHEJ in hematopoietic cells transduced with nonintegrating RGN expressing lentiviruses. According to the CYBBbase database, 24% of XCGD patients harbor CYBB frameshift mutations, suggesting that about one quarter of these patients could benefit from a template free RGN gene therapy approach.

Abstract No. T31

Visualization of the short-range Wnt gradient in the intestinal stem cell niche

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Mammalian Wnt proteins are believed to act as short-range signals, yet have not been previously visualized. Self-renewal, proliferation and differentiation are coordinated along a putative Wnt gradient in the intestinal crypt. Wnt3 is produced specifically by Paneth cells. We have now generated an epitope-tagged, functional Wnt3 knock-in allele. Wnt3 covers basolateral membranes of neighboring stem cells. In intestinal organoids, Wnt3-transfer involves direct contact between Paneth cells and stem cells. Plasma membrane localization requires surface expression of Frizzled receptors, which in turn is regulated by the transmembrane E3 ligases Rnf43/Znrf3 and their antagonists Lgr4-5/R-spondin. By manipulating Wnt3 secretion and by arresting stem cell proliferation, we demonstrate that Wnt3 is propagated away from its source in a cell-bound manner though cell division, and not through diffusion. We conclude that stem cell membranes constitute a reservoir for Wnt proteins, while Frizzled receptor turnover and 'plasma membrane dilution' through cell division shape the epithelial Wnt3 gradient.

Abstract No. T32

Clonal heterogeneity within the exocrine pancreas

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The most abundant cell type in the mammalian pancreas are the acinar cells accounting for ~ 80% of all pancreatic cells. According to data from mouse models this cell type is the origin for the most common type of pancreatic cancer, pancreatic ductal adenocarcinoma (PDAC). Interestingly not all acinar cells transform to preneoplastic lesions upon KrasG12D expression indicating that the acinar cell compartment might be more heterogeneous than previously considered.

Here we use multicolor lineage tracing to study the clonal contribution of cells within the acinar cell population. Long-term lineage tracing, organoid formation assay and computational modelling reveal the existence of previously neglected clonal heterogeneity among acinar cells. During homeostasis we find mononuclear acinar cells that generate binuclear cells unable to divide further and division-competent mononuclear cells. Chemically-induced pancreatitis leads to a transient gain in proliferation of acinar cells which is not constraint to self-renewing acinar cells. Altogether, our study identifies distinct acinar subpopulation with heterogeneous proliferative capacity and thus transforms our understanding of the acinar cell compartment as a pool of equipotent secretory cells.

Abstract No. T33

Single cell analysis of cellular mechanisms regulating adult neural stem cell fate

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Adult stem cells are maintained in specialized niches that regulate their activity and longevity. Unlike embryonic precursors that are continuously dividing, adult stem cells can enter a transient quiescent (resting) state. The adult mouse Subventricular zone (SVZ) of the lateral ventricles provides a model system where dividing and quiescent stem cells co-exist. While their anatomical organization and lineage hierarchy have been studied extensively, the dynamics of neural stem cell (NSC) number and activity remain poorly understood. We combine genetic lineage tracing with quantitative analysis to examine the functional behavior of NSCs in the adult mouse SVZ. We show that the Wnt target Tnfrsf19/Troy identifies NSCs that transit reversibly between quiescence and activity. Single cell global expression profiling identifies a proliferative NSC cluster and reveal high level of heterogeneity among stem and progenitor cells. We establish a clonal lineage-tracing paradigm to fate map individual NSCs and use the data to generate a mathematical model of NSC dynamics. Our results best fit in a model where active NSCs divide symmetrically in a closed niche before correlating their fate choices to the total number of stem cells. We corroborate our results using a novel Ki67iresCreER allele to fate-map proliferating cells and confirm that active NSCs follow neutral drift dynamics occasionally increasing the quiescent stem cell numbers. Our results point out a novel cellular mechanism to regulate stem cell numbers and provide a high-resolution molecular profile of adult neurogenesis.

Abstract No. T34

In vivo Ribotag analysis reveals translational control during neural stem cell differentiation

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It is well known that epigenetic modifications as well as regulation of transcription factors are closely associated to cellular identity, highly distinguishing between the pluripotent and committed status of cells. However, little is known about the importance of changes at the posttranscriptional level. Recent studies on hematopoietic stem cells emphasize that protein translation is tightly controlled in the stem cell compartment and changes in both directions severely deregulate the system and deplete the stem cell pool. Highly polarized cells including neurons are characterized by a significant degree of post-transcriptional regulation. Here we look at the gradual changes of the translatome while differentiation of subventricular zone neural stem cells (NSCs) to olfactory bulb interneurons.

Using Ribotag mouse lines which express a HA-tagged variant of a ribosomal protein in different cell compartments along the differentiation path of NSCs, we profiled both, the total mRNA as well as the ribosome-bound mRNA *in vivo*. By this approach, we show that total mRNA is an inaccurate measure for protein levels for a significant part of the genome. Several genes including key transcription factors show comparable levels in their mRNA expression over the investigated cell populations, their action however is limited to certain stages by actual translation into proteins. The degree of this uncoupling increases with differentiation and maturation of the cells, which is in line with the literature describing local and temporal protein synthesis in mature neurons. However, the fact that translation is already specifically regulated in NSCs indicates that these mechanisms already exist very early in cells which will later become neurons. This study adds a novel layer of complexity to the process of neuronal differentiation. Identification of upstream regulators as well as associated mechanisms will be key to further complete this picture.

Abstract No. T35/P048

The transcription factor GATA2 controls mesenchymal stem cell fate

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Mesenchymal stem cells (MSC) are pluripotent cells with an inherent potential to regenerate a broad array of tissues. MSC lineage commitment and differentiation are tightly regulated by a complex network of transcription factors. In this study, we focused on the hematopoietic transcription factor GATA2, which was found to be expressed in MSC, and its role in controlling MSC differentiation. By applying chromatin immunoprecipitation coupled with deep sequencing (ChIPseq), we found enriched binding of endogenous GATA2 to canonical binding motifs near genes involved in osteoblast differentiation. In contrast to hematopoietic stem cells, GATA2 binding motifs in MSC co-localized with motifs for transcription factor of the FOX and HOX family, known regulators of skeletal development. Consistently, ectopic GATA2 expression in primary MSC regulated many osteoblast-related genes and resulted in strongly reduced osteoblast differentiation. Interestingly, GATA2 partially blocked bone morphogenetic protein (BMP)-2 induced SMAD1/5/8 activity, a pathway that is known to drive osteoblastogenesis. Deletion of GATA2 by MSC-specific CRE recombinase expression in GATA2 floxed mice resulted in normal skeletal development. However, bone-residing osteogenic progenitors that lack GATA2 exhibited enhanced differentiation *in vitro*. In summary, our study identifies GATA2 as a novel regulator of MSC biology and osteoblast differentiation and warrants further investigation into diseases of the skeletal system.

Abstract No. T36

Attenuation of alloimmunity to human Endothelial Colony Forming Cells (ECFC) by interaction with Mesenchymal Stem/Progenitor Cells (MSPC)

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Vessel regeneration in injured tissues including heart, bone or ischemic limb could benefit from endothelial colony forming cell (ECFC) transplantation. However, ECFC damage during inflammation and immunogenicity/rejection of allogeneic ECFC limit their applicability and long-term persistence. We hypothesized that synergistic action with mesenchymal stem/progenitor cells (MSPC) regarding immunomodulation and regeneration may improve stable revascularization.

Pairs of HLA-identical ECFC/MSPC from the same human umbilical cord were surface marker phenotyped after pro-inflammatory IFNγ treatment. Induction of immune cell proliferation and cytokine release were measured by flow cytometry comparing cocultures to monocultures. Cytolysis by cytotoxic T cells was determined by a calcein-based assay (ECFC vs. MSPC vs. co-culture in all experiments). The vasculogenic capacity was histologically assessed in NOD.Cg-Prkdc^{SCID}IL2rg^{tm1Wjl}/SzJ mice.

In contrast to IFNγ-treated ECFC and MSPC monocultures, cocultures demonstrated significantly reduced HLA-expression levels. HLA-DR down-regulation was contact-dependent (abrogated by transwell coculture). IFNγ pre-treated ECFC-induced T cell proliferation was abolished by MSPC co-cultures and partially reversible by the Indolamin-2,3-Dioxygenase inhibitor 1-Methyltryptophan. ECFC lysis by HLA-specific cytotoxic T cells was significantly reduced in cocultures compared to ECFC alone. Otherwise, ECFC in cocultures did not prevent the suppressive capacity of MSPC regarding T cell responses. *In vivo* stable micro vessel formation was seen only in subcutaneous Matrigel-plugs containing ECFC and MSPC. In contrast ECFC implantation alone resulted in less organized vascular structures and plugs containing just MSPC were completely lacking blood vessels.

Crosstalk between ECFC and MSPC in an inflammatory microenvironment might lower the risk for inflammatory damage and allogeneic vascular rejection. MSPC/ECFC for coapplication could be rapidly available off-the-shelf from allogenic sources like umbilical cords. A combined cell therapy product consisting of ECFC and MSPC might not only be superior in terms of tissue vascularization but also concerning cell engraftment and behavior in inflammatory or allogeneic settings.

Abstract No. T37

Murine bone marrow derived mesenchymal stem cells as a putative therapy approach for influenza-virus induced pneumonia

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Mesenchymal stem cells (MSC) are multi-potent self-renewing stromal cells with anti-inflammatory and regenerative potential. Recently MSC were attributed a beneficial role in acute and chronic lung injury. Influenza virus (IV) infects the upper respiratory tract and occasionally spreads to the alveolar compartment causing primary IV pneumonia. This frequently progresses to acute respiratory distress syndrome (ARDS) with severe alveolar damage, lung edema and hypoxemia, requiring mechanical ventilation or extracorporal oxygenation procedures. Antiviral therapies are only effective in the very beginning of infection and specific treatment strategies for IV-induced ARDS are lacking. We investigate MSC delivery in the lung as a promising treatment strategy in IV-induced ARDS.

In this study we generated MSC from bone marrow (mBM-MSC) of C57Bl6 mice. Our mBM-MSC expressed stem cell-specific markers and demonstrated differentiation potential. In ex vivo infection experiments mBM-MSC decreased IV-induced alveolar epithelial cells (AEC) apoptosis, increased AEC regeneration and strongly diminished IV replication in paracrine manner. This was associated with expression of type I interferons, of an anti-apoptotic factor stanniocalcin-1 and of growth factors FGF10 (fibroblast growth factor 10) and VEGF (vascular endothelial growth factor) in mBM-MSCs after co-culture with infected AECs, suggesting a pathogen-/injury-specific priming of mBM-MSCs. *In vivo*, intratracheal instillation of mBM-MSC after IV challenge strongly increased IV clearance and decreased IV-induced apoptotic injury of AECs. Of note, mBM-MSCs also increased the regenerative response of epithelial stem/progenitor cell pool of the distal lung.

Taken together our experiments showed a beneficial role of mBM-MSCs in influenza infection model and confirmed the therapeutic potential of these cells in influenza induced ARDS.

Abstract No. T38/P047

Regenerative potential of human pluripotent stem cell-derived MSCs in a Gunn rat liver injury model

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The Crigler-Najjar syndrome type 1 is characterized by massively increased levels of unconjugated bilirubin in the plasma because of the lacking of hepatic uridine 5'-diphospho-glucuronosyltransferase activity (due to a mutated UGT1A1 gene) which is crucial for bilirubin conjugation and excretion. The Gunn rat is an *in vivo* model for this disease. Current treatments have several limitations therefore alternative therapy strategies like (stem) cell transplantations are needed. Transplantation of primary hepatocytes is limited due to the dependence on donated organs. An alternative approach would be the increment of functional hepatocytes by cell transplantation. Studies have shown that co-transplanting hepatocytes with bone marrow-derived MSCs improves homing-in, engraftment and survival of the donor cells and that MSCs can transdifferentiate into hepatocytes. These properties make MSCs attractive tools in the treatment of acute liver injuries. However, the downside of procuring MSCs is that the number of cells that can be generated from a single human donor is limited due to their restricted long-term proliferation. To circumvent these drawbacks iMSCs (induced MSCs) were generated from (i) iPSCs derived from human fetal bone marrow MSCs and (ii) human embryonic stem cells- line H1. These were compared to human fetal bone marrow-derived MSCs. The iMSCs and the parental MSCs were transplanted into the spleen of partially hepatectomized Gunn rats (without immunosuppression). After a regeneration time of 1 week to 2 months the organs and sera were examined. Molecular, immunohistochemical and immunofluorescent-based analysis of rat liver tissue showed integration of human cells. Expression of human HNF4α as well as measureable human albumin levels in the sera provides evidences of trans-differentiation into hepatocytes. Further analysis revealed reduced levels of bilirubin. To surmize, human iMSCs are an alternative source for treating inherited liver diseases such as Crigler-Najjar syndrome type 1.

Abstract No. T39

An inducible overexpression system for rapid and highly efficient induction of mature cell types from human pluripotent stem cells

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Human pluripotent stem cells (hPSCs) represent a virtually unlimited source of human cells, which, in theory, can give rise to all cells of the organism. However, for certain cell types that are of considerable interest for regenerative medicine, such as skeletal myocytes, neurons and oligodendrocytes, robust differentiation protocols are still lacking. Reprogramming technologies based on forced expression of key transcription factors offer an alternative route for the rapid and efficient generation of such cell types. We developed an improved method to conditionally overexpress transgenes in hPSCs and their progeny by using an optimised doxycycline-responsive inducible system. This method overcomes mechanisms of gene silencing and allows tightly controlled, dose dependent, and transient overexpression of transgenes in hPSCs. We applied this system to overexpress several transcription factors that act as key determinants of certain cell lineages. By combining it with timed exposure to signalling cues from developmental differentiation we derived chemically defined, extremely rapid and deterministic protocols for the generation of skeletal myocytes, neurons and oligodendrocytes. Our system is easily scalable which allows for the generation of these cell types in bulk quantities as required for potential translational applications.

Abstract No. T40/P105

Reprogramming triggers endogenous LINE-1, Alu and SVA retrotransposition in human induced pluripotent stem cells affecting host gene expression

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Human induced pluripotent stem cells (hiPSCs) are capable of unlimited proliferation, can differentiate into every cell type of the adult body, and their use holds great therapeutic promise for regenerative medicine. However, reprogramming and subsequent cultivation of hiPSCs can induce genetic and epigenetic abnormalities that can result in tumorigenic iPSCs. Thus, it is unclear if iPSCs or their derivatives are safe for administration. Genomic mutations may undermine their use in regenerative medicine. Activation of the human endogenous mobile retrotransposons LINE-1 (Long Interspersed Element-1, L1), Alu and SVA can cause such mutations. In differentiated cells, L1 is primarily suppressed by methylation of its CpG-rich promoter, but reprogramming triggers L1 transcription.

To investigate if the observed L1 activation in pluripotent stem cells leads to L1-mediated mobilization, we applied retrotransposon capture-sequencing (RC-seq) to multiple hiPSC lines, their parental cells and human embryonic stem cell (hESC) lines. We identified, mapped and validated individual L1, Alu and SVA de novo retrotransposition events that occurred during reprogramming into iPSCs and cultivation of hiPSCs and hESCs. We calculated that each hiPSC carried ~4 L1 de novo insertions that occurred during reprogramming or hiPSC cultivation. As ~50% of all de novo retrotransposition events occurred in protein-coding genes actively transcribed in hiPSCs, including genes playing roles in oncogenesis or development, we investigated effects of these intronic de novo insertions on host gene expression in hiPSCs. To exemplify consequences of even short intronic L1 de novo insertions for host gene expression, we analyzed the effects of a 390-bp L1 de novo insertion in CADPS2 intron 23 on CADPS2 transcription in hiPSCs. We specified the period during hiPSC cultivation when this de novo insertion occurred. Our experiments demonstrate incidence and potential functional impact of reprogramming-activated endogenous retrotransposition in hiPSCs and imply consequences for the biosafety of hiPSC-derived cell therapies.

Abstract No. T41

Efficient patterning of human pluripotent stem cells towards distinct mesendodermal subtypes simply by modulation of the bulk cell density

Henning Kempf[1,*], *Ruth Olmer*[1], *Emiliano Bolesani*[1], *Annika Franke*[1], *Falk Buettner*[1], *Gerald Dräger*[2], *Oliver Pötz*[3], *Thomas Joos*[3], *Ulrich Martin*[1], and *Robert Zweigerdt*[1]

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Human pluripotent stem cell (hPSC) differentiation can recapitulate early aspects of embryogenesis *in vitro* but the underlying processes are poorly understood and controlled. Here we introduce the bulk cell density (BCD; modulated by the medium volume) as a key regulatory factor directing early differentiation patterning of hPSCs. By modulating the BCD at fixed concentrations of the GSK3 inhibitor CHIR99021 (CHIR), hPSC were primed towards distinct primitive streak-like patterns. This effect was mediated by differential, BCD-dependent paracrine environments. For detailed systems analysis global gene expression profiling, lineage-specific flowcytometry as well as protein array- and mass spectrometry-based secretome assessment was performed.

Our results show that 'high BCD / low CHIR' conditions favor priming towards definitive endoderm. Conversely, 'low BCD / high CHIR' results in late/posterior presomitic mesoderm priming along a primitive streak-like axis but entirely suppresses cardiac mesoderm formation. Strikingly, efficient cardiomyogenic differentiation is supported at both, 'low BCD/low CHIR' and 'high BCD/high CHIR' conditions and is characterized by a balanced intermediate gene expression pattern, essentially lacking exclusive expression of lineage regulators. Importantly, we show that the observed effect of the BCD is independent of the applied differentiation formats and was applicable to all hPSC lines tested (ESC and iPSC). In addition, we identify specific paracrine signatures underlying the observed lineage specific priming, supporting the critical regulatory role of secreted factors in the process.

Together, our results highlight the yet largely overlooked but highly decisive impact of the bulk cell density on hPSC differentiation. Monitoring and control of the BCD represents a novel, simple, but effective method to control hPSC priming, particular at early stages of lineage commitment. Our findings have strong practical implications for the definition and reproducibility of hPSC differentiation processes and their envisioned scale-up, which is prerequisite for the envisioned therapeutic and industrial application of hPSC derivatives.

Abstract No. T42

Direct *in vivo* glia-to-neuron conversion in the postnatal mouse cerebral cortex

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One strategy to overcome the limited regeneration capacity of the central nervous system after injury relies on cell replacement based therapies. Among them, direct reprogramming without passing through a pluripotent state of resident brain cells into neurons has been proposed as a new innovative strategy and has gained significant momentum within the last few years. Previous studies from our lab have shown that forced expression of Ascl1 and Neurog2 can direct postnatal cortical astrocytes towards GABAergic and glutamatergic neurogenesis *in vitro* (Heinrich et al., 2010; Heinrich et al., 2011). Here, we aim at testing whether glia can be lineage converted in the postnatal mouse cerebral cortex *in vivo*.

Glial cells proliferate locally in the early postnatal cortex (Ge et al., 2012). To target these cells for lineage conversion we transduced proliferating glia with retroviruses encoding for various neurogenic transcription factors. We found that overexpression of the transcription factors Ascl1 and Neurog2 alone in the postnatal cortex *in vivo* hardly gives rise to doublecortin (DCX) expressing neuroblasts. This suggests that glial cells *in vivo* are less responsive and that additional obstacles may to be overcome to acquire a neuronal identity. Thus, we tested whether co-expression of Sox2 can facilitate reprogramming induced by Ascl1. Consistent with the previously observed synergism between Sox2 and Ascl1 on lineage reprogramming of adult cells (Karow et al., 2012; Heinrich et al., 2014), we found here that combined expression of these two transcription factors results in the lineage conversion of proliferative glia into DCX positive neuroblasts in the early postnatal cortex *in vivo*.

Together, our work demonstrates for the first time the feasibility of direct *in vivo* lineage reprogramming in the healthy cerebral cortex and emphasizes the influence of the *in vivo* milieu on the susceptibility of glial cells to be directed towards a neuronal identity.

Company presentations

3rd International Annual Conference
German Stem Cell Network
9 – 11 September 2015

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C4 Consistency of pluripotent stem cell culture systems and differentiation protocols for generation of functional human stem cell derived hepatocytes
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C9 GrowDex® hydrogel for 3D cell cultures
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Abstract No. C1

Robust expansion of human pluripotent stem cells: integration of bioprocess design with transcriptomic and metabolomic

Marta M. Silva, Ana F. Rodrigues, Cláudia Correia, Marcos F.Q. Sousa, Ana S. Coroadinha, Margarida Serra, and Paula M. Alves*

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Human pluripotent stem cells (hPSCs) have an enormous potential as a source for cell replacement therapies, tissue engineering, and *in vitro* toxicology applications. The lack of standardized and robust bioprocesses for hPSC expansion has hindered the application of these cells and their derivatives in clinical settings.

In this study, we developed a robust and well-characterized bioprocess for the expansion of human embryonic stem cells (hESCs) under fully defined conditions and explored the potential of transcriptomic and metabolomic tools for a more comprehensive assessment of culture system impact on cell proliferation, metabolism, and phenotype. Two different hESC lines (feeder-dependent and feeder-free lines) were efficiently expanded on xeno-free microcarriers using environmentally controlled stirred-tank bioreactors. Both hESC lines maintained the expression of stemness markers, namely Oct-4, Nanog, SSEA-4, and TRA1-60 and the ability to spontaneously differentiate into the three germ layers. Whole-genome transcriptome profiling revealed a phenotypic convergence between both hESC lines along the expansion process in stirred-tank bioreactor cultures, providing strong evidence of the robustness of the cultivation process to homogenize cellular phenotype.

Under low-oxygen tension, results showed metabolic rearrangement with up-regulation of the glycolytic machinery favoring an anaerobic glycolysis Warburg-effect-like phenotype, with no evidence of hypoxic stress response, in contrast to two-dimensional culture. Overall, we report a standardized expansion bioprocess that can guarantee maximal product quality. Furthermore, the “-omics” tools used provided relevant findings on the physiological and metabolic changes during hESC expansion in environmentally controlled stirred-tank bioreactors, which can contribute to improved scale-up production systems.

This study provided valuable insights into the metabolic hallmarks of hPSC expansion and new information to guide bioprocess design and media optimization for the production of cells with higher quantity and improved quality, which are requisite for translation to the clinic.

Abstract No. C2

A human iPS cell platform to study pancreatic disease

Alexander Kleger

University Hospital Ulm

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Human induced pluripotent stem (iPS) cells can be generated from easily accessible tissues such as skin or plucked hair. In particular keratinocytes can be reprogrammed faster and more efficient than fibroblasts. In case of applying this tool to patients who are classified into a disease group, it enables the generation of disease-specific iPS cells. iPS cells have proven a significant tool to elucidate pathophysiological mechanisms in various diseases such as diabetes, blood disorders, defined neurological disorders and genetic liver disease. However, differentiation of hiPS cells towards the pancreatic lineage remains inefficient and variable. Herein, we report the generation of plucked human hair derived induced pluripotent stem cells from healthy donors and genetically determined pancreatic disease. We successfully applied a novel and stepwise pancreatic differentiation protocol aiming to generate exocrine and endocrine pancreatic cells and apply this model to human genetically determined disease. This set up will provide the basis for future studies dissecting the pathophysiology of pancreatic disease in a dish but patient specific context.

Abstract No. C3

Advances in pluripotent stem cell culture and differentiation

David Kuninger

Thermo Fisher Scientific, Frederick, USA

The number of hiPSC and hESC lines currently available for research and therapeutic use continues to grow rapidly. Predictable expansion and differentiation of these lines requires reliable reagents that are fully-defined, qualified, and preferably manufactured from animal-origin–free raw materials under cGMP. Over the long-term, fully defined culture conditions are key to standardizing culture performance, ensuring reproducible outcomes between experiments (and laboratories) and improving our understanding of experimental results.

Essential 8 fulfills these requirements by providing a simple, fully defined, cost effective expansion medium that enables culture growth under serum-free and feeder-free conditions and that can be adapted to large-scale manufacture. The new Essential 8 Flex keeps that same promise, but allows researchers to avoid daily feeding schedules due to stabilization of temperature sensitive components within the media system.

Also the need for easy, reproducible and affordable methodology to create cells being derived out of the stem cells, drove efforts to create differentiation systems on a kit basis. In this presentation we will review a number of recent method developments using Essential 8, such as the ability to perform single cell passaging (clonal subculture), as well as the compatibility of the above mentioned media systems with the differentiation kits being used to differentiate iPSCs or ESCs into a variety of cells (neural stem cells, cardiomyocytes, endoderm cells for downstream differentiation into e.g. hepatic or pancreatic cells).

Abstract No. C4

Consistency of pluripotent stem cell culture systems and differentiation protocols for generation of functional human stem cell derived hepatocytes

Mia Emgård

Takara Clontech

The interest in human pluripotent stem cells, and their potential usage in diverse applications has increased dramatically over the last decade. Qualities such as an unlimited supply of cells with a natural high proliferation rate, the possibility to differentiate into all cell types in the body and obtaining cell lines derived from different polymorphic variants and from specific patients, are some of many factors that have contributed to this interest. With the ambition to differentiate stem cells to specific cell types, and the possibility to compare cells from different donors, the relevance of robust and consistent cell culture systems has become clear. In this presentation, we will show data on functional human induced pluripotent stem (iPS) derived hepatocytes from multiple donor origins, and the importance of reliable cell culture and differentiation media. Moreover, we will present how Cellartis® DEF-CS Culture System, and our robust differentiation protocols, contributed to functionally improved hepatocytes from multiple donors.

Abstract No. C5

Reprogramming of somatic cells to induced pluripotent stem cells using a novel synthetic self-replicating RNA vector in a feeder-free system

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Integration of exogenous factors into the genome of somatic cells used during induced pluripotent stem cell (iPSC) generation is undesirable because it can potentially affect differentiation capacity or lead to oncogenic transformation. To circumvent issues associated with genomic integration, we have developed a synthetic, self-replicating RNA reprogramming vector, ReproRNA™-OSKGM, which expresses reprogramming factors Oct4, Sox2, Klf4, c-Myc, Glis1, and a puromycin selection cassette. To generate iPSCs, 4 X 10^5 neonatal human foreskin fibroblasts (HFFs) or adult human dermal fibroblasts (HDFs) were transfected, just once, with ReproRNA™-OSKGM. Transfected cells were plated and selected by culture in medium containing puromycin for 7 days, after which they were harvested, dissociated and re-plated on inactivated mouse embryonic fibroblasts (iMEFs) in standard hES medium (DMEM/F12, 20% KOSR, bFGF), or on Matrigel™ in ReproTeSR™ Medium. Embryonic stem cell (ES)-like colonies arose within 2 weeks after transfection of ReproRNA-OSKGM with cells displaying high nucleus to cytoplasm ratio and by 3-4 weeks were large enough to be scored and manually selected for subculture in mTeSR™1. Reprogramming efficiencies based on percent colony number per cells plated, on iMEFs, for HFFs and HDFs, were 0.07 ± 0.02% (n=2) and 0.12 ± 0.05% (n=6), respectively. Reprogramming efficiencies in feeder-free conditions in ReproTeSR™ were similar, at 0.047 ± 0.004% (n=1) and 0.13 ± 0.07% (n=6) for HFFs and HDFs. Individual clones subcultured in mTeSR™1, expressed undifferentiated hPSC markers Oct4, SSEA-3, Tra-1-60, and exhibited the capacity to differentiate in STEMdiff™ differentiation kits, to cells of the 3 germ-layers. Finally, we present preliminary evidence demonstrating the applicability of ReproRNA™-OSKGM in generating human iPSCs with naïve-like properties, which can be expanded after reprogramming in an optimized maintenance medium. In summary, our results show the ease of reprogramming fibroblasts to iPSCs with a single transfection of ReproRNA™-OSKGM non-integrating, self-replicating RNA vector under feeder-free and defined conditions.

Abstract No. C6

High throughput electrospinning to generate scaffolds containing cells for regenerative medicine applications

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representing I&L Biosystems GmbH

Due to its flexibility and versatility, electro-hydrodynamic processing comprising electrospinning and electrospraying, has emerged as an interesting technology to produce micron, submicron and nanostructured fiber mats and powdered materials from natural and/or synthetic polymers. Coaxial and multi-axial structures, produced by multinozzle injectors or other proprietary spinning devices, are of great interest in many fields of application.

This presentation will highlight some recent advances carried out in our laboratories to develop 2D and 3D biomedical devices making use of functional biopolymers and nanobiocomposites ofinterest in regenerative medicine. In particular we have shown the advantages of using electrospun fibers as scaffolds for cell growth and demonstrated the feasibility of using coaxial electrospinning as a tool to generate scaffolds pre-seeded with human cells for application in regenerative medicine.

The development of coaxial multinozzle injectors enables this technology to be applied beyond laboratory research scale and provides a feasible method for the fabrication of scaffold materials for regenerative medicine applications in the future.

Torres-Giner, S., Martinez-Abad, A., Gimeno-Alcañiz, J.V., Ocio, M.J., Lagaron, J.M. Controlled delivery of gentamicin antibiotic from bioactive electrospun polylactide-based ultrathin fibers, (2012) Advanced Engineering Materials, 14 (4), pp. B112-B122.
Torres-Giner, S., Gimeno-Alcañiz, J.V., Ocio, M.J., Lagaron, J.M. Optimization of electrospun polylactide-based ultrathin fibers for osteoconductive bone scaffolds. (2011) Journal of Applied Polymer Science, 122 (2), pp. 914-925.
Torres-Giner, S., Gimeno-Alcañiz, J.V., Ocio, M.J., Lagaron, J.M. Comparative performance of electrospun collagen nanofibers cross-linked by means of different methods. (2009) ACS Applied Materials and Interfaces, 1 (1), pp. 218-223.
Torres-Giner, S., Ocio, M.J., Lagaron, J.M. Development of active antimicrobial fiber based chitosan polysaccharide nanostructures using electrospinning. (2008) Engineering in Life Sciences, 8 (3), pp. 303-314.

Abstract No. C7

An optimal serum-free, xeno-free culture system for the isolation, expansion and differentiation of clinical grade human mesenchymal stem cells

Orit Ecker-Cohen

Biological Industries

Human mesenchymal stem cells (hMSCs) are multipotent adult stem cells that can be isolated from a variety of human tissues, such as bone marrow, adipose, placenta, cord blood and Wharton jelly. hMSCs have the ability to differentiate into bone, cartilage and fat cells and are the subject of many clinical trials for a range of regenerative and inflammatory diseases.

hMSCs have advantages over other stem cell types due to the broad variety of their tissue sources, since they are immuno-privileged, and for their ability to specifically migrate to tumors and wounds in vivo. Due to these traits hMSC has become a desirable tool in tissue engineering and cell therapy. In most clinical applications hMSCs are expanded in vitro before use. The quality of the culture medium and its performance are particularly crucial with regard to therapeutic applications, since hMSC properties can be significantly affected by medium components and culture conditions.

The present study evaluated a novel XF culture system, comprising of MSC NutriStem® XF culture medium and all the required auxiliary solutions for the attachment, dissociation, and cryopreservation of clinical grade hMSCs.

Results show that the XF culture system for hMSC efficiently supports initial isolation and optimal expansion of hMSC from various sources, while maintaining MSC features: typical fibroblast-like cell morphology, phenotypic surface marker profile, differentiation capacity, self-renewal potential, and genetic stability.

In addition, innovative serum-free, xeno-free differentiation media were developed for the generation of mature adipocytes, osteoblasts and chondrocytes using hMSC from variety of sources.

Abstract No. C8

Explore what makes every stem cell unique from the inside out

Mark Lynch

Fluidigm Europe B.V.

Stem cell state and fate are controlled by the complex interplay of phenotype, genomic state and microenvironment. However, the stochastic nature of stem cell transition prevents traditional analysis methods from identifying all the genes and proteins involved. Single-cell biology enables deeper, more precise insights into the drivers of pluripotency, differentiation and self-renewal. With integrated single-cell genomics applications on the C1™ and Biomark™ HD systems, and proteomics on Helios™, a CyTOF® system, Fluidigm enables the most complete approach to single-cell analysis. Our newest instrument, Callisto™, takes the next step into cell biology, giving you the power to rapidly design, execute and optimize cell fate determination protocols with a few taps on a touchscreen.

Abstract No. C9

GrowDex® hydrogel for 3D cell cultures

Lauri Paasonen

UPM-Kymmene Corporation

GrowDex® is UPM's hydrogel for 3D cell cultures. GrowDex® is a wood-based cellulose nanofibril hydrogel with an ultrastructure that resembles ECM. The xeno-free product is highly biocompatible with human cells and tissues but does not contain any animal- or human-derived material. The mechanical properties of GrowDex® can be tuned to fulfill the requirements of different cell types. GrowDex® forms a continuous hydrogel, which allows diffusion of small molecules, such as nutrients and oxygen, but prevents large-scale cell movements. GrowDex® supports 3D cell growth and the hydrogel can also be completely degraded by enzyme treatment. The flexible 3D cell culture system GrowDex® hydrogel provides several benefits for 3D cell culture application. The benefits are supported with scientific research and publications.

Presentations strategic sessions

3rd International Annual Conference
German Stem Cell Network
9 – 11 September 2015

Presentations strategic sessions: S1 – S8

S1 Technologies in genetics engineering of stem cells
Ulrich Martin

S2 Genetic engineering of human pluripotent stem cells using TALENs nucleases
Sebastian Diecke

S3 Mammalian genome engineering in the era of CRISPR/Cas
Ralf Kühn

S4 How to apply in research and industry
Hanna Proner

S5 Regulatory aspects and classifications of stem cell based medicinal products
Egbert Flory

S6 Accelerating the clinical development of cell based therapies
Natalie Mount

S7 The ADIPOA-1/-2 trial - Adipose tissue derived stromal cells for osteoarthritis therapy
Oliver Pullig

S8 Cell therapy for bone defects: in vitro, in vivo, and first clinical results
Ingo Marzi

Strategic session: “Stem cell technologies”

Abstract No. S1

Technologies in genetics engineering of stem cells

Ulrich Martin[1]

[1]Medical School Hannover

Abstract not available

Abstract No. S2

Genetic engineering of human pluripotent stem cells using TALENs nucleases

Sebastian Diecke[1]

[1]Max Delbrück Center, Berlin

Abstract not available

Abstract No. S3

Mammalian genome engineering in the era of CRISPR/Cas

Ralf Kühn[1]

[1]Max Delbrück Center, Berlin

Abstract not available

Strategic session: "Career development"

Abstract No. S4

How to apply in research and industry

Hanna Proner[1]

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Abstract not available

Strategic session: “Clinical trial and regulatory affairs”

Abstract No. S5

Regulatory aspects and classifications of stem cell based medicinal products

Egbert Flory[1]

[1]Paul Ehrlich Institute, Langen

Abstract not available

Abstract No. S6

Accelerating the clinical development of cell based therapies

Natalie Mount[1]

[1]Cell Therapy Catapult Ltd., London, UK

Increasing numbers of cell based therapies are reaching the stage of translation into clinical development, the culmination of many years of scientific research work. However, the path to translate this promising science into successful medicines that meet the needs of patients is a challenging one. In this talk, the current cell therapy development landscape will be reviewed, particularly highlighting recent analysis of preclinical and clinical trends in the UK as well as important regulatory initiatives in this area. It will be introduced how the in the UK, the Government established the Cell Therapy Catapult to work with academic and industry researchers to meet the challenges in this developing field and accelerate the development of promising therapies.

Abstract No. S7

The ADIPOA-1/-2 trial - Adipose tissue derived stromal cells for osteoarthritis therapy

Oliver Pullig[1,], Christian Jorgensen[2], Lars Rackwitz[3], and Ulrich Nöth[3]*

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*Presenting author

Cell based therapies are mainly initiated by academia in the anticipation to develop effective therapies where only unsatisfying therapeutic options exists. Stem cell therapies have highly innovative potential but data on efficacy are limited. The ADIPOA trial MSC-therapy is targeting osteoarthritis (OA); the most widespread musculoskeletal disorder in adults resulting in progressive loss of articular cartilage and frequently in pain and disability.

The First-In-Human application of adipose-derived stromal cells (ASCs) in knee OA was mainly performed under safety aspects to ensure that adverse events are minor and serious adverse events are not related to ASC treatment. Preliminary data on efficacy were obtained to support the hypothesis of beneficial effects after ASC treatment. The bicentric, open phase I clinical trial was conducted in France and Germany upon approval by regulatory agencies. Between April 2012 and December 2013, eighteen consecutive patients with symptomatic and severe knee OA have been treated with a single intra-articular injection of autologous ASCs. The trial design comprised of three consecutive cohorts, six patients each, the first cohort received a low-dose (2 x 10^6 cells), the second a medium-dose (10 x 10^6) and the third a high dose (50 x 10^6), respectively. The primary outcome parameter was safety evaluated by recording adverse events throughout the trial and secondary parameters were pain and function.

After 12 months, the intra-articular injection ASCs was safe and no related serious AEs were reported. Four patients experienced transient knee pain and swelling after injection of high dosage. Interestingly, patients treated with the low-dose of ASCs significantly improved in pain and function compared to baseline.

Our data suggest that the intra-articular injection of ASCs is a safe alternative to treat severe knee OA patients. A placebo-controlled double-blind phase IIb study has been initiated to assess clinical and structural improvement on higher number of patients.

http://adipoa2.eu

Abstract No. S8

Cell therapy for bone defects: *in vitro*, *in vivo*, and first clinical results

Ingo Marzi[1]

[1]Goethe-University Hospital Frankfurt, Frankfurt/Main

Abstract not available

Poster presentations

3rd International Annual Conference
German Stem Cell Network
9 – 11 September 2015

Poster session I: P001 – P074

P001 – P024: Hematopoietic stem cells
P025 – P038: Stem cells in regenerative therapies
P039 – P048: Stem cells in regenerative therapies:
Mesenchymal stem/stroma cells
P049 – P074: Stem cells in disease modeling and drug development

Even numbers: Wednesday, 9 September from 16:30 - 17:30
Odd numbers: 17:30 - 18:30

Poster session II: P075 – P147

P075 – P093: Pluripotency and embryonic stem cells
P094 – P107: Programing and reprograming
P108 – P113: Somatic stem cells
P114 – P130: Stem cells in development
P131 – P143: Stem cells in disease: Cancer stem cells
P144 – P147: Computational stem cell biology

Even numbers: Thursday, 10 September from 16:30 - 17:30
Odd numbers: 17:30 - 18:30

Poster session I: P001 – P074

P001 – P024 Hematopoietic stem cells

P001 Interleukin-3 supports large-scale myeloid differentiation of human induced pluripotent stem cells (hPSC)
Mania Ackermann

P002 Dissecting the role of Setd1a-mediated histone methylation in adult hematopoietic stem cell function
Kathrin Arndt

P003 The Metabolic Demands of Haematopoiesis
Christian Böhme

P004 Metabolic alterations affect functional phenotype of hematopoietic stem and progenitor cells
Anja Derlet

P005 Specific Transcripts of DNMT3A Modulate Differentiation of Hematopoietic Stem and Progenitor Cells
Joana Frobel

P006 FUSE binding protein 1 is essential for hematopoietic stem cell self-renewal
Katharina Gerlach

P007 Identification of Key Regulators of Symmetric versus Asymmetric Cell Divisions During Human Hematopoietic Lineage Specification
André Görgens

P008 Connective tissue growth factor (CTGF) is an inducible stroma-derived regulator of hematopoietic stem cell maintenance and cell cycle progression
Rouzanna Istvanffy

P009 Transcriptome-wide profiling and post-transcriptional analysis of hematopoietic stem/progenitor cells during myeloid commitment
Daniel Klimmeck

P010 Screening for HSC Regenerating Genes using the Mpl-Deficient Mouse Model
Saskia Kohlscheen

P011 Phosphorylation of the angiotensin converting enzyme (ACE) blocks stem cell mobilization by granulocyte-colony stimulating factor (G-CSF)
Karin Kohlstedt

P012 The significance of material properties for stem cells: How matrix mechanics, nanostructure and 3D architecture impact hematopoietic stem cells
Cornelia Lee-Thedieck

T27/P013 CCND1/CDK4-mediated cell cycle progression provides a competitive advantage for human hematopoietic stem and progenitor cells *in vivo*
Nicole Mende

P014 Mechanism of robust human hematopoietic stem cell engraftment and expansion in a Kit-deficient mouse model.
Susann Rahmig

P015 The heterotrimeric G-proteins Gαq/11 are required for Flt3-ITD autophosphorylation and regulate normal and leukemic HSPC cell cycle progression
Maike Rehage

P016 The significance of 3D architecture and perfusion in a biomimetic model of the hematopoietic stem cell niche
Lisa Rödling

P017 WNT5A expressed by the niche is required to maintain migratory properties of hematopoietic stem cells through the planar cell polarity pathway
Christina Schreck

P018 Adrenergic stimulation alters the mechanical properties of bone marrow niche cells and interferes with the adhesion of hematopoietic progenitor cells
Tanja Seeger

P019 The Role of Neogenin in HSC Maintenance and Activation
Renders Simon

P020 Humanized mice – a suitable model to study human hematopoiesis and immunotherapeutics for cancer therapy *in vivo*
Maria Stecklum

P021 Hyperinflammation triggers an IL-1b-dependent activation of hematopoietic stem and progenitor cells
Maren Weisser

P022 The Gadd45 family as mediators of hematopoietic stem cell differentiation – from genotoxic stress to cytokine-induced differentiation
Susanne Wingert

P023 The phosphatases STS1 and STS2 regulate hematopoietic stem and progenitor cell fitness
Jing Zhang

P024 α-Catenin is a dosage-dependent tumor suppressor gene in MDS
Antje M. Zickler

Abstract No. P001

Interleukin-3 supports large-scale myeloid differentiation of human induced pluripotent stem cells (hPSC)

Mania Ackermann[1,*], *Nico Lachmann*[2], *Doreen Lüttge*[1], *Eileen Frenzel*[3], *Christine Happle*[4], *Olga Klimenkova*[5], *Sebastian Brenning*[1], *Sabina Janciauskiene*[3], *Julia Skokowa*[5], *Gesine Hansen*[4], and *Thomas Moritz*[1]

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[2]JRG Translational Hematology & RG Reprogramming and Gene Therapy
[3]Department of Respiratory Medicine, Hannover Medical School
[4]Department of Pediatric Pneumology, Allergology and Neonatology; Biomedical Research in Endstage and Obstructive Lung Disease (BREATH), Member of the German Center for Lung Research (DZL), Hannover Medical School
[5]Department of Molecular Hematopoiesis, Hannover Medical School
*Presenting author

Hematopoietic *in vitro* differentiation of hPSCs holds great promise for gene and cell therapy, and additionally represents a valuable model system of embryonic hematopoietic development. We have established an embryoid body (EB)-based differentiation protocol employing IL-3 in combination with G-CSF or M-SCF to continuously (up to 5 months) generate large numbers (2-10x106/week/6 well plate) of >95% pure granulocytes (iPSC-gra) or monocyte/macrophages (iPSC-MΦ) via an intermediate "myeloid cell forming complex (MCFC)". IPSC-gra and iPSC-MΦ revealed typical morphology, surface phenotype, and functionality. Thus iPSC-gra migrated towards an IL8 or fMLP gradient, formed neutrophil extracellular traps, and produced ROS. IPSC-MΦ phagocytosed latex beads and secreted cytokines upon LPS stimulation.
Interestingly, in our model production of myeloid cells was driven by a MCFC-resident, CD34+, clonogenic progenitor population. Furthermore, early embryonic events such as endothelial versus hematopoietic specification were recapitulated. In this line, analysis of MCFCs revealed expression of MIXL1, SOX17, KDR1, GATA2, and RUNX1. Moreover, an early endothelial CD34+/CD45-/CD144- cell-population undergoing transition to a hematopoietic CD34+/CD45+/CD144- and thereafter a CD34-/CD45+/CD144- phenotype were detected.
Of note, cultivation of MCFCs with IL-3 only resulted in generation of primarily myeloid progenitor cells, which retained the capacity of subsequent M- or G-CSF-driven terminal differentiation into iPSC-MΦ and IPSC-gra, respectively. Even more important, cells shedded from "IL3 only MCFCs" contained up to 15% CD34+ stem/progenitor cells and generated white and red colonies, which was not observed for cultures treated with IL3/M-CSF or M-CSF alone.
In summary, our *in vitro* PSC-differentiation model allows for the large-scale production of IPSC-gra and iPSC-MΦ for cell-therapy approaches. In addition, the protocol faithfully recapitulates key events in embryonic hematopoiesis and due to its minimal cytokine requirement appears highly suited to study early hematopoietic development. We further provide evidence that IL3 constitutes a key-cytokine driving the early hematopoietic specification of human PSC.

Abstract No. P002

Dissecting the role of Setd1a-mediated histone methylation in adult hematopoietic stem cell function

Kathrin Arndt[*], *Andrea Kranz*, *A. Francis Stewart*, and *Claudia Waskow*

TU Dresden
*Presenting author

Setd1a is one out of six histone methyltransferases (Mll1-4, Setd1a, Setd1b) placing methylation marks on histone H3 lysine 4 (H3K4), an epigenetic signature that is related to active transcription. Setd1a is essential for embryonic development through regulating the expression of genes ensuring maintenance of pluripotency in embryonic stem cells. Setd1a has a non-redundant regulatory role in B cell development but potential effects on the function of adult hematopoietic stem cells (HSCs) remain unknown. Due to early lethality (E7.5) of constitutive knock-out embryos, we here took advantage of conditional knock-out mice to analyze the functional role for Setd1a in adult HSCs. Ubiquitous deletion of Setd1a in adult mice results in severe hematopoietic defects and rapid death, suggesting a role for Setd1a in HSC function. Specific deletion in adult HSCs revealed that Setd1a is not essential for their maintenance but, instead, it plays a crucial role for continuous hematopoiesis. Consistently, the frequency of growth factor-responsive bone marrow progenitor cells is significantly reduced, pointing at the importance for Setd1a-mediated effects in the formation of progenitor cells. We suggest a non-redundant function for Setd1a to maintain hematopoietic homeostasis by regulating gene expression important for differentiation but not for cellular survival.

Abstract No. P003

The Metabolic Demands of Haematopoiesis

Christian Böhme[1,*], *Dietger Niederwieser*[1], *Tilo Pompe*[2], and *Michael Cross*[1]

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*Presenting author

Haematopoietic bone marrow produces erythroid and myeloid cells at different spatial locations relative to the blood supply, suggesting that the metabolic environment influences the migration of progenitor cells and/or lineage specific patterns of anabolic metabolism. In support of a decisive influence of the metabolic environment on haematopoiesis, it has recently been reported that glutamine concentration governs the commitment of multipotent progenitors to the erythroid lineage (Oburoglu et al., Cell Stem Cell, 2014, 15: 1-16).

Here, we have studied the effects of glutamine on the migration and colony formation of erythroid-myeloid (EMP) progenitors derived from umbilical cord blood. We first developed a procedure that enables the establishment and maintenance of metabolite gradients under otherwise standard colony assay conditions and found that the application of either glutamine or glucose gradients alone had no reproducible effect on the migration of colony forming cells. However, during the course of this work we noted that the maintenance of moderately high levels of glutamine (8mM) specifically blocked the development of erythroid colonies. This contrasts with the pro-erythroid effects of glutamine seen in other assays and indicates significant differences in amino metabolism between the myeloid and erythroid lineages.

Furthermore, we defined conditions under which the efficient colony development can be supported by lactate rather than glucose. Since glucose is normally considered to be essential for cell proliferation as a source of ribose-phosphate for nucleoside synthesis, this suggests that haematopoietic progenitors may be capable of significant gluconeogenesis from lactate – a pathway normally associated with the liver. We hypothesise that the lactate generated by glycolysis in the vicinity of arterioles is in turn used to supply glucose at deeper levels. In this way, the spatial compartmentalisation of alternative pathways of anabolic metabolism may act to support the high levels of cell proliferation throughout the haematopoietic marrow.

Abstract No. P004

Metabolic alterations affect functional phenotype of hematopoietic stem and progenitor cells

Anja Derlet[1,*], *Tina Rasper*[1], *Aaheli R. Choudhury*[2], *Michael Rieger*[2], *Andreas M. Zeiher*[3], *Stefanie Dimmeler*[1], and *Florian H. Seeger*[3]

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Modulation of energy metabolism allows cells to match the divergent demands of the environment and is involved in reprograming as well as differentiation and dedifferentiation of cells. Recent evidence implicates nutrient-responsive metabolites as mediators of crosstalk between metabolic flux, cellular signaling, and epigenetic control of cellular functions. Administration of bone marrow-derived mononuclear cells (BMC) in clinical cell therapy may increase cardiac function after myocardial ischemia. We recently showed that cardiovascular disease induces metabolic alterations that might influence the functional activity of BMC as well as human hematopoietic stem and progenitor cells (HSPC). Therefore, we examined the regulation of energy metabolism in BMC.

To examine the effect of metabolic changes on the functional phenotype of cells, we modulated the metabolism of BMC as well as $CD34^+CD38^-$ lin^- HSPC and $CD34^+CD38^-$ $CD90^+CD45RA^-$ lin^- HSC *ex vivo* by reducing glycolysis or respiration with 2-desoxy-D-glucose (2-DG) or antimycin A (AA), respectively. Reduction of glycolysis by 2-DG (68±8% control) reversibly reduces invasion (69±17% control) as well as colony forming capacity (1±0% control) without profoundly affecting ATP-production (80±6% control) in BMC, and profoundly reduces proliferation of HSPC and HSC. The abolished colony forming capacity was reversible as colony formation partly recovered after 2-DG wash out and repopulation of the cells (31±5% control). Inhibition of respiration by AA (31±3% control) without affecting total ATP production (95±1% control), leads to a compensatory increase in glycolytic capacity (150±17% control) correlating with increased colony formation (143±18% control) as well as invasion capacity (145±45% control). These metabolic changes were cell type-specific, as if sorted subpopulations showed different metabolic profiles.

Conclusively, this study shows that changing the balance between respiration and glycolysis affects functional phenotype of BMC and HSPC *ex vivo*. Future studies will address whether metabolic modulation might be a therapeutic option to improve patient-derived cells for clinical cardiac cell therapy.

Abstract No. P005

Specific Transcripts of DNMT3A Modulate Differentiation of Hematopoietic Stem and Progenitor Cells

Joana Frobel[1,*], *Tanja Božić*[1], *Annamarija Raić*[1], *Stefanie Heilmann-Heimbach*[2], *Andrea Hofmann*[2], *Tamme W. Goecke*[3], *Edgar Jost*[4], and *Wolfgang Wagner*[1]

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DNA methyltransferase 3A (DNMT3A) is a *de novo* DNA methyltransferase that is alternatively spliced in a tissue- and disease-specific manner, but the functional relevance of the different transcripts is hardly known. *DNMT3A* is frequently mutated in patients with acute myeloid leukemia (AML) and we have recently demonstrated that effects of these mutations can be mimicked by an epigenetic dysregulation within the *DNMT3A* gene: about 40% of AML patients revealed aberrant DNA hypermethylation which was associated with shorter overall survival. Notably, *DNMT3A* mutations and "epimutations" seem to have an impact on the expression of different *DNMT3A* transcripts. In this study we elucidated the functional role of individual *DNMT3A* transcripts (Tr.1+3, Tr.2, Tr.4) in hematopoiesis. Single *DNMT3A* transcripts were knocked down by lentiviral expression of short-hairpin RNAs in $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) that were isolated from cord blood.
Downregulation of either Tr.2 or Tr.4 reduced the proliferation rate of HSPCs significantly ($n=3$; $p<0.05$). Notably, HSPCs maintained CD34 expression for more cell divisions upon knockdown of Tr.2 ($n=3$; $p<0.05$). In colony forming unit assays the downregulation of Tr.4 resulted in a clear bias towards erythroid colonies.
These functional effects might be governed by specific regulation of DNA methylation patterns. In fact, DNA methylation analyzed with Illumina 450k BeadChip technology revealed significant differential methylation of several CpGs, particularly upon knockdown of Tr.2 and Tr.1+3 (8,905 and 352 CpGs, respectively; adjusted p-value<0.05). Notably, various CpGs in the promoter region of CD34 were significantly hypomethylated in HSPCs with downregulated Tr.2 and Tr.1+3. In contrast, knockdown of Tr.4, which does not exhibit methyltransferase activity, did not reveal any significant changes.
Our results demonstrate that specific *DNMT3A* transcripts have different effects on DNA methylation patterns and differentiation of HSPCs. This may also be relevant for disease progression in AML.

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Abstract No. P006

FUSE binding protein 1 is essential for hematopoietic stem cell self-renewal

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We identified the transcriptional regulator *FUSE* Binding Protein 1 (FUBP1) as an important factor required for hematopoietic stem cell (HSC) self-renewal. Impaired FUBP1 function in two different gene trap mouse models led to lethality around day E15.5 of embryonic development with an anemic phenotype, a strong reduction of total fetal liver cell numbers and the extinction of long-term repopulating HSCs (LT-HSCs).

Competitive transplantation experiments using E15.5-derived fetal liver cells from gene trap mice or adult LT-HSCs lentivirally transduced with *Fubp1* shRNA constructs revealed a significantly lower engraftment in primary transplants and no engraftment in secondary transplants of FUBP1-deficient cells. We then investigated the transcriptional network of FUBP1 by transcriptome profiling using *Affimetrix* array hybridization and via quantitative real-time PCR. Our results indicate that FUBP1, amongst others, inhibits the expression of the cell cycle regulator *p21* and the pro-apoptotic gene *Noxa* in adult LT-HSCs. Indeed, single cell tracking of *Fubp1* knockdown LT-HSCs demonstrated significantly prolonged generation times and increased cell death rates. *Ex vivo* culturing of these cells confirmed a drastic expansion defect as a consequence of FUBP1 inactivation. Our data reveal an intrinsic defect in the maintenance, expansion and long-term blood reconstitution of fetal as well as adult LT-HSCs lacking FUBP1, implying that FUBP1 is a central player in the regulation of fetal and adult HSC self-renewal.

Further studies of FUBP1 signaling will increase our understanding how FUBP1 is mechanistically implicated in the regulatory network that controls the development and maintenance of the hematopoietic system. Our future studies will also reveal whether FUBP1 plays an important role in hematologic disorders. We previously published that FUBP1 is required for hepatocellular carcinoma (HCC) tumor growth. As a crucial factor for LT-HSC self-renewal, FUBP1 may also function as an oncoprotein in leukemia initiating cells (LICs) in chronic (CML) and/or acute (AML) myeloid leukemia.

Rabenhorst, U., Thalheimer, F. B., Gerlach, K. et al. Single-Stranded DNA-Binding Transcriptional Regulator FUBP1 Is Essential for Fetal and Adult Hematopoietic Stem Cell Self-Renewal. Cell Reports 11 (2015)

Rabenhorst, U. et al. Overexpression of the far upstream element binding protein 1 in hepatocellular carcinoma is required for tumor growth. Hepatology 50, 1121-9 (2009)

Abstract No. P007

Identification of Key Regulators of Symmetric versus Asymmetric Cell Divisions During Human Hematopoietic Lineage Specification

André Görgens[1,*], *Stefan Radtke*[1], *Symone Vitoriano*[1], *Florian Murke*[1], *Michael Möllmann*[2], *Jan Dürig*[2], *Helmut Hanenberg*[3], *Peter A. Horn*[1], and *Bernd Giebel*[1]

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Hematopoietic stem cells (HSCs) contain lifelong potentials to self-renew and to create progenitors of all mature blood cells. According to the current view, HSC homeostasis is controlled by both, HSC-niches as well as asymmetric cell divisions. Our previous studies linked the process of asymmetric cell division of human hematopoietic stem and progenitor cells (HSPCs) to the expression of the stem cell surrogate antigen Prominin 1/CD133 (1). Furthermore, by characterizing human HPSCs subpopulations by means of their CD133 surface expression we gained evidence that CD133+ multipotent progenitors (MPPs) create CD133+ lymphomyeloid (LMPP) and CD133low erythromyeloid (EMP) daughter cells (2). Regarding lineage specification, we showed for the first time that under conventional culture conditions almost all MPPs divide asymmetrically to create a set of LMPP and EMP daughter cells, resulting in a loss of MPPs after the first cell division (3). Thus, our data suggest that under conventional culture conditions asymmetric cell divisions are rather lineage-instructive than self-renewing (4). Now, aiming to identify key factors regulating the MPP division mode, we study whether conditions reported to promote HSC/MPP expansion interfere with the outcome and symmetry of the HSC/MPP cell division. In this context we co-cultured human MPPs with murine and primary human stromal lines (human bona fide MSCs) and surprisingly observed that LMPPs are maintained and expanded but not MPPs. This contrary finding can be attributed to the former experimental definition of multipotent cells based on the classical model of hematopoiesis, according to which cells with dual lymphocyte and granulocyte (conventionally neutrophil) potentials can insufficiently be considered as multipotent. Currently, we test other culture conditions reported to expand human HSCs/MPPs *in vitro*. After confirming any of these conditions as HSC/MPP expansion condition, we will analyze its impact on the division mode of HSCs/MPPs.

(1) Beckman et al, Blood 2007.
(2) Görgens et al, Cell Reports 2013.
(3) Görgens et al, Stem Cell Reports 2014.
(4) Görgens et al, Cell Cycle 2013.

Abstract No. P008

Connective tissue growth factor (CTGF) is an inducible stroma-derived regulator of hematopoietic stem cell maintenance and cell cycle progression

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Hematopoietic stem cell (HSC) are regulated by their 'niche', which limits activation of HSCs, to ensure their long-term maintenance and self-renewal. In culture, HSCs can only be preserved with stromal cells, or their conditioned medium. To dissect the interactions involved during rapid activation in a co-culture model, we studied gene expression changes of UG26-1B6 stromal cells in response to the presence of Lineage- Sca-1+ Kit+ (LSKs). Clustering and candidate gene prioritization of stromal cell gene expression data identified connective tissue growth factor (Ctgf) as highly upregulated in stromal cells in response to LSK cells. Culture of HSCs on stroma with decreased Ctgf (shCtgf) expression impaired HSC engraftment, in particular myeloid engraftment, and long-term quality of co-cultured HSCs. As a possible underlying mechanism, we found that LSK cells from shCtgf stromal cocultures show a higher fraction of cells in G0 and impaired G0/G1 transition. Single cell cultures of CD34-, but not CD34+, CD48- CD150+ LSK cells in shCtgf stromal conditioned medium showed a delayed time to first division. To understand why a decrease of environmental Ctgf favors G0 retention, we assembled a CTGF signaling network model. Experimental validation of this model showed that CD34-, but not CD34+, CD48- CD150+ LSK cells express the TGFBR1, and co-cultures of these cells that demonstrated that diminished CTGF in the external space activates TGFbeta / Smad2/3 signaling, increases CDKN1B expression, and downregulates Cyclin D1. In summary, our data shows rapid stromal CTGF upregulation in response to LSK cells, which is required for proper cell cycle activation and the maintenance of long-term repopulating HSCs with myeloid engraftment potential.

Abstract No. P009

Transcriptome-wide profiling and post-transcriptional analysis of hematopoietic stem/progenitor cells during myeloid commitment

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Hematopoietic stem cells are critical due to their life-long self-renewal activity and generate multipotent progenitors that differentiate into lineage-committed and subsequently mature cells. In this study, we present a transcriptome-wide (RNA-seq) analysis of ex vivo isolated mouse multipotent hematopoietic stem/progenitor cells (LinnegSca-1+c-Kit+) and myeloid committed precursors (LinnegSca-1negc-Kit+), which covers coding and non-coding RNAs. Our data display the dynamic transcriptional networks and identify a novel stem/progenitor signature, which is characterised by cell adhesion and immune response components such as kallikrein-related proteases. We present a global signature of 498 lncRNAs which are potential regulators of self-renewal and/or multipotency. By integrating the transcriptome data with our recently reported proteome data (Klimmeck et al., 2012), we found post-transcriptional regulation of metabolism and response to oxidative stress. Finally, our study identifies a surprisingly high number of protein isoforms generated by alternative splicing upon lineage commitment. In summary, this in-depth molecular analysis outlines the enormous complexity of gene expression of coding and non-coding RNAs as well as post-transcriptional regulation during the early differentiation steps of HSCs.

Ref:
1. Klimmeck, D. et al. (2014). Stem Cell Reports, 3, 858–875.
2. Klimmeck, D. et al. (2012). Mol Cell Proteomics, 11, 1-54.

Abstract No. P010

Screening for HSC Regenerating Genes using the Mpl-Deficient Mouse Model

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Mpl represents the cytokine receptor for thrombopoietin (Thpo) and Thpo/Mpl-signaling regulates megakaryopoiesis, hematopoietic stem cell (HSC) maintenance and post-transplant expansion. Importance of Thpo/Mpl-signaling in HSC is demonstrated by MPL deficiency in human patients, causing thrombocytopenia and lethal aplastic anemia, called congenital amegakaryocytic thrombocytopenia. To further elucidate the molecular mechanism we performed transcriptome analysis of the stem cell enriched Lin-, Sca-1+ and c-Kit+ (LSK) cell population in two different transplantation models. In one model, the HSC defects of the Mpl-/- mouse model were corrected by lentiviral overexpression of Mpl. In the other model, expression of an intracellular-truncated, signaling-deficient Mpl receptor (termed dominant-negative (dn)Mpl) in wildtype mice induced thrombocytopenia and HSC defects. dnMpl mice had 4-fold reduced LSK cells and bone marrow cells did not engraft in secondary recipients. The expression profile of dnMpl LSK cells negatively correlated with known HSC stemness signatures and the Wnt-, Jak/Stat-, and PI3K/Akt-signaling pathways, while expression of genes involved in cell cycle progression were positively correlated. The lower percentage of quiescent HSC was confirmed by flow cytometry and the expression of mouse HSC markers (Tie2, EPCR, Esam1) was significantly reduced (**$p<0.005$) in dnMpl mice compared to control mice. *In vitro* experiments revealed receptor competition for Thpo binding and intracellular inhibition of wtMpl-signaling as the underlying mechanisms. By comparing the transcriptome of Mpl corrected Mpl-/- LSK cells and Thpo/Mpl-signaling inhibited LSK cells we could identify Thpo/Mpl-induced genes and pathways that are potential therapeutic targets for the regeneration of HSC. Candidate genes, including transcription factors and aforementioned surface marker with unknown function, are currently investigated in the Mpl-/- transplantation model for their effects on hematopoiesis. Lentiviral overexpression of selected candidates improved engraftment potential of Mpl-/- cells, altered progenitor cell proliferation or corrected thrombocytopenia confirming the Thpo target genes as potential therapeutic targets for the regeneration of HSC.

Abstract No. P011

Phosphorylation of the angiotensin converting enzyme (ACE) blocks stem cell mobilization by granulocyte-colony stimulating factor (G-CSF)

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The blood pressure regulating ACE has been proposed to be a stem cell marker influencing hematopoietic progenitor cell(HPC)-mobilization. ACE is not only a peptidase, it is also capable of signal transduction by a mechanism initiated by CK2-mediated ACE-phosphorylation (Ser1270). Aim of this study was to verify ACE-expression in HPCs and to determine the role of ACE-signaling in HPC-mobilization.

ACE was expressed by a small population of bone marrow cells, but more strongly in bone itself. To determine whether ACE played a role in HPC-mobilization, wild-type (WT) and ACE-deficient (ACE.4) mice were treated with vehicle or G-CSF (5 days) and HPC-mobilization was assessed by quantifying colony-forming units (CFU) in culture formed by peripheral blood derived mononuclear cells (PB-MNCs). The number of CFUs formed by G-CSF-stimulated ACE.4-derived PB-MNCs was significantly higher than from WT-derived PB-MNCs. Also, in WT mice the ACE-inhibitor Ramipril increased CFU-formation. Transplantation rescue experiments demonstrated that the loss of ACE in the HPC microenvironment rather than in the HPCs themselves increased mobilization. To assess how ACE-deletion affects mobilization, we focused on “ACE-signaling” and found that G-CSF elicited ACE-phosphorylation sensitive to CK2-inhibition. The role of ACE-phosphorylation in HPC-mobilization was addressed in transgenic mice expressing non-phosphorylatable ACE (ACES/A), which demonstrated a significant increase in G-CSF-stimulated CFU-formation. Mass spectrometry analysis of the proteins co-precipitated with ACE revealed that several proteins with putative links to HPC-mobilization colocalised with ACE from WT but not ACES/A mice.

These data demonstrate an important role for ACE in the HPC microenvironment and revealed that ACE-expression/phosphorylation negatively affects the G-CSF-induced HPC-mobilization, most likely via interaction with specific proteins.

Abstract No. P012

The significance of material properties for stem cells: How matrix mechanics, nanostructure and 3D architecture impact hematopoietic stem cells

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Human hematopoietic stem cells (HSCs) are the only stem cells applied routinely to treat patients suffering from hematological disorders. Their use in the clinics, however, is restricted by their limited availability. Therefore, multiplication of HSCs by *in vitro* cultivation would be of benefit for tens of thousands of patients. The fundamental problem in this purpose is the fast onset of differentiation of HSCs during *in vitro* culture. *In vivo* HSCs are mainly located in the bone marrow in stem cell niches. These niches possess unique microenvironments that allow the maintenance and self-renewal of HSCs. In recent years it turned out that not only the biochemical composition of the microenvironment but also its physical properties such as substrate nanostructure, elasticity and architecture influence cell behavior. We aim to understand the mutual biological and physical interactions of HSCs with their niches and to engineer artificial biomimetic stem cell niches that allow the targeted *in vitro* expansion or differentiation of HSCs.

By using materials sciences techniques to answer biological questions we study the influence of nanostructure, mechanical properties and architecture (2D versus 3D) of the environment on HSCs. We could show that HSC adhesion, signal transduction and gene expression depend on the precise nanometer-scaled distance between integrin ligands by applying nanopatterned, bio-functionalized gold nanoparticle arrays. Furthermore, we investigated the role of substrate stiffness in the HSC niche with the help of PEG hydrogels with tunable mechanical properties. The development of a biomimetic 3D scaffold allowed us to show how the three-dimensional architecture of the environment in synergy with other factors impacts HSC proliferation and differentiation. Our future studies will focus on the further development of 3D cell culture systems as well as multifunctional nanostructured biomaterials for HSCs with the vision to engineer -one day- an artificial stem cell niche for HSC proliferation.

Raic A, Rödling L, Kalbacher H, Lee-Thedieck C (2014). Biomimetic macroporous PEG hydrogels as 3D scaffolds for the multiplication of human hematopoietic stem and progenitor cells. Biomaterials 35: 929–940.

Altrock E, Muth C, Klein G, Spatz J, Lee-Thedieck C (2012). The significance of integrin ligand nanopatterning on lipid raft clustering in hematopoietic stem cells. Biomaterials 33: 3107-3118.

Abstract No. T27/P013

CCND1/CDK4-mediated cell cycle progression provides a competitive advantage for human hematopoietic stem and progenitor cells *in vivo*

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Hematopoietic stem cell (HSC) function is well maintained over time and it is thought that one main reason is a tight control on cell cycle entry and, inversely, HSC activation is measured by a G0 to G1 phase shift. Still, it remains unknown whether the transit time through distinct cell cycle phases itself influences HSC activity. Therefore, we tested the effect of a 'shortened' G1 cell cycle phase on fate choices of human HSCs, reasoning that a 'short' G1 phase may provide less opportunity for the delivery of differentiation-inducing signals compared to a 'long' G1 phase. To shorten G1 experimentally, we overexpressed the G1 phase regulator Cyclin D1 and its catalytic partner cyclin-dependent kinase 4 (CCND1/CDK4, termed 4D) in human $CD34^+$ HSPCs. As a result, the G1 phase of HSPCs was shortened while overall cell cycle length was unaffected. Consequently, there was no difference in HSPC proliferation and colony formation *in vitro*. In contrast, serial transplantation experiments revealed an increased HSPC engraftment in secondary recipients compared to controls, suggesting that an accelerated transit through G1 improves the maintenance and function of hematopoietic stem cells *in vivo*. Consistently, 4D transduced HSPCs were protected from differentiation-inducing signals evidenced by an increased preservation of HSC numbers upon stimulation with LPS or growth factors. These results suggest that a shortened G1 phase transit is a mechanism providing resistance for human HSPCs to hematopoietic stress signals and favors their long-term maintenance. Interestingly, overexpression of the second G1 phase regulatory complex CCNE1/CDK2 promoted a more accelerated transit through G1, but resulted in the rapid loss of functional HSPCs *in vivo*. Thus, our data suggests that a balanced transit time through early and late G1 cell cycle phase is a key regulator for human HSPC function and provides a new regulatory platform to ensure lifelong hematopoiesis.

Abstract No. P014

Mechanism of robust human hematopoietic stem cell engraftment and expansion in a Kit-deficient mouse model.

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Humanized mice could enable the in-depth analysis of human hematopoietic stem cell (HSC) function. However, currently available mouse models for xenotransplantation have difficulties in achieving stable engraftment of substantial numbers of human HSCs. Further, self-renewal of engrafted human HSCs is limited as poor engraftment in secondary recipients shows. We previously described a new mouse model that combines a loss-of-function Kit receptor with immunodeficiency. The NSG KitW41/W41 (NSGW41) model efficiently supports stable engraftment of human HSCs in the long-term without the need for previous irradiation and displays improved multilineage engraftment, especially of the myeloid and erythroid lineages. Transplanting titrated numbers of human HSCs into NSGW41 mice revealed that human HSCs expand in situ. Secondary transplants still show robust levels of engrafted human cells including stem and progenitor cells, suggesting that the functionality of expanded human donor HSCs is preserved in NSGW41 mice. Interestingly, we observe a decrease of endogenous mouse HSCs in transplanted NSGW41 mice that is dependent on the level of human cell engraftment. Thus, we hypothesize that endogenous stem cells carrying a defective Kit receptor are largely replaced by human KIT-proficient HSCs indicating that mouse and human HSCs may occupy the same niches in the murine bone marrow. Preliminary data suggests that NSGW41 mice may also support improved engraftment of malignant hematopoietic cells. We conclude that Kit-signaling regulates HSC engraftment across the human-mouse species barrier and that Kit deficient mice show great potential for the study of human HSC functions including self-renewal and differentiation as well as malignant hematopoiesis.

Cosgun, K.N., Rahmig, S., Mende, N., Reinke, S., Hauber, I., Schafer, C., Petzold, A., Weisbach, H., Heidkamp, G., Purbojo, A., et al. (2014). Kit Regulates HSC Engraftment across the Human-Mouse Species Barrier. Cell Stem Cell.

Abstract No. P015

The heterotrimeric G-proteins Gαq/11 are required for Flt3-ITD autophosphorylation and regulate normal and leukemic HSPC cell cycle progression

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Cell cycle control of hematopoietic stem and progenitor cells is instrumental for long-term blood regeneration. Heterotrimeric G-Proteins transmit signals of G-Protein coupled receptors and are important regulators of basic cellular functions. However, their role in normal and malignant hematopoiesis remains obscure. Previous data suggested the involvement of heterotrimeric G-proteins in Flt3-ITD-mediated leukemic transformation. FMS-like tyrosine kinase 3 with internal tandem duplication is an important oncoprotein in acute myeloid leukemia causing constitutive active STAT5 signaling.

Here, we examined conditional double KO mice for Gαq and Gα11, heterotrimeric G-proteins with high redundancy, in normal hematopoiesis and in Flt3-ITD positive leukemia. We could show that Gαq is important for Flt3-ITD mediated transformation, as Gαq activity is necessary to maintain the autophosphorylated status of FLT3-ITD. We further determined a direct physical interaction between FLT3-ITD and Gαq. Moreover, Gαq is required to transmit Flt3-ITD ligand independent proliferation and downstream signaling. Under non-leukemic conditions, hematopoietic stem and progenitor cells (HSPCs) deficient for Gαq/11 reconstitute primary recipients less efficient than controls, but remain more immature over time, shown by a higher repopulation in secondary recipients. This suggested a delay in cell cycle activation of HSCs in Gαq/11 KO mice. Indeed, Gαq/11 double KO HSPCs accumulated in the G0 dormant phase and showed a decreased fraction of cells in active cell cycle. Additionally, cell cycle related gene expression in HSPCs differed significantly in absence of Gαq, unravelling Gαq/11 as important regulators of HSC dormancy, proliferation and long-term maintenance. Furthermore, we observed decreased phosphorylation of the important regulators of proliferation AKT and STAT5 in Gαq/11 knockout mice.

These results show that Gαq/11 regulate dormancy and proliferation of HSPCs by influencing expression of cell cycle regulators. Under malignant conditions, the interaction between Gαq and Flt3-ITD is functionally relevant for FLT3-ITD-induced transformation and has potential as a target for antileukemic strategies.

Abstract No. P016

The significance of 3D architecture and perfusion in a biomimetic model of the hematopoietic stem cell niche

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Hematopoietic stem cells (HSCs) can differentiate into all blood cell types and hold great promise for the treatment of leukemia: when transplanted into patients HSCs reconstitute the entire hematopoietic system.

The demand on HSCs, however, is higher than their availability. Hence it is of great interest to elucidate the conditions required for *in vitro* HSC expansion. Their niche in the bone marrow is so far the only place known where HSCs expand while preserving their stemness.

In order to have a tool to study and identify critical factors among all stem cell – niche interactions, our group developed an artificial biomimetic HSC niche. This artificial niche consists of a macroporous PEG hydrogel and presents the minimal integrin recognition peptide RGD on its surface. When hematopoietic stem and progenitor cells (HSPCs) were cultured together with mesenchymal stem/stroma cells (MSCs) as supporting cell type in this artificial niche, significantly higher numbers of HSPCs compared to all control conditions that lacked MSCs or/and the 3D environment were produced [1]. Perfusion of the hydrogel was established to avoid shortage of nutrients and oxygen inside the hydrogel. Perfusion seemed to favor HSPC differentiation.

The most primitive HSPCs were found outside of the hydrogel. As a consequence, the necessity of the 3D environment as well as the direct contact of HSPCs and MSCs was tested separately. We found that MSCs might depend on the 3D environment in order to provide supporting signals for the maintenance of HSC phenotype and function. An indirect contact of both stem cell types seemed to be superior to direct cell-cell contacts for HSPCs expansion. These results indicate that compartmentalization of HSPCs and MSCs in a 3D environment might be the culture conditions to choose in order to obtain higher numbers of HSPCS during *ex vivo* expansion.

[1] A. Raic, L. Rödling, H. Kalbacher & C. Lee-Thedieck. Biomimetic macroporous PEG hydrogels as 3D scaffolds for the multiplication of human hematopoietic stem and progenitor cells. Biomaterials 2014

Abstract No. P017

WNT5A expressed by the niche is required to maintain migratory properties of hematopoietic stem cells through the planar cell polarity pathway

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We previously identified Wnt5a as a secreted factor that maintains HSCs *in vitro* (Buckley, et al., Exp Hematol. 2011) and as a factor responsible for decline of HSC function during aging (Florian et al., Nature. 2013). We here studied in detail how Wnt5a-deficiency affects the niche and its ability to maintain long-term repopulating HSC.

Chimeric transplantation experiments showed that Wnt5a+/- HSCs engraft similarly to wild-type Wnt5a+/+ (WT) HSCs up to tertiary transplantations. To assess whether niche Wnt5a affects engraftment, and/or self-renewal capacity of HSCs, wild-type (WT) HSCs were transplanted in Wnt5a-deficient recipients. We found that in primary recipients, engraftment was similar to that in WT recipients. Unexpectedly, however, WT donor LSK cells from the Wnt5a-deficient recipients completely failed to engraft in secondary recipients. Although the phenotype of the WT donor cells engrafted in either WT or Wnt5a+/- environments was similar, the sorted LSK cells from Wnt5a+/- recipients showed defective, apolar distribution of Cdc42 and F-actin, suggesting possible defects in migratory properties. Indeed, RNAseq of sorted donor LSK cells from Wnt5a+/- recipients showed highly divergent expression of molecules involved in heterotrimeric G-protein assembly and small GTPase-mediated planar cell polarity (PCP) signal transduction. Further experiments showed that WT donor LSK cells from Wnt5a+/- recipients showed defective lamellipodia formation, and only poorly homed to the bone marrow of recipient WT mice. In addition, mature T and B lymphocytes failed to migrate towards CXCL12.

In conclusion, our experiments show that a Wnt5a-deficient niche allows for initial engraftment, but fails to maintain the ability of engrafted HSCs to properly home and engraft secondary recipients, due to defective PCP-regulated migratory properties of HSCs and mature lymphoid cells.

Abstract No. P018

Adrenergic stimulation alters the mechanical properties of bone marrow niche cells and interferes with the adhesion of hematopoietic progenitor cells

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Hematopoietic stem and progenitor cells (HSPC) reside in the bone marrow in endosteal and vascular niches. The interaction with niche cells, especially with osteoblasts and mesenchymal stromal cells (MSC), is important for their maintenance. We have recently shown that human osteoblasts flatten and get stiffer after adrenergic stimulation which alters the adhesion of HSPC during their mobilization into the peripheral blood, indicating that mechanical properties of the niche are an important factor during HSPC mobilization. However, it remains unknown whether this effect is restricted to osteoblasts or whether it is a general mechanism in the niche.

Using scanning ion conductance microscopy (SICM) and atomic force microscopy (AFM), we studied the mechanical and morphological properties of human niche cells and their interaction with HSPC after adrenergic stimulation with the β2-adrenergic agonist clenbuterol. In addition to osteoblasts and the osteoblast-cell line CAL-72 we investigated primary bone marrow-derived MSC, the MSC cell line iMSC#3 (Skårn et al., Stem Cells Dev. 23: 2377, 2014) and the bone marrow stromal cell line HS-5.

The expression of at least one β-adrenergic receptor could be identified by RT-PCR analysis for CAL-72 cells, osteoblasts, MSC cells and HS-5 cells but not for the cell line iMSC#3. Stiffening and/or flattening after clenbuterol treatment was observed for most of the cells. The adhesion of CD34+ HSPC on the different niche cells notably decreased after clenbuterol treatment, as analyzed by AFM single cell detachment force measurement. High resolution SICM imaging indicated a smoothening of the cell surface of clenbuterol-treated niche cells.

Our data suggest that not only osteoblasts, but also other niche cells respond to adrenergic signaling by flattening and stiffening. The HSPC can respond to the altered mechanical properties by a reduced adhesive strength, which might be facilitated by the smoothening of the surface of the niche cells.

Abstract No. P019

The Role of Neogenin in HSC Maintenance and Activation

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Somatic stem cells harbor the highest self-renewal activity and generate a series of multipotent progenitors that differentiate into lineage-committed progenitors and subsequently mature cells. Recently, we explored the molecular signatures employed by hematopoietic-stem-cells (HSC) along differentiation by performing quantitative proteome, transcriptome (RNA-seq) and whole genome DNA methylation data analyses on five HSC and multipotent progenitors populations (MPP1-4) (Cabezas-Wallscheid et al., Cell Stem Cell 2014; Klimmeck et al., Stem Cell Reports 2014; Lipka et al., Cell Cycle 2014). We identified more than 6,000 expressed proteins, 27,000 genes and 15,000 differentially methylated regions.

Interestingly, the DCC-like transmembrane receptor Neogenin was among the most enriched genes in HSCs. Neogenin binds different neuronal guidance molecules in a variety of tissues and functions as a BMP co-receptor. Our hypothesis is that Neogenin (Neo) and its ligands might directly and indirectly influence HSC regulation and activation. To functionally address our hypothesis, we are using a Neogenin-deficient mouse model. Neo KO mice die 4-8, weeks after birth and show phenotypes in cerebral development and iron homeostasis. Upon competitive transplantation, Neogenin KO crowds out wildtype bone marrow, on the level of differentiated as well as Stem Cells. We propose that an altered baseline inflammatory state fine-tuned by BMP signaling might mediate this effect by interfering with the control of cell cycle, apoptosis and migratory behavior.

Abstract No. P020

Humanized mice – a suitable model to study human hematopoiesis and immunotherapeutics for cancer therapy *in vivo*

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Objective
The preclinical evaluation of novel checkpoint modulators is dependent on models with a functional human immune system. In previous experiments, we have demonstrated, that hematopoietic stem cells (HSC) can proliferate and differentiate *in vivo*. However, it has to be evaluated whether parallel xenotransplantation of patient-derived human tumors (PDX) in these mice would allow to generate a panel of personalized models for immuno-oncology research. We designed a study to investigate the differentiation and function of HSC in immunodeficient mice in the presence of transplanted PDX.

Methods
HSC, isolated from cord blood were transplanted intravenously into three week old irradiated nod scid gamma mice. After 4 and 8weeks, blood was collected and screened by FACS for human leukocytes (huCD45+). After 12-16weeks, PDX were subcutaneously transplanted into these humanized mice and followed for growth and tolerance. Functionality of the human immune system was evaluated by treatment of mice with the CTLA-4 checkpoint inhibitor ipilimumab.

Results
After 16weeks up to 50% of leukocytes in the blood were CD45+ cells and T-cell subpopulation reached up to 20%. Engraftment in different organs has been detected, with up to 10% CD45+ cells in thymus, 40% in spleen, and 60% in the bone marrow.

PDX (lung, renal, mammary cancer, melanoma) showed engraftment in the humanized mice. No significant difference was observed compared to the growth in immunodeficient mice demonstrating no innate immune response against the tumors. Treatment with ipilimumab led to a tumor growth delay and an increased percentage of T-cells in the blood and in the tumor (up to 75% of CD45+ cells).

Conclusion
Transplantation of HSC into immunodeficient mice generates a human immune system. PDX can be successfully transplanted into these humanized mice. Initial results revealed the function of our model as a tool to investigate the human hematopoiesis and the efficacy of immunotherapeutics.

Abstract No. P021

Hyperinflammation triggers an IL-1b-dependent activation of hematopoietic stem and progenitor cells

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Inflammation is a physiological response to infections and tissue damage. In immunodeficiency syndromes, like chronic granulomatous disease (CGD), hyperinflammation is a recurrent problem. Defects in the phagocytic NADPH oxidase 2 subunit gp91*phox* cause X-linked chronic granulomatous disease (X-CGD). We studied the effects of hyperinflammation on the hematopoietic compartment in X-CGD and wild-type mice. X-CGD mice displayed an expanded hematopoietic progenitor cell (HPC) population in the bone marrow, a decreased reconstitution capacity of hematopoietic stem cells in competitive transplantation experiments and increased levels of interleukin 1β (IL-1β) protein in bone lavages.

IL-1β treatment of wild-type mice induced an expansion of HPCs and a reduction of long-term reconstitution capacity of hematopoietic stem cells upon transplantation, mimicking the effects observed in X-CGD mice. Treatment of X-CGD mice with dexamethasone to control inflammation and the IL-1 receptor antagonist anakinra resulted in a significant decrease in the proportion of hematopoietic progenitor cells (LSK cells) in the bone marrow.

Treatment of human CD34+ cells with IL-1β induced a decrease in the frequency of phenotypically characterized HPCs, but increased the number of cells with colony-forming potential. IL-1β treatment of NSG mice transplanted with CD34+ cells reduced the frequency of hematopoietic stem/progenitor cells (CD34+/CD38- cells) in the bone marrow.

Thus, our experiments decipher the effects of IL-1β on murine hematopoietic stem and progenitor cells, and suggest a role for IL-1β on human hematopoietic homeostasis.

Abstract No. P022

The Gadd45 family as mediators of hematopoietic stem cell differentiation – from genotoxic stress to cytokine-induced differentiation

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The growth arrest and DNA-damage-inducible 45 (Gadd45) protein family is encoded by three genes, Gadd45a, b and g. They are early responders of cellular stress with tumor-suppressive function. Lately, we identified GADD45G as the molecular link of differentiation-promoting cytokines to induce differentiation in HSCs. Here, we unraveled the function of the genotoxic stress-induced Gadd45a in hematopoiesis. GADD45A has been implicated in cell cycle control, cell death and senescence, as well as in DNA damage repair. In general, GADD45A provides cellular stability by either arresting the cell cycle progression until DNA damage is repaired or, in cases of fatal damage, by inducing apoptosis. However, the function of GADD45A in hematopoiesis remains controversial.

We revealed the changes in murine HSC fate control orchestrated by the expression of GADD45A at single cell resolution. In contrast to other cellular systems, GADD45A expression did not cause a cell cycle arrest or an alteration in the decision between cell survival and apoptosis in HSCs. Though GADD45A strongly induced and accelerated the differentiation program. Continuous tracking of individual HSCs and their progeny via time-lapse microscopy elucidated that once GADD45A was expressed, HSCs differentiate into committed progenitors within 29 h. GADD45A-expressing HSCs failed to long-term reconstitute the blood of recipients by inducing multi-lineage differentiation *in vivo*. The differentiation induction by GADD45A was transmitted by activating p38 MAPK signaling, and allowed the generation of megakaryocytic-erythroid, myeloid and lymphoid lineages.

These data indicate that GADD45A expression in HSCs prevents their fatal transformation by directing them into differentiation and thereby clearing them from the system. As the differentiation induction is conserved throughout the GADD45 family our study establishes this cell fate as an HSC-specific DNA-damage escape mechanism. Comparative analyses of the three proteins will further dissect the induced mechanisms at the molecular level.

Abstract No. P023

The phosphatases STS1 and STS2 regulate hematopoietic stem and progenitor cell fitness

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FLT3 and c-KIT are crutial regulators of hematopoietic stem and progenitor cells. After ligand stimulation, their surface expression is tightly controlled by the E3 ligase CBL, promoting ubiquitination, endocytosis and signal termination. In this study, we use genetic tools to investigate the role of two important CBL-binding partners, STS1 and STS2, on FLT3 and c-KIT phosphorylation, activity and function in normal and stress-induced hematopoiesis. STS1/STS2 deficient mice show a profound expansion of multipotent progenitors and FLT3+/limphoid primed multipotent progenitors, with elevated colony-forming capacity *in vitro* and *in vivo*. Although long-term hematopoietic stem cells are not increased in numbers, lack of STS1 and STS2 significantly promotes long-term repopulation activity and recovery after cytotoxic stress, demonstrating a pivotal role of STS1/STS2 in regulating hematopoietic stem and progenitor cell fitness. Biochemical analysis identified STS1/STS2 as phosphatases of FLT3 and c-KIT. Loss of STS1/STS2 induces hyperphosphorylation of FLT3, leading to enhanced AKT signaling both *in vitro* and *in vivo*. Remarkably, depletion of STS1/STS2 provides a strong proliferative advantage that is dependent on FLT3 kinase activity. Therefore, our study reveals that STS1 and STS2 may serve as a novel pharmaceutical target to improve hematopoietic recovery after bone marrow transplantation and cytotoxic stress.

Abstract No. P024

α-Catenin is a dosage-dependent tumor suppressor gene in MDS

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Myelodysplastic syndromes (MDS) are a heterogeneous group of hematological disorders characterized by clonal expansion of hematopoietic stem and progenitor cells (HSPCs). Cytogenetic and high-resolution genomic profiling studies of a large patient cohort have defined two commonly deleted regions (CDRs) on chromosome 5q. However, the identity of the predicted haploinsufficient tumor suppressor gene within the proximal 5q CDR is still obscure. Here we employ a transduction/transplantation experiment to establish a mouse model of this specific genetic aberration using lentiviral-mediated RNA interference (RNAi). As donor cells, we used HSPCs obtained from *Tp53* knockout mice to implement the frequent co-occurrence of inactivating *TP53* mutations with 5q deletions in patients with MDS. We first tested the knockdown of α-Catenin (*Ctnna1*) and found a competitive advantage of HSPCs with loss of *Tp53* and reduced expression of *Ctnna1* leading ultimately to a myeloid malignancy and an earlier onset of death compared to control animals. To further specify the observed phenotype and the downstream effects of low *Ctnna1* levels, we cultivated primary wildtype bone marrow cells with RNAi-mediated knockdown of *Ctnna1*. Surprisingly, this genetic modification was sufficient for the survival, proliferation and immortalization of the cells *in vitro*. After 8 weeks in culture, the cells mainly showed a myeloid immunophenotype, and the culture contained a lineage-negative subpopulation of progenitor cells that appeared to maintain its growth. The re-expression of *Ctnna1* reversed this growth phenotype *in vitro* revealing the specificity of the RNAi-mediated effect. Currently, we perform a gene expression analysis to define the molecular pathway engaged by reduced expression of *Ctnna1*. Our studies determined the role of *Ctnna1* for the pathogenesis of MDS with 5q deletions and will uncover potential functions of *Ctnna1* in hematopoiesis.

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Abstract No. P025

The STAT3-Ser/Hes3 Signaling Axis: An emerging molecular player in stem cell biology

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The STAT3-Ser/Hes3 Signaling Axis regulates the number and function of various stem and progenitor cells, including neural, brain cancer, and adrenomedullary chromaffin progenitors, as well as pancreatic islet beta cells in paradigms of damage and regeneration: (a) When Hes3 is knocked out from the Hes1:Hes5 double mutant mouse line, neuroepithelial cells prematurely differentiate into neurons during development; (b) Delta4, a pathway activator, increases the number of endogenous progenitors in several areas of the adult brain, resulting in profound neuroprotection; (c) Macrophage inhibitory factor induces Hes3 expression and promotes neural stem cell (NSC) / progenitor cell proliferation and maintenance; (d) A phosphomimetic STAT3-serine construct promotes prostate tumorigenesis; (e) Hes3 knockdown opposes the growth of cultured glioblastoma cancer stem cells; (f) The anti-tumor efficacy of a small molecule inhibitor of gamma-secretase can be predicted by the level of Hes3 expression in breast cancer xenograft models; (g) Notch-dependent STAT3-serine phosphorylation contributes to the growth of embryonic stem cell-derived NSCs following induction of Hoxb1 expression. More recently, the pathway has been implicated in different paradigms of reprogramming, and specifically in decisions towards iPS state and transitions to the NSC lineage: (1) hES cells express Hes3 whose expression transiently increases in the early neural specification stage; (2) Different combinations of transduced genes induce the direct reprogramming of adult mouse cells into NSCs; all successful gene combinations include Hes3; (3) Pharmacological upregulation of Hes3 greatly improves the yield and quality of neurons derived from iPS cells. We present work on the involvement of this pathway in reprogramming (*in vitro* and *in vivo*), cancer stem cell growth, and brain and pancreas regeneration and its use in identifying drug targets against the elusive cancer stem cell population. This signaling pathway is operational in many aspects of stem cell biology and deserves a broader investigation.

Hes3 is expressed in the adult pancreatic islet and regulates gene expression, cell growth, and insulin release. J. Biol. Chem. PMID: 25371201
Hes3 regulates cell number in cultures from glioblastoma multiforme with stem cell characteristics. Sci Rep. PMID: 23393614

Abstract No. P026

Hepatic differentiation of hiPSC in a perfused 3D multicompartment bioreactor

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The hepatic differentiation of human induced pluripotent stem cells (hiPSC) holds great potential for applications in regenerative medicine, pharmacological drug screening and toxicity testing. However, full maturation of hiPSC into functional hepatocytes has not yet been achieved.

In this study, we investigated the potential of a perfused 3D hollow fibre membrane bioreactor technology to improve the hepatic differentiation of hiPSC in comparison to static 2D cultures. A total of 100 Mio. hiPSC were seeded into each bioreactor (n=3). After a proliferation phase of three days with mTeSRTM1 differentiation into definitive endoderm was induced by adding activin A, Wnt3a and sodium butyrate to the culture medium. For further maturation hepatocyte growth factor and oncostatin M were added. The same differentiation protocol was applied to hiPSC in 2D cultures.

During hepatic differentiation a stable metabolism, assessed by glucose consumption and lactate production, was observed in 3D as well as in 2D cultures. Secretion of alpha-fetoprotein (AFP), a marker for definitive endoderm, was significantly ($p<0.05$) higher in 2D cultures, while albumin secretion was higher after hepatic differentiation of hiPSC in 3D bioreactors. Functional analysis of different cytochrome P450 isoenzymes (CYP) showed activity of CYP1A2, CYP2B6 and CYP3A4, with a significantly ($p<0.05$) higher activity of CYP2B6 in 3D bioreactors as compared with 2D cultures. Immunofluorescent staining showed that the majority of cells were positive for albumin, cytokeratin 18 and HNF at the end of the differentiation process. In addition, CK 19 staining revealed the formation of bile duct like structures similar to native liver tissue in 3D bioreactors.

The results indicate a better maturation of hiPSC in the bioreactor system compared to 2D cultures and emphasizes the value of dynamic 3D culture systems in stem cell differentiation approaches especially regarding the formation of tissue like structures.

Hay DC, Zhao D, Ross A, Mandalam R, Lebkowski J, Cui W (2007) Direct differentiation of human embryonic stem cells to hepatocyte-like cells exhibiting functional activities. Cloning Stem Cells. 9(1):51-62. Erratum in: Cloning Stem Cells. 2009 Mar;11(1):209

Miki T, Ring A, Gerlach J (2011) Hepatic differentiation of human embryonic stem cells is promoted by three-dimensional dynamic perfusion culture conditions. Tissue Eng Part C Methods 17:557-68. doi:10.1089/ten.TEC.2010.0437

Abstract No. P027

Directed cardiac differentiation of cynomolgus monkey induced pluripotent stem cells for tissue engineering

Monica Jara-Avaca[*], *Stephanie Wunderlich*, *Henning Kempf*, *Ingrid Schmidt-Richter*, *Robert Zweigerdt*, *Ulrich Martin*, and *Ina Gruh*

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Induced pluripotent stem cell (iPSC)-derivatives possess great therapeutic potential for regenerative medicine. Following myocardial infarction, a promising treatment option to prevent heart failure is the replacement of fibrous connective tissue by in-vitro generated iPSC-derived cardiomyocytes and engineered tissue thereof. Indeed, the efficiency of such an approach still needs to be determined. Transplantation of cardiomyocytes or bioartificial cardiac tissue (BCT) into ischemic cynomolgus monkeys represents a promising strategy to confirm the therapeutic potential prior to translation to humans, as non-human primates closely match with humans in anatomy, physiology and complexity of the immune system.

We previously reported the generation of cynomolgus iPSC (cyiPSC) and their general potential to form cardiomyocytes applying a spontaneous, serum-based differentiation protocol. However, as clinical applicable grafts require defined expansion and differentiation, we report here the successful establishment of a feeder-free cultivation process maintaining 90% Oct4+ cells in fully-defined medium. The cells were subsequently differentiated in a 2D system applying small molecule Wnt-signaling modulators resulting in beating areas around day 10. Since replacement therapies will require large quantities of de novo formed cardiomyocytes, we adapted a 3D protocol with optimized compound concentration and timing. We show efficient cardiac differentiation by emerging beating aggregates, immunofluorescence analysis for sarcomeric proteins and quantification by flow cytometry with cardiomyocyte yields of up to 51%. Furthermore, casting of BCT by mixing beating aggregates with extracellular matrix was feasible.

Overall, we show that a fully-defined, feeder-free system can be used to cultivate, expand and differentiate cyiPSC towards cardiomyocytes. Noteworthy, we found that cyiPSC culture and differentiation requires specific adjustments compared to their human counterparts to enable robust large-scale production needed for transplantation purposes.

Abstract No. P028

Fully integrated, adherent, closed-system cultivation of pluripotent stem cells

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Pluripotent stem cell (PSC) derived cell products hold great promise for future clinical use in a variety of diseases like Type I diabetes, macular dystrophy or Parkinson's disease. Constantly raising regulatory requirements for such advanced-therapy medicinal products (ATMPs) imply the need for standardized reagents and highly reproducible production procedures. Consequently, automation of PSC expansion and differentiation, and potentially product optimization through cell sorting may contribute to successful and cost-effective innovative therapies.

Using our highly versatile and integrated GMP cell processing platform CliniMACS Prodigy® we previously set up a variety of complex cell manufacturing protocols in a closed system. Likewise, we have started exploring solutions for iPS-derived cell therapy candidates such as dopaminergic progenitors. As a first step we sought to establish automated PSC expansion within the closed system.

To this end, we have optimized protocols allowing adherent cultivation of human iPSC under feeder- and serum-free conditions either within the Centrifugation and Cultivation unit (CCU) or sterilely connected cell-cultivation plastic ware using different cultivation matrices. Matrigel was used for initial proof of principle experiments before turning to the recombinant proteins Vitronectin and Laminin-521 which are more suitable for future clinical applications. Analysis of the iPSCs after the complete process from seeding to harvest, revealed that the cells remained viable and highly proliferative, showed a characteristic undifferentiated morphology as well as expression of Tra-1-60, SSEA-3 and SSEA-4 and absence of the differentiation marker SSEA-1. Proliferation rates were comparable to the laboratory scale.

The established process is highly flexible and compatible with enzymatic single cell or non-enzymatic cluster passaging. Furthermore, the system provides the possibility for magnetic isolation of target cells.

Taken together, we have developed a method for adherent, closed-system cultivation and expansion of PSCs. As a next step, differentiation and magnetic separation of mesencephalic dopaminergic progenitor cells will be assessed.

Abstract No. P029

Pulmonary Macrophage Transplantation employing HSC- or iPSC-derived cells as an innovative gene therapy approach in Pulmonary Alveolar Proteinosis

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Pulmonary alveolar proteinosis (PAP) caused by mutations in the granulocyte/macrophage–colony stimulating factor (GM-CSF- alias CSF2-) receptor α chain (*CSF2RA*) represents a rare, life-threatening lung disease characterized by the accumulation of phospholipids and proteins in the lungs secondary to a functional insufficiency of alveolar macrophages. Whereas current therapeutic options for this disease are limited, we recently demonstrated that a cell replacement therapy based on the endotracheal application of healthy or gene-corrected hematopoietic-stem cell derived macrophages (Pulmonary macrophage transplantation, PMT) maybe feasible and effective (*Happle C, Lachmann N, et al. 2014*). We now have evaluated the genetic correction of human HSC- as well as iPSC-derived macrophages from PAP patients as a potential source for such a cell replacement therapy and employed a lentiviral construct expressing a codon-optimized human CSF2RA-cDNA from an EFS1α promoter (EFS.CSF2RA.iGFP). In PAP patient-derived $CD34^+$ HSCs, transduction with the EFS.CSF2RA.iGFP vector rescued hGM-CSF dependent colony formation as well as monocyte/macrophage (M/M) differentiation, while healthy donor $CD34^+$ cells overexpressing CSF2RA exhibited no aberrations in growth, colony formation or *in vitro* differentiation towards $CD14^+CD11b^+CD68^+CD163^+$ macrophages. As an alternative, iPSC lines from CSF2RA deficient individuals were established, yielding M/Ms of typical morphology and phenotype (CD14, CD11b, CD45) using a large-scale hematopoietic differentiation protocol (*Lachmann N, Ackermann M, et al. 2015*). 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. Following genetic correction by EFS.CSF2RA.iGFP overexpression, GM-CSF dependent functionality including CD11b activation, GM-CSF uptake, phagocytosis, and CSF2R-downstream signalling were restored. Thus, in summary we here demonstrate efficient and functional genetic correction of PAP-patients derived macrophages and introduce HSC- but also iPSC-derived M/Ms as a potential source for innovative, PMT-based therapy in this life-threatening disease.

Happle C, Lachmann N, Skuljec J, Wetzke M, Ackermann M, Brennig S, Mucci A et al (2014) Pulmonary transplantation of macrophage progenitors as effective and long-lasting therapy for hereditary pulmonary alveolar proteinosis. Science Transl Med 6: 250ra113
Lachmann N, Ackermann M, Frenzel E, Liebhaber S, Brennig S et al (2015) Large-scale hematopoietic differentiation of human induced pluripotent stem cells provides granulocytes or macrophages for cell replacement therapies. Stem Cell Reports 4: 282-296

Abstract No. P030

IPSC as a cell source to generate a 3D alveolar and tracheobronchial tissue models

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The opportunities to study the human lung *in vitro* and *in vivo* are limited. The current 3D models are built with primary cells that are difficult to obtain and have a high donor dependency. However, according to European health report of 2012 of the WHO, lung disease are the fourth highest cause of death in the European Union and 3D models for research and new drug development are highly needed. Induced pluripotent stem cells (iPSCs) bear the chance to built reproducible 3D human airway models. In our studies, we aimed to build 3D human alveolar and tracheobronchial tissue models based on the differentiation of iPSCs into alveolar type II (AET II) and multiciliated cells (MCC).

To engineer most realistic models, we tested different approaches: The iPSCs were differentiated into anterior foregut endoderm (AFE) under 2D culture conditions, and seeded on (1) a small intestinal submucosa derived from the biological vascularized scaffold (BioVaSc-TERM®), (2) decellularized rat lungs or (3) Corning Transwells® for further differentiation. The models were tested regarding vitality and further analyzed with immune fluorescence and RT-qPCR. The results were compared with already established models built with primary cells.

After 21 to 42 days differentiated iPSCs expressed AET II and MCC specific marker. The cells could be subcultured on well plates as well as on 3D ECM matrices. Both undifferentiated iPSCs and the cells differentiated into AFE were able to adhere and grow on the matrices. Also cells differentiated on the matrices were vital and expressed the AET II and MCC specific marker.

We succeeded to differentiate iPSCs into AET II and MCC-like cells, which bears the chance, in combination with our decellularized matrices, to create 3D airway models. In subsequent studies, we will further characterize these models regarding their ability to reflect the physiological airway properties.

Ghaedi et al., "Human iPS cell – derived alveolar epithelium repopulates lung extracellular matrix," J. Clin. Invest., vol. 123, no. 11, pp. 4950–4962, 2013.
Firth et al., "Generation of multiciliated cells in functional airway epithelia from human induced pluripotent stem cells," Proc. Natl. Acad. Sci. U. S. A., vol. 111, no. 17, pp. 1723–30, Apr. 2014.

Abstract No. P031

Small subchromosomal variants in early passage iPSCs largely pre-exist in parental cells and their frequency does not depend on the donor age

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It is currently presumed that the alarming mutational load observed in iPSCs is in part derived from parental cells, which might limit the therapeutic value of iPSCs from aged donors. Other mutations may be generated de novo during reprogramming, which would further impact the general clinical usefulness of iPSCs.

We have derived 37 human iPSC lines from neonatal and aged individuals under comparable conditions. Applying high accuracy exome and amplicon sequencing we demonstrate the pre-existence of all analysed 'true' SNPs and INDELS in their parental cells. iPSCs from aged donors did not contain increased numbers of SNPs or INDELs. Array CGH revealed CNVs at frequencies similar or lower than in clinical control samples.

Our data indicate that subchromosomal abnormalities in iPSCs are largely source cell derived but are not enriched in iPSCs from aged donors, and call into question the significance of reprogramming-induced de novo mutations in iPSCs.

Ji et al. 2012 Elevated coding mutation rate during the reprogramming of human somatic cells into induced pluripotent stem cells. Stem Cells 3,435-440.
Gore et al. 2011 Somatic coding mutations in human induced pluripotent stem cells. Nature 471,63–67.

Abstract No. P032

A systems approach to identify mechanisms of muscle stem cell self-renewal

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Satellite cells are a distinct and small population of skeletal muscle resident stem cells responsible for regeneration and maintenance of mature skeletal muscle. Transcription factors from the Pax gene family are critical for satellite cell biogenesis, survival and self-renewal. Pax7 expression persists in activated, proliferating satellite cells but is rapidly down-regulated in cells that commit to terminal myogenic differentiation. These facts suggest that an active mechanism exists in satellite cells to sustain Pax7 expression for stem cell maintenance or to mediate its degradation for entry into myogenic differentiation. We aim to find out if such a pathway exists and, if so, to identify the molecular determinants that govern Pax7 protein stability.

Our data indicate that Pax7 is degraded via the ubiquitin-proteasome system. To isolate the molecular machinery that mediates Pax7 ubiquitylation we will perform a multi-parametric high-throughput, loss-of-function lentiviral RNAi screen against all known ubiquitin related genes. The ultimate goal of this project is to identify the mechanism and the putative factors underlying Pax7 stability and the therapeutic potential of targeting these for muscle development and regeneration related disease.

Abstract No. P033

Immunogenicity of induced pluripotent stem cell – derived differentiated renal cells

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With their ability of unlimited self-renewal and their potential to differentiate into all cell types of the body human induced pluripotent stem cells (hiPSCs) hold large promise for cell-based therapies. Additionally, since these cells are reprogrammed from adult cells, hiPS lines in principle could be generated from every patient and further, autologous or HLA-matched transplantation of the cell type of need would not require immunosuppressive drugs. However, in mouse models it was shown that syngeneic iPS-derived cells may elicit an immune response, perhaps because of aberrant gene expression. Consequently, it is essential to test the immunogeneic potential of every hiPSC progeny even from autologous origin.

The aim of this study was to define the immunogenicity of hiPSC-derived renal cells in autologous and allogeneic recipients. For these experiments, hiPSCs were differentiated into renal progenitor cells (intermediate mesoderm) and into terminal proximal tubular cells, respectively. Human leukocyte antigen I (HLA) expression is very low in renal differentiated hiPSCs and can be increased by stimulation with the pro-inflammatory cytokine interferon-gamma (IFNγ). Prolonged *in vitro* cultivation of hiPSC-derived proximal tubular cells restores HLA class I expression and IFNγ additionally leads to the expression of HLA class II molecules. Differentiated renal progenitor and proximal tubular cells do not provoke T cell proliferation in autologous and allogeneic donors, although they induce pro-inflammatory cytokine secretion. Long-term cultivated differentiated proximal tubular cells in contrast induce T cell proliferation in allogeneic recipients. Primary kidney cells from the iPSC-donor induced a T-cell response, which was stronger than that of iPSC-derived renal cells. Our data indicate that diminished HLA expression in hiPSC-derived immature renal cells may lead to immuntolerance, which is not maintained upon progressive terminal differentiation.

Abstract No. P034

A high-throughput RNAi screen identifies chromatin modifiers regulating muscle stem cell self-renewal and differentiation

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Satellite cells are myogenic stem cells responsible for the post-natal growth, repair and maintenance of skeletal muscle. The ability of stem cells to self-renew and/or to differentiate is tightly controlled by epigenetic modifications. Significant knowledge gap exist on factors that regulate epigenetic modifications and transition of chromatin states in adult muscle stem cells. We aim at understanding the crucial role of epigenetic modification that governs cell fate determination of adult muscle stem cells. In a more general context, we have generated a novel and high-throughput platform for the generation and analysis of muscle regenerative cells, yielding fundamental insights into the mechanisms of skeletal muscle development and cell fate specification. We have established a robust pipeline of high-throughput platforms for genetic screens, transcriptomic and proteomic approaches to identify factors that regulate muscle stem cell regeneration. We use a transgenic Pax7 reporter mouse line combined with FACS sorting for efficient isolation of a large and pure population of muscle stem cells. These cells can then be efficiently transduced with recombinant lentiviruses to knockdown desired gene of interest. We performed a multi-parametric high-throughput, lentiviral RNAi screen to identify chromatin modifiers that regulate muscle stem cell self-renewal and differentiation. Our preliminary data identifies known and putative chromatin modifiers previously not characterized in muscle stem cells, playing a crucial role in maintenance of quiescence and differentiation. The significant outcomes of our experiments will be the identification of the functional pathways that may be useful for (1) the maintenance or expansion of muscle stem cells outside the body, or (2) the *in vivo* rescue or expansion of a failing muscle stem cell population within the body (3) the identification of genes that regulate global chromatin changes and epigenetic modifications that determine muscle stem cell fate.

Abstract No. P035

Differentiation of human pluripotent stem cells into airway epithelial cells

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Pluripotent stem cells (PSCs), like embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), offer promising new therapeutic options to treat lung diseases, such as cystic fibrosis or surfactant deficiencies, based on cellular/tissue replacement therapies, disease modelling and drug screening. These regenerative approaches crucially rely on the efficient *in vitro* production of lung epithelial (progenitor) cells from PSCs which is the aim of our present study.

We made use of the human (h)ESC reporter cell line hES3 NKX2.1-eGFP (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.

Using the commercially available STEMdiff™ Definitive Endoderm (DE) Kit (TeSR™-E8™ Optimized) by Stemcell Technologies resulted in the robust generation of a highly enriched DE population of > 93 % CXCR4pos/c-Kitpos cells from two different hESC lines and one iPSC line demonstrated by flow cytometric analysis. Definitive endoderm generation was additionally verified by coexpression of the transcription factors FOXA2 and SOX17 based on immunofluorescence staining. Subsequent differentiation via anterior foregut endoderm induction resulted in the generation of up to 28 % NKX2.1-eGFPpos cells related to the entire differentiation culture. Verification of the lung epithelial phenotype of purified NKX2.1-eGFPpos cells is currently ongoing as well as further maturation experiments. For this purpose we successfully generated a traceable hES3 NKX2.1-eGFP AAVS1-CAGRedStarnuc reporter cell line by TALEN (transcription activator-like effector nucleases) technology as a useful tool for lung epithelial maturation experiments e.g. in co-culture/transplantation attempts.

In summary, we were able to generate a highly enriched DE population followed by the induction of a distinct NKX2.1-eGFPpos population. Future work will focus on additional optimization of the differentiation strategy and further maturation of NKX2.1-eGFPpos cells into lung epithelial cell types, like e.g. Club cells or ciliated cells.

Abstract No. P036

Generation of a NKX2.1/CCSP knockin human induced pluripotent stem cell reporter line for monitoring the generation of respiratory cells

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One promising therapeutic option to cure hereditary pulmonary diseases like cystic fibrosis or surfactant deficiencies might be a cell replacement therapy comprising the generation of patient specific autologous induced pluripotent stem cells (iPSCs), followed by the correction of the underlying genetic mutation, *in vitro* differentiation into the needed airway epithelial cell type and replacement of the endogenous diseased cells. For long term restoration, most likely airway progenitor or stem cells like club cells, basal cells or submucosal gland (SMG) stem cells will be required. A prerequisite of this strategy is the development of an efficient and robust protocol for the generation of the desired airway progenitor or stem cells from human iPSCs (hiPSCs). The transcription factor NK2 homeobox1 (NKX2.1) expressed by lung epithelial progenitor cells represents a suitable marker for optimizing differentiation protocols towards lung epithelial cells. Combination with the club cell secretory protein (CCSP) should allow for monitoring of club cell generation in sequential differentiation protocols. The aim of the present study was the generation of a hiPSC double transgenic reporter line targeting the NKX2.1 and CCSP locus. Therefore we designed one targeting vector for a non-disruptive integration of an eGFP coding sequence into the NKX2.1 locus and one targeting vector for the non-disruptive integration of nuclear localized Venus coding sequence into the CCSP locus. Furthermore, the CCSP targeting vector introduces a Neomycin selection cassette under control of the endogenous CCSP promoter by the use of a 2A-site located behind the Venus coding sequence. This established hiPSC NKX2.1/CCSP reporter line represents an optimal tool for the improvement of protocols for the differentiation of hiPSCs into club cells and enables their selection which is essential for further *in vitro* and *in vivo* analysis.

Abstract No. P037

Establishment of a 3D culture system based on a biological scaffold to differentiate human iPSCs into pancreatic β-cells

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Insulin-secreting β-cells derived from human induced pluripotent stem cells (hiPSCs) are an interesting cell source for transplantation strategies treating Diabetes mellitus type 1. However, the low frequency of functional and mature β-cells is a key limitation of current monolayer differentiation protocols. Several reports demonstrated so far, that extracellular matrix (ECM)-components improve the survival and function of pancreatic islets or single cells *in vitro*. Here in this work, we hypothesize that a three-dimensional (3D) culture system based on a biological scaffold could improve the generation of functional and mature β-cells from hiPSCs. To this end, we generated two 3D culture systems for pancreatic lineage differentiation of hiPSCs based on either decellularized porcine pancreas or small intestinal segment (SISser). Decellularization of both scaffolds was verified by histochemical analysis. Immunofluorescence analysis demonstrated that hiPSCs proliferated (KI67 expression) and maintained characteristics of pluripotent cells (expression of SOX2, NANOG) when cultured on both scaffolds. For differentiation of hiPSCs towards pancreatic hormone expressing cells (PHECs) in monolayer cultures an already published protocol was established in our lab. RT-PCR analysis during differentiation revealed expression of pancreatic lineage-specific marker genes. Further, expression of hormones characteristic for pancreatic endocrine cell types was verified by immunofluorescence. Preliminary data from differentiation experiments on decellularized pancreas or SISser showed that cells survived on both scaffolds during differentiation and further underwent morphological changes. In addition, RT-PCR analysis demonstrated increased gene expression levels for hPDX1 and hINS in comparison to monolayer cultures at d22 of differentiation. Taken together, our data indicate that our 3D culture system based on decellularized pancreas or SISser represent a promising strategy to improve pancreatic lineage determination of hiPSCs *in vitro* resulting in increased frequencies of functional and mature β-cells compared to standard monolayer cultures.

Abstract No. P038

Cell Replacement Therapy with BDNF-overexpressing Neural Stem Cells in Mouse Models of Chorea Huntington

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Huntington's Disease (HD) is an inherited neurodegenerative disease with fatal motoric failures and is characterized by the loss of striatal medium spiny neurons. Neuronal cell death has been linked to impaired expression and axonal transport of the neurotrophin BDNF (brain derived neurotrophic factor). Several studies aimed to add BDNF exogenously to the striatum, which was either virus-mediated or with BDNF overexpressing cell lines. In fact, neuroprotection via BDNF has been demonstrated in Huntington mouse models, but failed to show long-term motor behaviour improvement. HD still lacks effective therapy, but embryonic stem cell derived neural transplantation may provide an effective treatment. Recently, a murine embryonic stem cell line overexpressing BDNF has been produced in our lab, which shows enhanced neuronal and specifically GABAergic differentiation. In the study presented here, the two therapeutic approaches, exogenous supply of BDNF and cell replacement therapy with embryonic stem cells, are combined. Pre-differentiated ES cells were used for grafting to assure *in vivo* differentiation into medium spiny neurons. Cells were sorted via MACS to remove the tumorigenic potential of early progenitors, and grafted into the striatum of HD mouse models (R6/2, N171-82Q and quinolinic acid-lesioned mice).

Behavioral results of the motor tests are presented. Neuronal differentiation, the number of GABAergic neurons, integration into the host tissue and migration of transplanted cells is analyzed via immunhistology.

P039 – P048 Stem cells in regenerative therapies: Mesenchymal stem/stroma cells

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Abstract No. P039

Trapping and Culture of Human Mesenchymal Stromal Cells in a 3D Gel of Human Platelet Lysate

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Human platelet lysate (HPL) is a commonly used supplement for cell culture of mesenchymal stromal cells (MSCs) – in contrast to fetal calf serum it does not harbor the risk of xenogeneic immune reactions or transmission of bovine pathogens. Coagulation of HPL-medium is usually prevented by addition of heparin. Otherwise, it forms an HPL-gel within one hour. Cells cultured on this gel reveal significantly higher proliferation rates in comparison to MSCs cultured on tissue culture plastic (TCP). In this study, we followed the hypothesis, that MSCs can also be trapped within the HPL-gel to facilitate three-dimensional growth and reorganization with secreted extracellular matrix (ECM) proteins.

MSCs were trapped in HPL-gel by rapid polymerization with thrombin (less than 2 minutes) and retained by short-term fibrinolysis with trypsin or plasmin to analyze their immunophenotype. Cells trapped in HPL-gel were evenly distributed, and capable of proliferation over 15 days. There was no significant effect on apoptosis or immunophenotype. Notably, cells grown in HPL-gel revealed higher adipogenic and osteogenic differentiation than on TCP. We are concurrently analyzing ECM proteins that are secreted and structured by MSCs within the HPL-gel.

Taken together, HPL-gel is not only a suitable matrix but it can also be used as 3D scaffold for culture of MSCs. These growth conditions appear to be more physiological than TCP and facilitate faster culture expansion. Furthermore, MSCs trapped in HPL-gel may be directly transplanted together with their ECM-components to facilitate better engraftment than MSCs that are injected in solution.

Hemeda H, Giebel B, Wagner W. Evaluation of Human Platelet Lysate versus Fetal Bovine Serum for Culture of Mesenchymal Stromal Cells. Cytotherapy. 2014; 2:170-80.
Hemeda H, Kalz J, Walenda G, Lohmann M, Wagner W. Heparin concentration is critical for cell culture with human platelet lysate. Cytotherapy. 2013; 15:1174-81.

Abstract No. P040

Characterization and Comparison of Mesenchymal Stem Cells from Osteoporotic and Healthy Patients

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Introduction: Osteoporosis is defined as a complex, systemic age-related deterioration of bone mass leading to fragility fractures. Osteoporosis is a major growing medical and socioeconomic burden. Bone development and its constant remodeling is a well-regulated biological process orchestrated by mesenchymal stem cells (MSCs)-derived osteoblasts forming bone and hematopoietic stem cells (HSCs)-derived osteoclasts resorbing bone. The spine is the most commonly affected and subsequently vertebral compression fractures are the most frequent osteoporotic fractures.

The aim of the current study was to isolate, characterize and compare MSCs from osteoporotic and healthy patients.

Materials/Methods: Osteoporotic patients: MSCs were harvested from vertebral bodies. Healthy patients: MSCs were isolated from adipose tissue, femur head, vertebral bodies. MSCs were characterized via their differentiation towards adipo-chondro-osteogenic lineages, surface marker expression, proliferation behaviour and immunomodulatory capacity. Moreover, calcification process was evaluated by measuring the optical density (OD) and free phosphate ions in the supernatants during the osteogenic differentiation.

Results: All investigated MSCs showed typical fibroblast-like morphology, expressed MSCs surface marker. Tri-lineage differentiations were confirmed via corresponding staining. Calcification and proliferation of MSCs from healthy patients were increased when compared to MSCs from osteoporotic patients. Amounts of free phosphate ions measured were clearly elevated in osteogenic induced MSCs compared to controls. Vertebral body-derived MSCs from osteoporotic and healthy patients showed immunomodulatory capacity by suppressing lymphocytes proliferation.

Conclusion: We successfully isolated MSCs from vertebral body bone marrow aspirate treated for an osteoporotic compression fracture. MSCs from osteoporotic patients showed delayed calcification and proliferation in comparison to MSCs from healthy patients. Otherwise all investigated characteristics were similar and no impairment was detected. Nevertheless, they could provide a useful tool for further studies to investigate their contribution in the pathogenesis of osteoporosis. Furthermore, because of their unique regenerative potential MSCs from osteoporotic patients could be possibly used in cell-based therapy approaches.

Abstract No. P041

Introducing an animal serum-free potency assay to monitor immune modulation capacity of human mesenchymal stem/progenitor cells (MSPCs)

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The observed variability of immune response modulation efficiency by MSPCs due to donor variations, influences of organ-of-origin and/or freeze/thaw-related functional impairment hampers the generation of a stable MSPC product for clinical use. To generate a reliable potency assay for testing immune modulatory function of MSPCs we hypothesize that pooling responder leukocytes as well as MSPCs could contribute to the compensation of individual donor variation.

PBMCs from 10 different donors were pooled and pre-labeled with CFSE before cryopreservation. To determine donor and organ variation human MSPCs from 5 independent bone marrow (BM), white adipose tissue (WAT) and umbilical cord (UC) donors were tested either freshly thawed (off-the-shelf) or cultured for 3 days with pooled human platelet lysate (pHPL) replacing fetal bovine serum. The potential of these cells to inhibit phytohaemagglutinin (PHA)-mediated proliferation of CD3+ cells (day 4) or to prevent the allo-response of the pooled PBMCs (day 7) was examined by flow cytometry.

By pooling PBMCs a 'general responder' could be created showing more stable stimulation of CD3+ cells than PBMCs derived from single donors. All analyzed MSPCs displayed immune modulatory potential at day 4 in particular at a 1:3 and 1:10 modulator:responder ratio with a profound degree of variability. Significant differences between the 5 donors were found in each organ-derived MSPC group which could be compensated by pooling. Impaired immune modulation immediately after thawing was donor-variable and could be corrected by 'rescue culture'. Inhibition of the allo-response was generally less potent compared to inhibition of mitogenesis.

There is an urgent need for predictive potency assays for autologous MSPC therapies but also for selection of potent pre-tested donors for off-the-shelf allogeneic immunotherapy. Pooling responder as well as modulator cells could help to gain higher degree of constancy in these assays.

Abstract No. P042

Expression Of Glucose Transporters In The Human Amnion Derived Mesenchymal Stromal Cells Under Hyperglycemic Conditions

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Objective: In this study, we aimed to investigate the presence of GLUT proteins in these cells and the regulation of GLUT proteins in response to glucose.

Methods: Mesenchymal stromal cells were isolated from amniotic membranes of human term placentas. Characterization of the isolated cells was performed using flow cytometry. GLUT1 and GLUT3 proteins were identified in these cells with immunofluorescence stainings. Characterized cells were cultured in normoglycemic and hyperglycemic conditions for 24 and 48 hours and the presence of GLUT1 and GLUT3 proteins and mRNA were identified in these cells by Western blot method and Q-PCR.

Results: Isolated cells were positive with MSCs markers CD44, CD90, CD73, CD105 and negative with hematopoietic markers CD34, CD11b, CD19, CD45, CD14 and HLA-DR. The expression of GLUT1 and GLUT3 proteins was detected in the MSCs. Normoglycemic (5 mM D-glucose) and hyperglycemic (25 mM D-glucose) culture of MSCs did not change the expression of GLUT1 and GLUT 3 proteins expression in a time and dose-dependent manner. However, GLUT1 and GLUT3 mRNA levels altered time and dose dependently.

Conclusions: Glucose is an important molecule for the proliferation and differentiation of mesenchymal stem cells. Therefore, the clarification of the relationship between glucose and mesenchymal stem cells may provide a better understanding for the relationship between stem cells and glucose for the glucose-related pathologies such as diabetes during pregnancy. In addition, the clarification of this relationship is necessary for the clinical applications of stem cells and for ensuring optimization of culture conditions in stem cell amplification.

Abstract No. P043

Challenges in Human Mesenchymal Stem Cell Expansion Process According to Good Manufacturing Practice

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Cell therapy has evolved into a distinct healthcare sector observable by an increasing number of clinical trials and FDA-approved commercial products. As stem cells are a complex and viable product, their manufacturing has to meet several requirements. Production in a Good Manufacturing Practice (GMP) environment and compliance with Process Analytical Technology (PAT) over the whole expansion process is challenging. One important cell source for clinical application are hMSCs (human mesenchymal stem cells). To enable their use as advanced therapy medicinal products (ATMP), high quantities of undifferentiated cells with high viability are required.

Stem cell expansion systems using microcarrier are presented. Expansion processes up to 2.4 L were established using stirred tank glass reactors and a disposable bioreactor. Upstream from small spinner flask to bioreactor was performed via bead-to-bead transfer without enzymatically detachment of cells. Stem cell quality was confirmed according to the minimal criteria of the International Society for Cellular Therapy (ISCT). To ease the approval of hMSC as ATMP, an alternative to serum-containing media, a fully chemically defined medium is needed. The new developed growth medium showed promising results in hMSC attachment and growth on protein-coated surfaces. New enzymes for cell harvest and defined expansion media further promote the production of these cells in an ATMP production approved environment. To go along with the quality by design (QbD) approach, online monitoring of viable cell mass in stirred bioreactor was performed with dielectric spectroscopy.

Performing the whole hMSC manufacturing process, including inoculation, expansion under GMP and PAT is challenging. Dynamic bioreactor systems offer a good opportunity to achieve large amounts of high-quality hMSCs under controlled and monitored process conditions.

Elseberg C L, Salzig D, Czermak P: Bioreactor expansion of human mesenchymal stem cells according to GMP requirements, Methods in Molecular Biology (2015) 1283: 199-218

Abstract No. P044

Mesenchymal stromal cells can inhibit platelet activation

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Mesenchymal stromal cells (MSCs) possess promising therapeutic capacities like multilineage differentiation or immunomodulation already evaluated in numerous clinical trials. In general, Phase I trials proved the safety of systemic MSC injection. However, there were rare incidences suggesting a risk of thromboembolic events after MSC infusion. Recent studies suggest both promoting and inhibiting influences of MSCs on plasmatic coagulation. As more evidence is needed to ensure the safety of MSC infusion we aimed to investigate possible interactions between platelets and MSCs asking whether MSCs can promote or inhibit platelet activation. Effects of MSCs and MSC supernatant were studied by assessing platelet activation using flow cytometry (CD62P, CD63, PAC1-binding) and impedance aggregometry (Multiplate; Roche Diagnostics). Platelets were used unstimulated or stimulated with different agonists (ADP, TRAP) in the presence of MSCs (either from lipo aspirate (LA), cord blood (CB) or bone marrow (BM)), human umbilical vein endothelial cells (HUVECs) or HeLa tumor cells, respectively. In flow cytometry HeLa cells and CB-MSCs were capable of inducing activation of unstimulated platelets, whereas, activation marker expression of stimulated platelets was either unaffected or increased compared to controls without cells. BM-MSCs, LA-MSCs and HUVECs caused a dose related decrease in activation marker expression of stimulated platelets independent of the agonist used.The results from impedance aggregometry generally indicated a decrease in platelet aggregation in the presence of any cell type, which suggests that the method is probably not suitable in the present context.

The data clearly demonstrate that MSCs derived from BM and LA are able to inhibit platelet activation in a dose-dependent manner. This inhibition of primary hemostasis supports the clinical safety data. Interestingly,whereas both LA- and BM-derived MSC depicted this anti-coagulatory phenotype, CB-derived MSCs differed in this respect. Further experiments should help to understand the underlying mechanisms to ensure safety of MSC therapies.

P045

Preconditioning enhances the therapeutic potential of adipose-derived stem / stromal cell-conditioned medium in cisplatin-induced acute kidney injury

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Objective: The development of new strategies to improve renal regeneration capacity and to preserve renal function after acute kidney injury (AKI) is necessary. The organ-protective effects of mesenchymal stem / stromal cells (MSCs) and their conditioned medium (CM) have been investigated demonstrating that either infused cells or their conditioned medium promoted renal tubular recovery and the outcome of AKI by paracrine actions. Strategies to optimize the potential of transplanted cells or their CM must be the final goal of further studies to enhance the renal regeneration process.

Material and methods: MSCs were isolated from adipose tissue from C57BL/6 mice. Cells were either cultured under standard conditions (controls) or preconditioned by incubation in a hypoxic environment (hyp; 0.5% O2) or in the presence of tumor necrosis factor-alpha (TNFa) or epidermal growth factor (EGF). The expression of 308 proteins in the supernatant was measured by a commercially available protein array. Selected readouts were further verified by qPCR analysis. Then, medium of hyp-preconditioned MSCs (pCM) was used to enhance renal regeneration in a mouse model of cisplatin-induced AKI. Renal function was assessed by measurements of specific markers for AKI and serum cytokine levels.

Results: From the 308 assayed proteins the expression of 64 proteins was found to be more than two-fold increased after incubation in a hypoxic environment whereas 44 and 62 proteins were increased after incubation with EGF or TNFα, respectively. Thereafter, hyp was chosen as preferred precondition regime and the pCM was used in the *in vivo* model of cisplatin-induced AKI. pCM significantly ameliorated serum creatinine and serum N-GAL values, and also serum cytokines levels (IL-1b and IL-6) of mice with AKI.

Conclusion: Preconditioning by hypoxia significantly increases the release of regenerative factors in MSCs and enhances the therapeutic effects of CM in cisplatin-induced AKI in mice.

Abstract No. P046

Optimizing conditions to differentiate human iPS cells into functional mesenchymal stem/progenitor cells

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Human mesenchymal stem/progenitor cells (MSPCs) are currently tested in multiple clinical trials evaluating their immune modulatory and tissue regenerating capacity. Restricted availability and a limited life cycle of MSPCs confine both clinical applicability and mechanistic studies that are urgently needed to better understand their mode of action.

In this study induced pluripotent stem cell (iPSC) lines have been generated from healthy parental MSPCs derived from human bone marrow (BM) and umbilical cord blood (UCB) in order to optimize and study mechanisms of their differentiation into iPSC-derived mesenchymal progenitor cells (iP-MPCs) and function.

MSPCs were established as published under animal serum-free conditions. After primary large scale culture, purity and identity of the cells were characterized by clonogenicity (CFU-F), immune phenotype and three-lineage differentiation capacity, before reprogramming into induced pluripotent stem cells (iPSCs) by Sendai virus-mediated transient transfection of Oct3/4, Klf-4, Sox-2 and Myc. Absence of Sendai-virus, diploid chromosome content and teratoma formation were confirmed before subsequent experiments. The differentiation of iPSCs along the mesenchymal lineage into iP-MPCs was initiated in media containing 10% pooled platelet lysate (pHPL). In order to promote the differentiation into functional iP-MPCs, we additionally treated the iPSCs with small molecules targeting different signaling pathways involved in stem cell self-renewal, oxygen sensing, immune modulation and cell adherence. Immune phenotype, clonogenicity, differentiation capacity and immune modulatory potential of iP-MPCs were compared to their parental MSPCs.

Under feeder-free conditions, iPSCs differentiated into CD73+/CD90+/Tra-1-81- MSPC-like cells which displayed immune suppressive competence comparable to their parental MSPCs. Small molecules further modified the MSPC-like phenotype of iP-MPCs depending on the target. Their entire immune phenotype and functional repertoire need to be established in order to comply with the functionality of their parental MSPCs. This strategy builds the basis to study mechanisms of mesenchymal specification and function.

Abstract No. T38/P047

Regenerative potential of human pluripotent stem cell-derived MSCs in a Gunn rat liver injury model

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The Crigler-Najjar syndrome type 1 is characterized by massively increased levels of unconjugated bilirubin in the plasma because of the lacking of hepatic uridine 5'-diphospho-glucuronosyltransferase activity (due to a mutated UGT1A1 gene) which is crucial for bilirubin conjugation and excretion. The Gunn rat is an *in vivo* model for this disease. Current treatments have several limitations therefore alternative therapy strategies like (stem) cell transplantations are needed. Transplantation of primary hepatocytes is limited due to the dependence on donated organs. An alternative approach would be the increment of functional hepatocytes by cell transplantation. Studies have shown that co-transplanting hepatocytes with bone marrow-derived MSCs improves homing-in, engraftment and survival of the donor cells and that MSCs can transdifferentiate into hepatocytes. These properties make MSCs attractive tools in the treatment of acute liver injuries. However, the downside of procuring MSCs is that the number of cells that can be generated from a single human donor is limited due to their restricted long-term proliferation. To circumvent these drawbacks iMSCs (induced MSCs) were generated from (i) iPSCs derived from human fetal bone marrow MSCs and (ii) human embryonic stem cells- line H1. These were compared to human fetal bone marrow-derived MSCs. The iMSCs and the parental MSCs were transplanted into the spleen of partially hepatectomized Gunn rats (without immunosuppression). After a regeneration time of 1 week to 2 months the organs and sera were examined. Molecular, immunohistochemical and immunofluorescent-based analysis of rat liver tissue showed integration of human cells. Expression of human HNF4α as well as measureable human albumin levels in the sera provides evidences of trans-differentiation into hepatocytes. Further analysis revealed reduced levels of bilirubin. To surmize, human iMSCs are an alternative source for treating inherited liver diseases such as Crigler-Najjar syndrome type 1.

Abstract No. T35/P048

The transcription factor GATA2 controls mesenchymal stem cell fate

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Mesenchymal stem cells (MSC) are pluripotent cells with an inherent potential to regenerate a broad array of tissues. MSC lineage commitment and differentiation are tightly regulated by a complex network of transcription factors. In this study, we focused on the hematopoietic transcription factor GATA2, which was found to be expressed in MSC, and its role in controlling MSC differentiation. By applying chromatin immunoprecipitation coupled with deep sequencing (ChIPseq), we found enriched binding of endogenous GATA2 to canonical binding motifs near genes involved in osteoblast differentiation. In contrast to hematopoietic stem cells, GATA2 binding motifs in MSC co-localized with motifs for transcription factor of the FOX and HOX family, known regulators of skeletal development. Consistently, ectopic GATA2 expression in primary MSC regulated many osteoblast-related genes and resulted in strongly reduced osteoblast differentiation. Interestingly, GATA2 partially blocked bone morphogenetic protein (BMP)-2 induced SMAD1/5/8 activity, a pathway that is known to drive osteoblastogenesis. Deletion of GATA2 by MSC-specific CRE recombinase expression in GATA2 floxed mice resulted in normal skeletal development. However, bone-residing osteogenic progenitors that lack GATA2 exhibited enhanced differentiation *in vitro*. In summary, our study identifies GATA2 as a novel regulator of MSC biology and osteoblast differentiation and warrants further investigation into diseases of the skeletal system.

P049 – P074 Stem cells in disease modeling and drug development

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Jan M. Bruder

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P070 Angelman syndrome iPSCs form immature teratomas *in vivo* containing derivatives of all three germ layers
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T15/P071 Modelling reactive gliosis in mESC-derived retina organoids
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P072 Differentiation of Midbrain Floor Plate Progenitors and DA Neurons from Human PSCs
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P073 Using iPSC-derived midbrain dopamine neurons for rapid phenotyping of LRRK2 mutant-associated alterations
Beatrice Weykopf

P074 Effects of induced pluripotent stem cell long term passaging on pluripotency, neuronal differentiation and stress response
Susanne Zach

Abstract No. P049

Identifying Novel Drugs for ALS and FTD by High Throughput Screening with a Human Stem Cell-based model of C9ORF72-mediated Neurodegeneration

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In an aging society, human neurodegenerative diseases pose a major logistical, ethical, and financial challenge in the next few decades. Mutations in the locus C9ORF72 are the first genetic link to be identified between fronto-temporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) and thus are a causative co-factor in two of the most abundant familial cases of neurodegenerative diseases of our time.

Over the last few years, stem cell-based approaches have enabled us to build human neural disease models *in vitro*. We have matured our techniques and protocols to a degree that allows the use of large-scale high throughput screening approaches to identify small molecule entities that mitigate C9ORF72-associated cell death in human motor neurons.

We have designed a reproducible phenotypic disease model with a Z' of >0.5 and demonstrate high throughput handling and analysis by screening 50,000 compounds for their ability to alleviate stress-induced neurodegeneration in cells harboring critical repeat expansion in C9ORF72.

Using this screen, we have identified 50 compounds with the potential to ameliorate C9ORF72 dependent cell death, which are now undergoing further testing.

Both, the protocols developed for this project, and the newly identified compounds will help propel basic and applied science towards identifying novel drug candidates for human neurodegenerative diseases as well as help uncover the underlying molecular mechanisms.

Abstract No. P050

Disease in the Dish: Reprogramming into iPS cells and renal differentiation of urine cells from patients with polycystic kidney disease

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Polycystic kidney disease (PKD) is the most common monogenetic hereditary disease. Since the pathomechanisms are not fully understood yet, no causal therapy exists. Standardized protocols for long term cultivation and effective renal differentiation of human pluripotent cells need to be established to create a “disease in the dish” model for PKD.

To this end, urine cells from PKD patients were collected and reprogrammed into induced pluripotent stem (iPS) cells by retroviral transduction of the transcription factors Oct4, Klf4, Sox2 and c-Myc. On days 5-17 after transduction, colonies were picked and cultivated on mouse embryonic feeders (MEFs). The expression of pluripotency markers c-Myc, Oct-3/4, GKLF and Sox-2 was verified by immunostaining. For renal differentiation, human iPS cells were sequentially treated with CHIR99021, fibroblast growth factor-2, retinoic acid, fibroblast growth factor-9 and activin. Cells formed tubule-like ring structures which expressed the renal marker nephrin and the epithelial marker cytokeratin as demonstrated by immunostaining.

We conclude that urine cells from PKD patients can be reprogrammed into iPS cells and differentiated into tubule-like kidney structures under standardized conditions. Our protocol enables the non-invasive generation of a disease in the dish model for PKD and opens the way to analyze pathogenesis and possible therapeutic interventions.

Abstract No. P051

Implementing a human induced pluripotent stem cell (hiPSC) platform for drug discovery in Alzheimer´s disease

Roberta De Filippis[1,*], *Katharina Janssen*[1], *Mariya Vasileva*[1], *Bettina Liebel*[1], *Katharina Spieler*[1], *Daniela Gomm*[1], *Claudia Kiefer*[1], *Jeroen Van Bergeijk*[1], *Volker Nimmrich*[1], *Martine Geraerts*[2], *Viktor Lakics*[1], *Georg C. Terstappen*[1], and *Margot H.M. Bakker*[1]

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Human induced pluripotent stem cell (hiPSC) technology enables a promising approach to model neurodegenerative disorders *in vitro*. In addition, this technology offers the possibility to build a drug discovery platform for target identification, validation and generation of lead molecules for therapeutic intervention employing human neurons, directly derived from healthy controls or patients, promising better translation.

In this study we developed and optimized two neuronal differentiation protocols for the generation of specific neuronal subtypes (i.e. cortical and midbrain) and to set up phenotypic *in vitro* models for Alzheimer´s disease (AD). These protocols were further optimized for application with an automated cell culture system to increase reproducibility and to allow for a medium- to high-throughput scale. Molecular characterization studies were carried out during several stages of the differentiation process employing RT-PCR and immunofluorescence staining to demonstrate an increase in expression of typical neuronal markers (e.g. vGLUT1/2, GAD65, Tuj1 and Map2), as well as the presence of synapses (e.g. synaptophysin and PSD95). Furthermore, the regional identity of these hiPSC-derived neurons was also confirmed by using a customized RT-PCR gene expression array. Whole cell patch clamp analyses 6 weeks after neuronal induction detected sodium and potassium like currents. Furthermore, action potentials and spontaneous synaptic activity were indicative of appropriate neuronal functioning.

To set up disease-relevant phenotypic assays we used neuronally differentiated hiPSCs, derived from aged healthy controls and familial AD patients. In a time course experiment we compared the production of Aß40 and Aß42 and investigated neurite outgrowth in the presence or absence of different growth promoting or growth inhibiting compounds employing live cell and high content imaging technologies.

Taken together, our studies demonstrate the utility of patient-derived, differentiated iPSCs for the implementation of relevant *in vitro* human disease models for drug discovery, which might ultimately lead to the development of new effective therapies.

Abstract No. P052

Modeling axonal degeneration in Parkinson's disease using human iPSC derived neurons

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Parkinson's disease (PD) is the second most common neurodegenerative disease. It is characterized by typical motor symptoms, primarily linked to the loss of dopaminergic neurons in the substantia nigra. The neuronal dysfunction is most likely caused by intraneuronal α-synuclein (α-syn) accumulation. Although the effect of α-syn aggregation on neuronal cell function is not yet resolved, there is persuasive evidence suggesting the onset of pathogenic events within the axonal-dendritic compartment. Neuronal projections require constant metabolic support that is provided by fast axonal transport. Thus, we analyzed axonal transport of mitochondria in human induced pluripotent stem cell (iPSC) derived neurons of controls and PD patients in order to investigate the pathogenic effect of α-syn on the axonal-dendritic compartment. Forced overexpression of wildtype α-syn in control iPSC derived neurons resulted in a severely impaired anterograde axonal transport. In order to study axonal pathology in iPSC derived neurons of sporadic PD patients we generated neural progenitor lines using an embryoid body based protocol and further differentiated them into dopaminergic neurons by application of dual SMAD inhibition. PD patient derived neurons were successfully cultivated in microfluidic chambers and will be analyzed regarding axonal transport of mitochondria. A better understanding of the initial cellular effects of α-syn pathology, resulting in a disruption of neuronal circuitry is of great importance for the development of new therapy strategies to interfere with disease progression.

Abstract No. P053

Differentiation of human embryonic and induced pluripotent stem cells into CFTR expressing cholangiocytes for drug screening and disease modeling

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Introduction:
Cystic fibrosis (CF) is the most common lethal monogenic recessive disease in the caucasian population. While the majority of patients die of respiratory failure, hepatobiliary disease is the third leading cause of death in CF.
Over 1900 mutations are known so far in cystic fibrosis transmembrane conductance regulator (CFTR) gene, but the most common is the ΔF508 mutation, resulting in enhanced degradation of the CFTR protein.
Currently approved CFTR modulators are only available for a minority of CF patients. In addition, these modulators possess varying potentials. Therefore finding new modulators is of exceptional importance and might be achieved by the establishment of new cell culture based platforms for drug screening and disease modeling.

Materials and Methods:
Human embryonic stem cell (hESC) and induced pluripotent stem cell (iPSC) are utilized for differentiation approaches. Cells are monitored for definitive endoderm and hepatic endoderm generation by flow cytometric analysis. Maturation of hepatoblasts into cholangiocytes is achieved by the addition of growth factors. CFTR expression is monitored via qRT-PCR and can be directly observed by application of hESCs and hiPSCs carrying a CFTR Tomato reporter construct. CFTR function was assessed by patch clamp analysis.

Results:
The generation of definitive endoderm with hESCs as well as hiPSCs is with up to 97% highly efficient. Endodermal cells were further differentiated into hepatoblast and early cholangiocytes. Subsequently cholangiocytes were enriched and further maturated to gain up to 15% tomato-positive cells expressing CFTR. Patch clamp analysis confirmed functional CFTR in tomato-positive cells.

Conclusion:
The protocol is a first step for hPSC derived cholangiocytes, which might represent a suitable basis for disease modelling and drug screening approaches of liver associated diseases. These results might be translated to other organs e.g. the lung and facilitate CF research in the respiratory system.

Abstract No. P054

Using human pluripotent stem cell derived hippocampal PROX1 granule cells to investigate the functions of metabotropic glutamate receptor 2

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Human pluripotent stem cells represent basic research tools for developmental biology and molecular medicine. These self-renewable human cell lines can be used to generate various neuronal types *in vitro* and to study their morphological, electrophysiological and pharmacological properties. Besides the potential of generating individual cell lines from patients with neurological and psychiatric disorders, these *in vitro* derived neurons can also be used to investigate the role of various receptor subtypes. Group II metabotropic glutamate receptors (mGluRs) have been extensively studied as possible targets for the treatment of schizophrenia and other psychiatric disorders.

Using established protocols we generated hippocampal dentate gyrus granule cells. The dentate gyrus protocol is based on dual SMAD inhibition, Wnt- and SHH inhibition in embryoid bodies made from pluripotent stem cells. The differentiation results in forebrain specific Sox2 and Nestin positive neuronal progenitor cells (NPCs). This intermediate cell type can be further differentiated into PROX1 and MAP2 expressing mature neurons. Using this protocol we compared the Wnt-antagonist effects of recombinant DKK1 and XAV939, a small molecule used in other protocols. Both Wnt antagonists are sufficient to generate PROX1 neurons. The mature neurons start expressing mGluR2 along the differentiation process at subcellular compartments corresponding to the receptors *in vivo* localization. Using calcium-imaging and single cell electrophysiology it is possible to characterize the pharmacology of these receptors. mGlu2 and mGlu3 have been shown to modulate hippocampal circuitry *in vivo*, therefore these receptor subtypes warrant further investigation. The results will be discussed in the context of IPS cell based *in vitro* disease modeling.

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Abstract No. P055

Patient-specific iPS cell-based modeling of Transthyretin-Related Familial Amyloid Polyneuropathy

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Human pluripotent stem cells hold great promise in regenerative medicine and are a valuable tool for research on disease models and drug screening. Since somatic cells can be reprogrammed to induced pluripotent stem (iPS) cells, disease-relevant cell types can be obtained by directed *in vitro* differentiation of iPS cells.

In our study, we aimed for modeling Transthyretin-Related Familial Amyloid Polyneuropathy (TTR-FAP) using patient-specific iPS cells. TTR-FAP is a rare autosomal dominant disease caused by the aggregation of mutated TTR protein, thereby forming amyloid fibrils. These are accumulating as amyloid deposits in peripheral tissues, leading to an impairment of autonomic organ function. Importantly, TTR-FAP shows genotype to phenotype variations between the affected patients, making iPS cell-based disease modeling to a promising tool for this multisystemic disease.

We generated patient-specific iPS cells and differentiated these towards hepatic cells. Investigating the mRNA expression levels of hepatic marker genes (Albumin, HNF4α, AFP and TTR), we detected comparable expression levels in the differentiated TTR-FAP-iPS cells and in control hES or iPS cells. The TTR-FAP hepatic cells also showed functional characteristics like secretion of TTR and Albumin as well as Cytochrome P450 1A1 activity. By Mass Spectrometry analyses TTR protein with wildtype sequence as well as protein with Valin30Methionin substitution was found in supernatants of TTR-FAP hepatic cells. Most importantly for the disease modeling approach, the supernatants of TTR-FAP hepatic-differentiated cells contained significant amounts of TTR in an insoluble form. This insoluble TTR could be identified as amyloid fibrils by staining with intercalating dyes such as Congo Red and ThioflavinT.

In conclusion, reprogramming of fibroblasts from TTR-FAP patients resulted in iPS cells capable of being differentiated towards hepatocyte-like cells that express and secrete mutated TTR protein. Importantly, we were able to investigate the disease-causing amyloid fibrils formed by the mutated TTR in our *in vitro* system.

Abstract No. P056

Analysis of neurodevelopmental defects in Cockayne Syndrome B (CSB) by employing human neurospheres derived from CSB-deficient iPSCs

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Cockayne Syndrome (CS) is a rare autosomal recessive disease which, amongst others, leads to severe neurodevelopmental defects. The most frequent mutations causing CS are mutations in the Cockayne syndrome group B (CSB) gene. How CSB mutations impair brain development is so far enigmatic. Available CSB-deficient animal models are of limited use to address this question, because they show only a very mild neurological phenotype.

In the present study we have therefore employed human induced pluripotent stem cells (hiPSCs) derived from two different patients with two different CSB mutations (p.0 and p.Arg683x). Specifically, we differentiated hiPSC lines derived from these two CSB patients and two healthy controls into neural progenitor cells (NPCs) cultured as three-dimensional neurospheres. Proliferation of neurospheres was monitored via sphere growth over time.

During 14 days of proliferation, sphere growth was reduced in the CSB p.Arg683x neurospheres, while CSB p.0 NPC's proliferation was not impaired. Strikingly, the CSB p.Arg683x mutation is accompanied by a stronger neurological disease phenotype in the respective patient than CSB p.0. These data imply that (i) hiPSC-derived neurospheres might be suited for neurodevelopmental disease modeling and (ii) impaired NPC proliferation might contribute to certain CSB neurodevelopmental phenotypes.

Abstract No. P057

Generation of astrocytes from human induced pluripotent stem cells to investigate astrocyte biology in neurodegenerative diseases

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For more than 100 years the neuron was accepted as the most important cell type for cognitive function in the brain. Astrocytes were discussed to have only supportive functions in the central nervous system “filling the gap” between neurons. However, there is growing evidence that the contribution of astrocytes is far more complex and critical for brain function in physiology and pathology. Astrocytes are important regulators of brain homeostasis metabolism and synaptic activity thus play a critical role to achieve normal brain function. Astrocyte dysfunction is also indicated to be critical in neurodegenerative disorders like Alzheimer´s Disease (AD). A better understanding of astrocyte biology and their interplay with neurons will help to develop disease modifying treatments for these conditions. In this study, we differentiated mature human astrocytes from control and AD-patient derived iPSCs to study their role in disease in greater detail.

To uncover the potential role of human astrocytes in synaptic maturation hiPSC-derived astrocytes were used in co-culture experiments with hiPSC-derived neurons. In addition the extracellular matrix (ECM) of these cells was used as a substrate for hiPSC-derived neurons in the same context. Synaptic marker expression and excitotoxicity were measured to assess neuronal maturity. Both, direct co-culture as well as astrocyte-derived ECM lead to increased neuronal survival. Furthermore, astrocyte ECM had a proliferative effect on neuronal precursor cells. In a next step we successfully differentiated human astrocytes from AD-patient derived hiPSCs. Immunofluorescence staining and qPCR revealed the expression of astrocyte markers (e.g. GFAP, S100ß).

Using a next generation sequencing approach we aim to identify novel disease relevant genes/ pathways in hiPSC-derived astrocytes from AD patients. These cells will also be used to build *in vitro* models for AD dissecting their role in the disease process, ultimately identifying patient-relevant astrocyte pathology.

Abstract No. P058

Patient-specific iPSCs as a model system for inherited metabolic diseases

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Human induced pluripotent stem cells (hiPSCs) represent an invaluable tool for regenerative therapy, drug screening and toxicity testing. Moreover, they can serve as model system to identify pathomechanisms in rare human diseases where patient material is often not easily available. Especially for the study of rare inherited metabolic diseases affecting the central nervous system, hiPSCs are very helpful as they can be generated from patient´s cells and differentiated into affected cell types (in this case neurons) allowing for the molecular and biochemical analysis of the deficiencies *in vitro*.

Here, we report the generation of hiPSCs from patients with deficiencies in neurotransmitter biosynthesis and metabolism to study the underlying pathomechanisms of the diseases. Congenital defects of tyrosine hydroxylase (TH), aromatic amino acid decarboxylase (AADC), GTP cyclohydrolase I (GTPCH) or sepiapterin reductase (SR) lead to severe central deficiency of dopamine, serotonin, epinephrine, norepinephrine and tetrahydrobiopterin (BH4). Depending on the underlying enzyme defects patients present with neurological symptoms reaching form dopa-responsive dystonia to severe infantile Parkinsonism or seizures. Expression and activity of the genes involved have been characterized so far in healthy (wt) and diseased fibroblasts using classical biochemical methods, Western Blot analysis, and RT-PCR. After reprogramming, hiPSCs have been characterized for pluripotency marker gene expression and normal karyotype as well as transgene silencing and differentiation potential. Affected hiPSCs will now be differentiated into dopaminergic neurons to investigate how deficient cells differ from healthy ones and how the clinically observed phenotype can be retraced to the cellular phenotype. Thus, hiPSCs and differentiated derivatives are an invaluable tool to study metabolite profiles as well as expression and activity of TH, AADC, GTPCH and SR. This will improve the understanding of these rare inherited diseases and enable the development of better treatment regimens with improved patient´s outcome.

Abstract No. P059

Highly Efficient Media System for Definitive Endoderm Induction from Human Pluripotent Stem Cells

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Human pluripotent stem cells (hPSCs) provide the potential to differentiate into multiple cell types, thus are useful tools for both basic and therapeutic research. Paramount to reaching their potential is the simple and robust differentiation of stem cells to the desired end cell type. Definitive Endoderm (DE) derived from hPSCs provide a valuable source of cells for hepatic and pancreatic biology basic research as well as applied studies in pharmacological drug discovery, and cellular therapies. Development of a highly pure population of DE is critical to downstream differentiation to multiple lineages including liver, pancreas, and gut. However, optimizing cultures to obtain sufficient differentiated cell yields has been limited by complex steps with multiple component additions, lengthy protocols and high variability between hPSC lines. We have developed a fully defined, ready-to-use, xeno free media system that allows efficient induction of human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) into DE. We demonstrate that our PSC Definitive Endoderm Induction kit generates >90% CXCR4+/PDGFRα- cells within 48 hours of induction which is accompanied by loss of pluripotency markers and gain of definitive endoderm markers. Differentiation is highly efficient and reproducible across multiple hESC and iPSC cell lines, yielding cells that express markers of definitive endoderm including CXCR4, SOX17, and FOXA2. In addition, these cells maintain their ability to subsequently differentiate towards anterior, posterior, and mid/hind gut lineages. This simple and highly robust induction of hPSCs to definitive endoderm is an important step to achieve highly functional and pure populations of terminal endodermal lineages which can enable research and therapies.

Abstract No. P060

Simplified culture system for cardiomyocyte differentiation of human pluripotent stem cells in basic and translational research

David Kuninger[*], *Mohan Vemuri*, *Shayne Boucher*, *Stacy Jones*, *Daniel Beecham*, *Kevin Chambers*, and *Prasad Siddavatam*

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A simplified and reliable media system for generating cardiomyocytes from donor- or disease-specific human pluripotent stem cells (PSC) would provide a valuable source of cells for basic and translational research. Current protocols have led to heterogeneous results with varying purity and long lead times for generation of cardiomyocytes.

As a result, we developed, tested and manufactured a GMPgrade culture media system that is scalable and can be used to generate large numbers of continuously maintained or cryopreserved cardiomyocytes. PSC derived cardiomyocytes were analyzed by morphology, immunocytochemistry, flow cytometry, electrophysiology, gene expression and RNA-Seq.

Individual beating cells were observed as soon as day 10 and contracting syncytia by day 14. There was an over 100-fold increase in cell number from time of plating on day 0 to contracting syncytia on day 14. Quantitative flow cytometry detected populations of 90% and higher troponin T type 2 (TNNT2)-immunoreactive cells. Key to high percentage of TNNT2-immunoreactive cells is optimal seeding density, which was PSC line-dependent. PCR and RNA-Seq studies confirmed expression of mesoderm, cardiac mesoderm and mature cardiomyocyte genes as well as presence of atrial, ventricular and sinus-nodal genes. Immunocytochemistry studies verified expression of early and late cardiac markers.

Calcium fluor and microelectrode array studies supported the presence of electrically active cardiomyocytes sensitive to known cardioactive agents.

A simple and robust differentiation media system can serve as a standardized culture system for generating large numbers of consistent, spontaneously active cardiomyocytes for basic and translational research studies.

Abstract No. P061

Impairment of mitochondrial calcium handling in iPSC-derived neural progenitors carrying a MT-ATP6 mutation

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Mitochondrial DNA (mtDNA) mutations cause rare genetic diseases predominantly affecting the nervous system and for which no effective treatment exists. Animal models are scarce, due the difficulties in mtDNA engineering, and current cellular models lack the metabolic features of neural cells and the patient-specific mitochondrial-nuclear matched genome. The development of relevant model systems is therefore critical.

We explored the possibility to employ iPSC-derived neural progenitor cells (NPCs) as a novel model system for mtDNA disorders. NPCs were efficiently generated from human iPSCs and hESCs using a direct small molecule-based approach. Global transcriptomics confirmed their neural identity with respect to published NPCs and human brain atlas repositories. We previously demonstrated that somatic mitochondria are dramatically reconfigured upon iPSC derivation, leading to a switch towards glycolytic metabolism. However, little was known about the mitochondrial state of neural-committed cells. Electron microscope studies, quantitative calcium imaging, and bioenergetic profiling demonstrated that NPCs exhibit mitochondrial maturation and a metabolic shift away from glycolysis. Moreover, deep sequencing of mtDNA established that the parental mtDNA profile is maintained during reprogramming (both using retroviruses and episomal plasmids) and upon neural induction to NPCs.

We next reprogrammed to iPSCs patient-derived fibroblasts carrying a homoplasmic m.9185T>C mutation within the MT-ATP6 gene. Patient iPSC-derived NPCs relied on mitochondrial respiration and maintained the original parental mtDNA profile. Unlike fibroblasts, patient NPCs could not survive in glucose-free galactose media. Moreover, mutation-associated phenotypes could be identified in patient NPCs, including mitochondrial hyperpolarization, defective calcium release, and plasma membrane depolarization. This suggests that the mutation may have more detrimental effects in NPCs than in fibroblasts, thereby possibly interfering with correct neural functionality. Patient-derived NPCs, exhibiting the correct genetic and metabolic features, might therefore represent a novel model system allowing the personalized discovery of innovative treatment strategies for debilitating mtDNA encephalopathies.

Abstract No. P062

Preventing brain contribution of iPSC in primate-to-pig chimeras: Knockout of Foxg1 & evaluation of its functionality with *in vitro* differentiation

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The growth of human tissue in pigs is a promising solution to combat the growing shortage of human donor organs for transplantation. However, in pig-to-human chimeras any contribution of human cells to the brain has to be prevented. Therefore, the goal of this study is to induce a knockout of FOXG1, a Forkheadbox transcription factor critical for mammalian telencephalon development, in primate iPSC and to study the resulting phenotype after *in vitro* differentiation to telencephalon progenitor cells. The protocol is established first in mice, in which the results of *in vitro* differentiation can be verified *in vivo*.

The Foxg1 knockout was established using the CRISPR/Cas9 system in murine iPSC ubiquitously expressing the fluorescent protein Venus. After two rounds of limited dilution and clonal expansion, one line homozygous for an 80 bp deletion ($Foxg1^{-/-}$) was identified. To validate the functionality of this Foxg1 knockout, miPSC were differentiated to telencephalon progenitor cells using the SFEBq method and collected after 10-25 days to analyze mRNA expression of several telencephalic markers. Quantitative real-time PCR revealed that in $Foxg1^{-/-}$ cells ventral telencephalic markers such as Nkx2.1 and Gsx2 were less upregulated compared to wildtype miPSC after 25 days (NKX2.1: 1.9±2.1 to 5.6±1.1, Gsx2: 1.2±0.2 to 11.2±2.3, respectively; data as fold change to undifferentiated iPSC). Additionally, knockout cells expressed higher levels of the marker for postmitotic neurons Tuj1 after 20 days of differentiation (20.9±4.1 to 6.6±6.8).

These results are in accordance with $Foxg1^{-/-}$ effects *in vivo*, where ventral telencephalic areas are not specified and dorsal telencephalic neurons differentiate prematurely at the expand of the progenitor pool. Thus, we could partly recapitulate the *in vivo* phenotype with this *in vitro* differentiation protocol. Next, the contribution of our $Foxg1^{-/-}$ miPSC in mouse chimeras will be investigated and knockout and differentiation will be established for non-human primate iPSC.

Abstract No. P063

Development of a high-content platform to characterise large numbers of induced pluripotent stem cell lines

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The Human Induced Pluripotent Stem Cell Initiative (HipSci), funded by the Wellcome Trust and Medical Research Council (MRC), focuses on the generation and characterisation of hundreds of induced pluripotent stem (iPS) cell lines. These come from both healthy individuals and patients with selected diseases and will become an open resource for the scientific community, bringing together clinical genetics with data on genomics, proteomics and cell biology. At King's College London, the HipSci Cell Phenotyping group evaluates and quantifies responses of these cells to chemical, physical and biological stimuli using novel assays and artificial microenvironments. We have developed a 96-well format end-point assay exposing iPS cells to varying concentrations of fibronectin. The live image data are acquired for 24 hours prior the fixation. Our end-point read-out is based on a cytochemistry assay with DAPI and EdU to identify proliferating cells and CellMask to label the cellular plasma membrane. This allows phenotypes such as cell density, morphological features and proliferation to be detected, as well as complex cell-to-cell behavior (e.g. clumping). Importantly, phenotypic features can be quantified, compared and collated with genomic data. Here we present data obtained from a panel of over 70 iPS cell lines derived from healthy individuals. This approach is proving useful to benchmark healthy iPS cell lines and will likely be of great value in the future to establish relevant assays for disease modeling and drug discovery.

Abstract No. P064

Neural progenitor development, *in vitro*, from iPSCs generated from an Angelman Syndrome patient carrying a defined mutation in UBE3A

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Angelman Syndrome (AS) is a neurodevelopmental disorder characterized by the absence of speech, happy demeanor, seizures, movement disorders and developmental delay. The disease is caused by a lack of functional E3-Ubiquitin Ligase 3A (UBE3A) in the brain due to a defective maternal allele and silencing of the paternal allele during development which is mediated by the expression of a long non-coding antisense RNA (UBE3A-ATS or SNHG14), overlapping the whole UBE3A gene. However, how the complete lack of UBE3A alters the function of brain neurons ultimately leading to development of this disease is not known. Since primary neurons of AS patients are not amenable to detailed studies on the pathogenesis of the disease, we seeked to generate such cells by directed differentiation of patient-specific iPSCs, *in vitro*. For this purpose, we reprogrammed dermal fibroblasts derived from a patient carrying a defined 3bp deletion in the maternal copy of the UBE3A gene to iPSCs (AS_Δ3bp-iPSCs) together with fibroblasts from a healthy control person (Ctrl-iPSCs). After in-depth characterization of 4 AS_Δ3bp-iPSC clones and 2 clones of Ctrl-iPSCs we differentiated them towards neural progenitor cells in a stepwise fashion to first monitor the onset of UBE3A-ATS expression and subsequent start of monoallelic expression of the maternal UBE3A allele. UBE3A-ATS expression was upregulated during early NPC development, *in vitro*, whilst UBEA expression remained unaltered. During this rosette-forming progenitor stage, we did not yet detect imprinted, monoallelic expression of UBE3A, as monitored by single nucleotide primer extension analysis suggesting that silencing of transcription from the UBE3A-promoter occurs during subsequent terminal differentiation and maturation of neurons. Results of this ongoing work will be presented.

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Abstract No. P065

Examining the role of de novo mutations in LRRC7 postsynaptic density protein and KHSRP by parallel reprogramming of a schizophrenia case-parent trio

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De novo mutations (DNMs) have been implicated in the etiology of schizophrenia, a chronic debilitating psychiatric disorder. The large scale identification of DNMs has become feasible with the advent of next generation sequencing, primarily whole exome sequencing. While several DNMs have been demonstrated by examining schizophrenia cases and their unaffected parents, moreover the DNMs can be evaluated by bioinformatics tools with regard to their disease-causing effects, in most cases their biological significance remains inconclusive. To overcome this limitation we have developed an approach of using somatic cell reprogramming to generate induced pluripotent stem cell (IPSC) lines from each member of a schizophrenia case-control trio, in order to investigate the effects of DNMs in progenies of interest, particularly in forebrain glutamatergic neurons.

Here we describe a schizophrenia case-patient trio, whre the proban is a carrier of 3 non-synonymous DNMs in genes leucine rich repeat containing 7 (LRRC7), KH-Type Splicing Regulatory Protein (KHSRP), and Killer Cell Immunoglobulin-Like Receptor, Two Domains, Long Cytoplasmic Tail, 1 (KIR2DL1). LRRC7 encodes densin-180, a postsynaptic density protein in glutamatergic synapses, KHSRP is an RNA-binding protein implicated in axonal growth and dendritic spine development, while KIR2DL1 encodes killer cell immunoglobulin-like receptors (KIRs) that are transmembrane glycoproteins. IPSC lines were generated from his and his parents peripheral blood mononuclear cells using sendai virus based reprogramming. IPSCs were characterized using alkaline phosphatase staining, qPCR and immunostainig. The DNMs were validated in the IPSC lines by sequencing. After characterization the IPSCs will be used to investigate various neuronal phenotypes including morphology, neurite outgrowth, synaptic connectivity and electrophysiological activity. The approach of reprograming trios represents a means for investigating disease causing mutations and comparing cell lines with similar genetic background, without the need for genome editing technologies.

This study is funded by the National Brain Research Program (NAP) of Hungary.
Bird CW, Gardiner AS, Bolognani F, Tanner DC, Chen CY, Lin WJ, Yoo S, Twiss JL, Perrone-Bizzozero N: KSRP Modulation of GAP-43 mRNA Stability Restricts Axonal Outgrowth in Embryonic Hippocampal Neurons (2013). Plos ONE 8(11): e79255.

Abstract No. P066

Generation of disease-specific iPSCs and development of transgenic reporter cell lines for cystic fibrosis disease modelling and drug screening

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Cystic fibrosis (CF) is a genetic disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene coding for a cAMP-activated chloride-channel expressed in different epithelia. So far, immortalized cell lines overexpressing mutant CFTR-variants have been used to screen compound libraries. In fact, CFTR-protein-modulators have been identified, but show modest effects at best. Obviously, the complexity of the mutant CFTR-maturation and turnover kinetics including the influence of genetic modifiers require the use of advanced personalized cellular models that closely recapitulate the properties of the most clinically affected organs. To address these unmet needs we focus on the generation of induced pluripotent stem cell (iPSC) lines from patients with the most common trafficking mutation (F508del), but with different phenotypes. Therefore, CF-iPSCs were generated via reprogramming of CD34+ cells isolated from small volumes of non-mobilized peripheral blood. The resulting CF-iPSCs were analysed regarding their karyotype, pluripotency status and potential to differentiate. Moreover, different transgenic iPSC and embryonic stem cell (ESC) lines overexpressing a halide sensitive yellow fluorescent protein (YFP) or an artificial CFTR or containing a knock-in of a tomato-fluorescence-reporter in the endogenous CFTR locus were generated to monitor CFTR-function. The established CF-iPSCs showed the expression of pluripotency markers and differentiated into cell types of all three germlayers. Furthermore, we differentiated CFTR-tomato-reporter and YFP overexpressing iPSCs into cholangiocytes and obtained double-positive cells. We were also able to generate YFP and CFTR overexpressing ESCs and used them for first functional measurements to test the halide sensitivity of the YFP. To conclude, the stable integration of the halide reporter into CF-patient-specific iPSCs in combination with integration of the CFTR-tomato-reporter should enable disease modelling of F508del-based CF with regard to the individual genetic context and the implementation of high-throughput screening for novel correctors and potentiators of CFTR-trafficking mutations.

Abstract No. P067

Exploration of Nrf2 pathway activation as potential human neurotoxicity reporter

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Human pluripotent stem cells (hPSC) can be triggered to differentiate into any cell type of the body and are thus ideally suited to address predictive tissue-specific toxicity *in vitro*. In this context, repeated-dose toxicity testing is particularly desirable for predicting adverse effects on target tissues/organs, on dose-response relationships and on reversibility of any adverse effects observed. There is strong evidence that various cellular toxicity pathways share the Keap1-Nuclear factor (erythroid-derived 2)-like 2 (Nrf2)-antioxidant response element (ARE) pathway as common denominator for sensing of oxidative stress and for the induction of protective cellular responses. Exploitation of this pathway as an indicator of cell stress-associated events could thus provide a valuable tool for *in vitro* toxicity assay development.

In order to establish an hPSC-based platform for repeated-dose neurotoxicity testing, we made use of hPSC-derived long-term neuroepithelial stem cells (lt-NES; Koch et al. PNAS, 2009). To establish a high-throughput neurotoxicity assay platform we developed a semi-automated, standardized, and scalable method for the generation of mature lt-NES-derived neurons in 96-well plates. To assess the toxicological relevance of the Nrf2 pathway we first evaluated its basal and inducible activity in lt-NES and lt-NES-derived neurons at different maturation stages. QRT-PCR analysis of the Nrf2 downstream targets SRXN1, NQO1 and HMOX1 revealed increased expression levels with the progression of neuronal maturation. In accordance with the qRT-PCR results, dose-response curves for repeated-dose toxicity revealed an increased resistance of more mature neurons to Rotenone as determined by AlamarBlue assay. Complete removal of antioxidants from the cell culture medium revealed that Nrf2 pathway activation is strongly increased in response to Rotenone treatment. These observations indicate that setting up an hPSC-based neurotoxicity assay platform demands multiple considerations, including the maturity of the neurons and media conditions.

Koch, P., Opitz, T., Steinbeck, J.A., Ladewig, J. and Brustle, O. (2009) A rosette-type, self-renewing human ES cell-derived neural stem cell with potential for in vitro instruction and synaptic integration. Proc. Natl. Acad. Sci. U S A., 106, 3225-3230

Abstract No. P068

Cytokine-directed differentiation and 3D organoid-based maturation of hepatic hESC-derivatives

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Pluripotent stem cell derived hepatic cells might serve as transplants for metabolic or acute liver diseases as well as cell source for drug screening. So far, hepatic differentiation of hiPSCs and hESCs is well studied, but efficiencies and enrichment of functional active hepatic derivatives and strategies ensuring a homogenous hepatic cell population need further attention.

In this study, we investigated the generation of hepatic cells display a fetal phenotype. The cells were obtained applying a two-stage protocol to differentiate hESCs The first stage of this protocol is a cytokine-directed monolayer differentiation which is followed by an organoid-like based 3D suspension culture stage. In this stage a prolonged terminal maturation and propagation of the hepatic cells occur. To omit beside differentiation different media compositions, organoid-forming strategies and time frames of suspension culture were tested. To obtain a reasonable amount of hepatic cells the protocols were adapted for upscale the culture under Bioreactor conditions. Furthermore the ability of these organoids to serve as a source for transplantation was analyzed.

Quantitative real time PCR for progenitor (FOXA2, SOX17, AFP) and mature (HNF4a, ABCC2, TTR) hepatic markers were performed to analyse the efficiency of this maturation process. Immunhistochemical stainings (PAS, Albumin, CK19 and FAH) were performed to visualize the homogeneity of the organoids. Moreover, metabolic assays (Cyp1A1, Cyp3A4) were performed to validate the extent of hepatic maturation.

In conclusion, our two-stage differentiation protocol allows an efficient and reproducible generation of hepatic cells displaying a fetal phenotype after the first differentiation stage. During a second maturation stage, the cells obtained a more mature hepatic phenotype. Moreover, it was feasible to seed these organoids, as single cells as well as intact organoids for upstream transplantation studies.

Abstract No. P069

Introduction of an iPS cell-derived human monocyte cell culture model for studying pathomechanisms of Parkinson's Disease associated LRRK2 mutations

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Mutations in Leucine-rich repeat kinase 2 (LRRK2) are strongly associated with familial Parkinson's Disease (PD). LRRK2 is highly expressed in innate immune cells and may be linked to neuroinflammatory processes seen in PD patients.

We previously reported that activated primary microglial cells from adult mice overexpressing LRRK2(R1441G) show an increased secretion of pro-inflammatory cytokines compared to wildtype control cells (Gillardon et al. 2012). Additionally, conditioned medium from activated LRRK2(R1441G) microglial cell cultures induces significant cell death when added to primary neuron cultures. These findings indicate that dysregulation of immune cells leads to enhanced neuroinflammation and thus may have a detrimental effect on neuronal survival in PD patients carrying LRRK2 mutations.

In order to extend our findings from mouse models, we now established a human cell culture model. We differentiated human stem cell-derived monocytes/macrophages from LRRK2(G2019S) induced pluripotent stem (iPS) cell lines and isogenic controls expressing LRRK2 at endogeneous levels. Our ongoing work focusses on profiling of changes in gene expression and cytokine secretion in LRRK2 mutant iPSC-derived monocytes/macrophages and the potential pathophysiological relevance for Parkinson's disease.

Gillardon et al. (2012) Neuroscience Apr 19; 208:41-8

Abstract No. P070

Angelman syndrome iPSCs form immature teratomas *in vivo* containing derivatives of all three germ layers

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Angelman syndrome is an imprinting disorder associated with severe intellectual disability, absence of speech, ataxia and other specific symptoms. It is caused by the disruption of the maternal copy of the *UBE3A* gene, resulting in the absence of a functional UBE3A protein in the brain. Imprinted expression of *UBE3A* is controlled by the differential methylation at PWS-SRO, which locates to the promotor and exon 1 of *SNURF-SNRPN*. This methylation is set in the female germline. On the paternal chromosome, the unmethylated PWS-SRO serves as a neuron-specific promoter for a non-coding RNA, which overlaps the *UBE3A* gene in antisense direction, resulting in silencing of the intact paternal *UBE3A* gene.

We generated and characterised iPSCs of a patient with Angelman syndrome and a healthy control person using various methods. In order to verify the pluripotency of the generated iPSCs with another independent approach, we employed the teratoma formation assay as the most stringent test. iPSCs were injected into hind limbs of immunodeficient mice. The teratoma formation occurred with 100% efficiency. Sections of paraffin-embedded tumours were stained with hematoxylin and eosin and the origin of different tissues present in the teratomas was determined. Neural rosettes of the ectodermal layer, fat cells or loose mesenchyme stemming from mesoderm, and glandular tissue with epithelia of endodermal origin were identified. Staining with antibodies against the derivatives of the three respective germ layers was also conducted supporting our previous findings. In some cases, ectodermal pigmented cells, and capillaries of mesodermal origin were present in the sections, as well as endodermal mucinous epithelia, as shown by PAS staining. All of the iPSCs subjected to the analysis proved the ability to develop into derivatives of all the three germ layers, confirming their pluripotency.

Abstract No. T15/P071

Modelling reactive gliosis in mESC-derived retina organoids

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Pluripotent stem cell derived retinal organoids are a new experimental model system for studies on retinal degeneration and regeneration. Here we started to develop a retinal organoid-based model to study reactive gliosis, a glial response frequently associated with neuronal damage and disease.

We first characterized the developmental and molecular state of radial Müller glia (MG), the major type of glia in the retina, in the organoid system. We observed the first MG in developing retina organoids on day 18 and postmitotic MG by day 22. Next, we characterized cell death in the mESC-derived retina organoid system using TUNEL assay. Cell death increases with ongoing neurogenesis, and photoreceptors and bipolar cells (8% ± 5 SEM) are less affected compared to amacrine and ganglion cells (25% ± 1 SEM). Strikingly, MG in retina organoids do not express glial fibrillary acidic protein (GFAP), a hallmark of reactive gliosis, or other known gliosis associated markers in response to the organoid system immanent cell death.

Reactive gliosis does not occur due to ontogenetic cell death *in vivo*, but is induced upon retina explant culture. We hypothesized that the organoid system offers a unique opportunity to study reactive gliosis ex vivo. Thus, we asked if reactive gliosis can be induced in the retina organiod system. Strikingly, application of neuronal damage-related signaling factor TNF on day 20 induced GFAP expression in MG, increased cell proliferation, as well as a loss of retinal stratification within 5 days.

In sum, our data suggests that MG in the retinal organic system become quiescent and non-reactive at the end of retinogenesis. Stimulation of retinal organoids with TNF is sufficient to induce MG reactive gliosis and a retinal pathologic phenotype. We conclude, the organoid system offers a new model system and unique opportunity to study the mechanisms of reactive gliosis *ex vivo*.

Abstract No. P072

Differentiation of Midbrain Floor Plate Progenitors and DA Neurons from Human PSCs

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We developed culture medium system for the induction and maturation of authentic midbrain DA neurons from hPSCs. The system has broken the process down into 3 distinct steps:

(1) specification of hPSC to midbrain floor plate (mFP) cells,
(2) expansion and cryopreservation of mFP, and
(3) maturation to DA neurons.

Characterization of floor plate cells and mature DA neurons was performed by immunostaining for the presence of specific markers including Lmx1, Otx2, FoxA2 and TH, additional qPCR analysis included an expanded list of genes to help define these cell populations. Electrophysiological activity of these DA neurons was assessed by Multi-electrode array evaluation. In comparison to published protocols, our new system has several advantages including ease of use, significant expansion and preservation of progenitors in relatively short culture duration. This efficient system will benefit researchers with increased scale and flexibility in targeted studies.

Abstract No. P073

Using iPSC-derived midbrain dopamine neurons for rapid phenotyping of LRRK2 mutant-associated alterations

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Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting about 2% of the population above the age of 60 years. It is characterized by progressive loss of A9 midbrain dopamine (mDA) neurons in the substantia nigra pars compacta (SNpc). Current therapeutic approaches for PD provide only symptomatic relief but cannot change the disease course. Although most of PD cases are idiopathic and occur sporadically, a minority of 5-10% can be linked to monogenic causes. Notably, the autosomal dominant G2019S mutation in the large, multifunctional kinase LRRK2 leads to clinical symptoms typically found in common sporadic PD cases. Thus, in principle, mDA neurons derived from induced pluripotent stem cells (iPSCs) of LRRK2G2019S patients should be highly valuable for drug discovery screens. However, assessment of LRRK2G2019S-associated phenotypes in iPSC-derived mDA neuronal cultures typically requires weeks to months of *in vitro* differentiation, which makes these cell systems unsuitable for high-throughput applications. Here we set out to establish a culture system based on a standard mDA progenitor population amenable to replating and phenotypic read-out within a 48 hour time period – a time frame acceptable for scale-out and HTS. Our standard cell population is pre-differentiated for 3-5 weeks and shows expression of characteristic mDA markers such as FOXA2, LMX1A, PITX3, NURR1 as well as TH. Replating of these cells enables read-out of a 20% reduction in the length of TH+ neurites in LRRK2G2019S neurons already after 24 hours. Cell survival assays following autophagy inhibition with 3-methyladenine yield a LRRK2G2019S-specific increase in neuronal cell death already 48 hours after treatment. We expect these rapid phenotypic assays to expedite the study of PD-related pathomechanisms and enable the identification of therapeutic compounds via high-throughput drug screening applications.

Abstract No. P074

Effects of induced pluripotent stem cell long term passaging on pluripotency, neuronal differentiation and stress response

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iPS cells have a large potential for the development of disease relevant cellular models for academic research as well as in the pharmaceutical industry. However, working with iPSCs is technically demanding and the differentiation state of the cells needs to be monitored continuously.

In this study we monitored the effects of long time cultivation of stem cells on pluripotency, neuronal differentiation and rotenone sensitivity by immunohistochemistry for marker proteins, functional assays and next generation sequencing (NGS). The study was performed with three iPSC clones originating from one fibroblast donor, which have additionally been divided into two daughter cell lines each. Neuronal progenitor cell lines (NPCs) were generated from these six lines and cultured in parallel. The iPSC lines and the NPC lines were cultivated over 30 passages and samples were taken on a regular basis. Furthermore, the NPCs were differentiated into dopaminergic midbrain neurons at an early, middle and late time point of the study. Pluripotency was verified by staining the cells with typical stem cell pluripotency markers; NPCs and differentiated neurons were characterized using neuronal progenitor, pluripotency and neuronal markers. Differentiated neurons were treated with rotenone at different concentrations to induce oxidative stress by inhibiting mitochondrial respiration.

The six iPSC lines expressed typical pluripotency markers over 30 passages and maintained their proliferation rates. Similarly, the six NPC lines stable expressed neuronal precursor markers over the course of the study and could be differentiated into midbrain dopaminergic neurons with comparable percentages of TH positive neurons and morphology. However, rotenone sensitivity was highly variable between individual experiments. The gene expression profile is currently under evaluation. We expect that these data will give insight into the stability of iPSC and NPC cultures over time and guide us to create a stable cell culture model in future.

Reinhardt P, M Glatza, K Hemmer, Y Tsytsyura, CS Thiel, S Hoing, S Moritz, JA Parga, L Wagner, et al. (2013). Derivation and expansion using only small molecules of human neural progenitors for neurodegenerative disease modeling. PLoS One 8:e59252

Poster session II: P075 – P147

P075 – P093 Pluripotency and embryonic stem cells

P075 Abstract withdrawn

P076 Nitric oxide synthesis and Zeb1 transcription factor inactivation characterize very early mesoendoderm precursors in mouse embryonic stem cells
Chiara Cencioni

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Katja Hufschmid

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Christian Freund

T06/P080 Ground state conditions induce rapid reorganization of core pluripotency factor binding before global epigenetic reprogramming
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P081 Linking high order chromatin reorganization to stem cell homeostasis by H3K56ac and Sirt6
Angelina Georgieva

T09/P082 Decoding the heterogeneity of pluripotent stem cells at the single cell level
Simon Hastreiter

P083 Novel nuclear specific regulatory role of LIN28A protein in pluripotent stem cell lineage acquisition
Sabina Kolar

P084 Expansion of human pluripotent stem cells in perfused bioreactors under xeno-free and chemically defined conditions
Christina Kropp

P085 Fetal bone marrow derived MSCs as an alternative and better source for propagating pluripotent cells
Peggy Matz

T07/P086 TDP-43 safeguards pluripotency by regulation of alternative polyadenylation
Miha Modic

P087 A modular open platform for systematic functional studies under physiological conditions
Sebastian Bultmann

P088 Metabolic implications of retinoid-sequestering blue fluorescent lipid bodies in 'primed' pluripotent stem cells
Thangaselvam Muthusamy

P089 Screening of cell-type-specific histone-bound long noncoding RNAs
Yuliya Ponomareva

P090 The immunoproteasome complex – a superior gatekeeper of protein homeostasis in human pluripotent stem cells.
Friederike Schröter

P091 Loss of Myc Activity Induces Cellular Dormancy in Embryonic Stem Cells Mimicking the Status of Diapause Embryos
Roberta Scognamiglio

P092 Implementation of a pluripotent stem cell nomenclature in the Human Pluripotent Stem Cell Registry hPSCreg
Stefanie Seltmann

P093 Development of a Two Part System Providing Enhanced Post-Thaw Recovery of PSCs & Reducing Cellular Stress Associated with Broad Range of Applications
Tim Wessel

Abstract No. P076

Nitric oxide synthesis and Zeb1 transcription factor inactivation characterize very early mesoendoderm precursors in mouse embryonic stem cells

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BACKGROUND: Exogenous nitric oxide (NO) induces chromatin remodelling and decreases stemness gene expression leading to mesendoderm and cardiomyocyte differentiation. Specifically, NO negatively regulates the zinc-finger E-box binding homeobox 1 and 2 (ZEB1, 2) factors via upregulation of miR-200 family members. This work aims to understand the mechanism by which NO contributes to mesendoderm commitment of mouse embryonic stem cells (mES). METHODS & RESULTS: mES were cultured according to the 3 inhibitor protocol (3i) which includes leukemia inhibitory factor; an ERK inhibitor and a GSK3 inhibitor. Alternatively, cells were left into spontaneous differentiation or exposed to 250 µM DETA/NO for 24 hours. The effect of NO donor was examined by a combination of RNA-seq and ChIP-seq showing the presence of lineage specific mesendodermal markers and the concurrent down modulation of neuroectodermal markers following ZEB1 detachment from the same genome loci interested by active transcription of NO-dependent genes. Mechanistically, a switch between ZEB1 and p53 on the target gene promoter regions accounted for mesendodermal marker expression. During the progress of these experiments, we found that a NO-positive mES ($ESNO^+$) population could be detected as early as 2 hours from release. These cells expressed eNOS and nNOS, and spontaneously generated endogenous NO paralleled by a significant production of cGMP. In addition $ESNO^+$ cells revealed the presence of nitrosylated HDAC2, a modification which inactivates HDAC2 function. FACS sorting of the NO^+ population showed, in these cells, the expression of same mesendodermal markers detected after exogenous NO treatment. CONCLUSION: Our data provide the first evidence of a cell subpopulation able to synthesize NO during very early stages of mES differentiation. $ESNO^+$ efficiently differentiated into mesendoderm via the coordinated action of ZEB1, HDAC2 and p53.

Abstract No. P077

A Novel Essential 8 Media System for Robust Weekend-Free Culture of Pluripotent Stem Cells

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Pluripotent stem cells (PSCs) are powerful tools for developmental biology, regenerative medicine, and the study of debilitating human diseases. While the development of feeder-free culture systems has simplified routine PSC culture significantly, additional hurdles continue to challenge today's stem cell scientists. Prominent among these is the daily media exchange required to replenish unstable media components. This daily feeding not only necessitates seven day workweeks for scientists but also increases the potential for contamination and introduces variability resulting from multiple users processing a single culture. The requirement for daily feeding is driven largely by the heat sensitivity of growth factors crucial to the maintenance of pluripotency in culture, with activity loss beginning immediately upon warming of growth medium and deteriorating upwards of 80-90% after 24 h at 37°C. In this work we have developed a new Essential 8 (E8)-based cell culture media that allows for long-term, feeder-free, healthy PSC culture without the need for weekend feeding. In this new culture medium virtually no loss of bioactivity is observed in heat-labile growth factors at 37°C for periods of 72 h or greater. The medium has been used with equal success in embryonic and induced pluripotent stem cell culture and does not require low cell densities or other significant changes to current culture workflows. Furthermore, our data indicate that PSCs cultured in this formulation using a weekend-free culture schedule (1) maintain expression of canonical pluripotency markers, including Nanog and Tra-1-60, (2) retain the capacity for trilineage differentiation, and (3) exhibit a normal karyotype over long-term passaging. In addition to simplifying and securing day-to-day PSC culture, enhanced stability of key factors will have added benefits in large-scale cultures for regenerative medicine, where large volume media exchanges are impractical and consistent performance is critical.

Abstract No. P078

Systems dissection of epiblast stem cells reveals shared and diverse Oct4 attributes of pluripotent cells

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Epiblast stem cells (EpiSCs) are pluripotent cells that can be isolated and cultured from post implantation embryos. In contrast to embryonic stem cells (ESCs), systematic studies to investigate the genes that maintain pluripotency in EpiSCs have not been reported. Here we combine a genome-scale RNAi screen with genetic interaction, protein localization and protein-level dependency studies to delineate connectivity between factors that control Oct4 expression in EpiSCs and compare the role of these factors to their function in ESCs. We demonstrate the power of this integrative approach by the identification of Tox4 as an interactor of Paf1C, a complex that acts in multiple facets of RNA polymerase II (RNAPII) regulation. Our results indicate that Tox4 cooperates with Paf1C for maintenance of Oct4 expression and pluripotent cell identity.

Abstract No. P079

Assessment of human pluripotency: A comparison of teratomas and *in vitro* assays

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The teratoma assay is considered as the gold standard to test pluripotency of human pluripotent stem cells (PSCs) *in vivo*. Nevertheless the assay is animal-dependent, time-consuming, expensive, difficult to quantify and experimental procedures are not standardized. Recently *in vitro* assays for the assessment of pluripotency have been developed: whereas PluriTest analyses the gene expression profile of undifferentiated PSCs the Scorecard test evaluates their short-term differentiation potential. Whether teratomas can be replaced by the *in vitro* assays is currently a matter of debate. Here we tested a validated human embryonic stem cell line (H9), an induced PSC line with reactivated transgenes (RT-hiPSCs) and a differentiation-defective embryonal carcinoma (EC) line in the various assays. H9 and RT-hiPS cells were cultured on vitronectin in TESR-E8 media. When analysed by Pluritest both H9 and RT-hiPSCs had comparable pluripotency scores indicating a similar pluripotency status. The EC cells scored less but were still considered pluripotent. For the teratoma assay 1x 106 cells were injected with matrigel subcutaneously. As expected EC-derived teratomas consisted mainly of undifferentiated cells expressing OCT3/4 and Nanog as shown by immunofluorescent (IF) staining. Interestingly the RT-hiPSC teratomas also contained significant amounts of undifferentiated cells whereas those cells were absent in H9 teratomas. IF staining of markers representing ectoderm (βIII-tubulin), mesoderm (smooth muscle actin (SMA)) and endoderm (α-fetoprotein (AFP)) revealed derivatives of the three germ layers in H9 and RT-hiPSC teratomas. By contrast EC-teratomas only contained SMA-expressing cells. For quantification of gene expression teratomas are currently being analysed by microarray. In addition all cell lines are being tested in *in vitro* differentiation assays for their capacity to generate ectoderm, mesoderm and endoderm. By comparing the results we will evaluate the potential of the *in vitro* assays to replace the commonly used *in vivo* method.

Abstract No. T06/P080

Ground state conditions induce rapid reorganization of core pluripotency factor binding before global epigenetic reprogramming

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Mouse embryonic stem cells (mESCs) cultured under serum/LIF conditions exhibit heterogeneous expression of pluripotency-associated factors that can be overcome by two inhibitors (2i) of the MEK and GSK3 pathway, respectively. Several studies have shown that the "ground state" induced by 2i is characterized by global hypomethylation and specific transcriptional profiles, but little is known about the contributing effectors. Here we show that 2i conditions rapidly alter the global binding landscape of OCT4, SOX2 and NANOG. The dynamic binding influences enhancer activity and shows enrichment for regulators linked to Wnt and Erk signaling. Epigenomic characterization provided limited insights to the immediate transcriptional dynamics suggesting these are likely more secondary effects. Likewise, knock-out of the PRC2 component EED to prevent H3K27me3 deposition had minimal effect on the transcriptome, rendering it largely dispensable for continued repression of bivalent genes and de novo silencing in 2i.

Abstract No. P081

Linking high order chromatin reorganization to stem cell homeostasis by H3K56ac and Sirt6

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High order chromatin reorganization is characterized as an epigenetic phenomenon associated with stem cell differentiation and lineage specification. *In vitro* embryonic stem cell differentiation into neuronal lineage is correlated with transition from euchromatin to heterochromatin structure whereas *in vivo* adult muscle stem cell differentiation is coordinated with loss of condensed heterochromatin and gain of euchromatin in differentiated myocytes. Surprisingly both stem cells are enriched with histone lysine 56 acetylation (H3K56ac), an epigenetic mark associating with transcriptional activation by inducing histone variant H2A.Z eviction. To understand the underlying cellular and molecular mechanism regulating the opposite re-organization of chromatin through H3K56ac, we focused on Sirt6, the main eraser of H3K56ac in mammalian cells, in control of chromatin structure and cell homeostasis of both embryonic and adult muscle stem cells. Depletion of Sirt6 in ES cells led to an increased H3K56ac, inhibited cell differentiation without apparent cell proliferation defect whereas inactivation of Sirt6 in satellite cells led to loss of heterochromatin, promoted stem cell proliferation without influencing differentiation. This suggests that Sirt6 mediated removal of H3K56ac might be involved in regulating the re-organization of chromatin structure irrespective to the cellular differentiation or proliferation state. Further molecular analyses revealed that in the absent of Sirt6 ES cells regain a significant number of new H3K56ac binding sites at the regulatory region such as enhancers within intergenic region and lose H2A.Z incorporation within chromatin, which is correlates with transcriptional activation of a fraction of genes. We are currently testing the hypothesis that Sirt6 regulates H3K56ac level thereby influencing H2A.Z incorporation, gene expression and cell homeostasis in ES cells and muscle stem cells *in vitro* and *in vivo*.

Abstract No. T09/P082

Decoding the heterogeneity of pluripotent stem cells at the single cell level

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Pluripotent stem cells have the unique ability to both self-renew indefinitely and to differentiate into all cell lineages of the embryo. The maintenance of pluripotency is controlled by a network of transcription factors and epigenetic modifiers centered around Oct4, Sox2 and Nanog which in turn is regulated by extracellular signals. It has been shown that the expression levels of key pluripotency factors like Nanog are highly heterogeneous at the single cell level in mouse embryonic stem cell (ESCs) cultures in standard Serum+LIF conditions. In contrast, dual inhibition of GSK3 and MEK signaling (2i) converts the culture into a more homogeneous ground state.

So far, it is still elusive how pluripotency factors regulate each other in single cells and what the dynamics and significance of their heterogeneity are. In this study we found two functionally distinct subpopulations of low Nanog expressing cells and distinguish selective and inductive effects of the 2i condition on Nanog heterogeneity. We further aim to decipher the regulatory logic in single cells by manipulating the pluripotency network and linking cellular dynamics to future fate choices. Long-term continuous time-lapse imaging of knock-in reporter cell lines enables us to quantitatively measure endogenous protein levels of key pluripotency factors in single ESCs over many days and microfluidic devices will allow us to precisely stimulate single cells and observe their immediate responses.

With our approach the cellular dynamics of ESC self-renewal and differentiation can be analysed at an unprecedented resolution and the data provide a rich resource for the development of accurate computational models of network dynamics.

Abstract No. P083

Novel nuclear specific regulatory role of LIN28A protein in pluripotent stem cell lineage acquisition

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LIN28A is a conserved RNA-binding protein important for pluripotency and reprogramming. Although originally identified as a cytoplasmic, LIN28A has been observed to shuttle between the nucleus and cytoplasm, and recently, the methyl-transferase Setd7 has been implicated in methylation of LIN28A, resulting in nuclear retention with preferential localisation to the nucleoli. Importantly, little is known with regard to the nuclear function of LIN28A.

Using CRISPR/Cas technology, we stably integrated eGFP into the locus of *LIN28A* in human embryonic stem cells, generating a fused protein. The LIN28A-eGFP maintains pluripotency, and is competent in producing differentiation progeny. GFP is readily detected by microscopy, enabling to quantify LIN28A localisation during early differentiation. Exposure of the cells to differentiation conditions did not lead to rapid reduction of the reporter, but as reported previously we observed nucleolar translocation. To elucidate nuclear role of LIN28A we performed co-immunoprecipitation and iCLIP (individual resolution Crosslinking and ImmunoPrecipitation) using nuclear and cytoplasmic subcellular fractions. Experiments revealed distinct cytoplasmic vs. nuclear protein interaction network of LIN28A and novel RNA targets of LIN28A, indicating that this protein obtains upon differentiation nuclear specific regulatory roles governing early pluripotent stem cell transition.

Abstract No. P084

Expansion of human pluripotent stem cells in perfused bioreactors under xeno-free and chemically defined conditions

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Human pluripotent stem cells (hPSCs), including human induced pluripotent stem cells (hiPSCs) and embryonic stem cells (hESCs), and their progenies are considered promising cells sources for cell based therapies, *in vitro* disease modelling and advanced drug discovery strategies. All these therapeutic and industrial applications will require the constant supply of billions of lineage-specific cells generated by robust, economically viable and well defined processes. The expansion of hPSCs as aggregates in 3D suspension provides a feasible and potentially superior solution for achieving the cell numbers needed for their envisioned applications.

Recently, the expansion of single cell inoculated hPSCs as cell-only-aggregates in stirred tank bioreactors was shown by us (Olmer et al., Tissue Eng Part C, 2012). Subsequently, this technology was combined with cardiomyogenic differentiation using chemical Wnt pathway modulators (Kempf and Olmer et al., Stem Cell Reports, 2014; Kempf and Kropp et al., Nature Protocols, 2015, in press).

As most media currently used for hPSC expansion in suspension culture are not chemically defined but complex and relative expensive, they are of limited use for the development of large-scale processes. In 2011, Chen et al. reported “essential 8” (E8), a xeno-free and chemically defined culture medium for hPSC maintenance (Chen et al. Nature Methods, 2011). E8 was shown to support hPSC maintenance in suspension culture (3D; Wang et al., Stem Cell Research, 2013) and its suitability for cell expansion ahead of cardiac differentiation in conventional 2D culture was demonstrated (Burridge et al., Nature Protocols, 2015).

Using E8 in perfused stirred tank bioreactors we will demonstrate a novel, more effective and homogenous hiPSC expansion process yielding up to 3.8 x 10^6 cells/mL in a working volume of 100 mL in 7 days, supporting clinical translation of hPSCs at reduced cost.

Olmer R, Lange A, Selzer S, Kasper C, Haverich A, Martin U, et al. Suspension Culture of Human Pluripotent Stem Cells in Controlled, Stirred Bioreactors. Tissue Eng Part C 2012;18:772-784
Kempf H and Olmer R, Kropp C, Rückert M, Jara-Avaca M, Robles-Diaz D, et al. Controlling Expansion and Cardiomyogenic Differentiation of Human Pluripotent Stem Cells in Scalable Suspension Culture. Stem Cell Reports 2014;3:1-15.

Abstract No. P085

Fetal bone marrow derived MSCs as an alternative and better source for propagating pluripotent cells

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Routine culture of embryonic stem cells (ESCs) as well as induced pluripotent stem cells (iPSCs) employs mouse embryonic fibroblasts (MEFs) as feeder layers and/or conditioned medium (CM). The main disadvantage of using MEFs is the potential contamination with mouse cells and pathogens. Several studies have shown the successful use of human cells as feeders e.g. human dermal fibroblasts, human amniotic epithelial cells or human adult bone marrow mesenchymal stem cells. In the current study, we investigated the use of fetal femur derived mesenchymal stem cells (fMSCs) as an alternative to MEFs. We comparatively cultured over several passages hESCs and iPSCs on fMSCs and MEFs as feeder layers and also conditioned medium (CM) produced from both. Immunofluorescence-based detection of expression of pluripotency associated proteins as well as qRT-PCR for the corresponding transcripts showed comparable levels. ACTIVIN-A is a major component in CM and is required for maintaining self-renewal. ELISA-based measurements revealed higher levels in fMSC-CM which was sustained over longer periods compared to compared to MEF-CM.

Furthermore, fMSCs secrete numerous growth factors and cytokines, amongst these are FGF2, FGF19, VEGF, PDGF-AA, IL-11 and CYSTATIN-C which are involved in proliferation processes. In summary, we have demonstrated that human fMSCs have several advantages compared to MEFs and can be used as an alternative for routine propagation of pluripotent stem cells and thus obviating the unnecessary need for breeding mice solely for the production of MEFs.

Abstract No. T07/P086

TDP-43 safeguards pluripotency by regulation of alternative polyadenylation

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Alternative polyadenylation (APA) of RNAs is increasingly being recognized as important posttranscriptional mechanism governing gene dosage that is essential for pluripotent stem cell (PSC) differentiation. Over half of mammalian genes harbor multiple polyadenylation sites that regulate translation and RNA localization. APA-pattern is dynamically regulated during early PSC differentiation and reprogramming. However, the factors regulating APA are still poorly understood. We elucidated a novel TDP-43 function in global regulation of APA, role of APA in paraspeckle formation and its connection to the differentiation of PSCs.

We show that unique subnuclear structures termed paraspeckles are formed independent of lineage type during exit from the pluripotency and are important for stabilization of the early commitment state. The long nonpolyadenylated isoform of non-coding RNA NEAT1 serves as the scaffold for paraspeckles assembly produced only upon differentiation. Undifferentiated PSCs express only the short NEAT1 isoform that doesn't polymerize paraspeckles. Importantly, we present evidence that TDP-43 maintains production of nucleoplasmic NEAT1 short isoform by directly regulating APA of nascent NEAT1 transcripts in undifferentiated cells, and that TDP-43 itself is sequestered in paraspeckles upon differentiation leading to formation of NEAT1 long isoform and paraspeckle assembly. This indicates the existence of a feed-forward feedback mechanism underlying paraspeckle assembly during onset of differentiation. Furthermore, we analyzed how paraspeckle assembly regulates exit from pluripotency; we show that cells lacking NEAT1 and paraspeckle proteins exhibit severe developmental delay. Together, this work implicates developmental regulation of APA in RNA-mediated nuclear restructuring leading to stabilization of the early differentiation state and pluripotency breakdown.

Abstract No. P087

A modular open platform for systematic functional studies under physiological conditions

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Any profound comprehension of gene function requires detailed information about the subcellular localization, molecular interactions and spatio-temporal dynamics of gene products. We developed a novel strategy for genome engineering based on a CRISPR/Cas assisted in-frame insertion of an *attP site*, which we refer to as the multifunctional integrase (MIN) tag. At the genetic level, the MIN-tag serves as an attachment site for the serine integrase Bxb1 that can be used to introduce a broad range of prefabricated functional cassettes into the genomic locus with high specificity and efficiency. At the protein level, the MIN-tag functions as a novel epitope tag that can be detected with a highly specific monoclonal antibody and used for immunoprecipitation as well as immunofluorescence experiments. To demonstrate the versatility of the strategy, we generated MIN-tagged murine embryonic stem cell (mESC) lines for a variety of major epigenetic factors, including Dnmt1, *Dnmt3a*, *Dnmt3b*, *Tet1*, *Tet2*, *Tet3* and *Uhrf1*. We created a toolbox of vectors for Bxb1-mediated recombina- tion to generate isogenic cell lines harboring knockout cassettes, fluorescent protein fusions, enzymatic tags and specific mutations; all derived from a single entry cell line en- suring maximal biological comparability. We demonstrate the power of this strategy using proximity-dependent protein labeling to identify novel interactors of TET1 in mESCs as well as to systematically study the subcellular localization, binding kinetics and protein expression dynamics of the *de novo* methyltransferase DNMT3B during epiblast differentiation.

Mulholland, CB, Smets, M, Schmidtmann, E, Leidescher, S, Markaki, Y, Hofweber, M, Qin, W, Manzo, M, Kremmer, E, Thanisch, K, et al. (2015). A modular open platform for systematic functional studies under physiological conditions. Nucleic Acids Res.

Abstract No. P088

Metabolic implications of retinoid-sequestering blue fluorescent lipid bodies in 'primed'pluripotent stemcells

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We had established a novel blue fluorescence as a non-invasive endogenous marker characteristic of 'primed' pluripotent stem cells. The blue fluorescence shows high correlation with the expression of pluripotency markers and also appears very early during reprogramming. The fluorescence is localized to perinuclear cytoplasmic lipid bodies or droplets which sequester retinoids. This property was also used to flow-sort pluripotent stem cells from differentiated cells, with high efficiency[1].

Multiple studies indicate that 'naïve' and 'primed' pluripotent stem cells exist in different metabolic states. Since the lipid bodies and retinol are involved in energy metabolism and affect cellular metabolic states, we hypothesize that lipid bodies in stem cells could reflect the metabolic state of pluripotent stem cells. We have observed that the presence of lipid bodies is influenced by the presence of retinol or retinyl esters and lipids in the culture media. In defined media lacking retinol such as E8, pluripotent stem cells do not have the lipid bodies. Interestingly, addition of exogenous retinol or retinyl esters causes the lipid bodies to appear, allowing us to establish pluripotent stem cell states with and without the lipid bodies. The appearance of these lipid bodies indicate changes in lipid biosynthesis, which is tightly linked to mitochondrial metabolism. Hence, we have sought to look for differences in various aspects of basal, glycolytic and mitochondrial metabolism in pluripotent stem cells with and without lipid bodies. Additionally, in these cells we are also examining the reactive oxygen species (ROS) levels and mitochondrial potentials.

Our results should contribute to a better understanding of the novel differences between the 'primed' and 'naïve' pluripotent stem cells. These differences indicate the existence of inherently different metabolic pathways which underlie the biology of naïve and primed pluripotent stem cells.

Thangaselvam Muthusamy, Odity Mukherjee, et al. A Method to Identify and Isolate Pluripotent Human Stem Cells and Mouse Epiblast Stem Cells Using Lipid Body-Associated Retinyl Ester Fluorescence. Stem Cell Reports 3, 169–184 (2014).

Abstract No. P089

Screening of cell-type-specific histone-bound long noncoding RNAs

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A recent study demonstrated that some lncRNAs bind specifically to chromatin to control its compactness (Mondal, 2010). Existing evidence suggests that such lncRNAs can regulate gene expression in cis or trans by modulating chromatin structure to influence epigenetic changes (e.g. histone modifications) in the mammalian genome (Caudron-Herger, 2011). LncRNAs have also been shown to play an important role in the pluripotency and differentiation of ES cells by regulating the chromatin structure by interacting with chromatin-modifying complexes (Dinger, 2008). Given that the expressions of lncRNAs are known to be tissue- and cell-type-specific, we performed screening of chromatin-bound lncRNAs using RNA immunoprecipitation (RIP) assay. The RIP samples from various tissues and cell types were analyzed by Affymetrix exon arrays (RIP-Chip) using our web interface "noncoder" (http://noncoder.mpi-bn.mpg.de) to identify tissue/cell-type-specific chromatin-bound lncRNAs. By combining RIP-Chip data, we uncovered that although the components of chromatin (e.g. histones) are the same from one cell type to another, those that bind to them for their integrity differ among cell types. In order to prove the cellular functionality of chromatin-bound-lncRNAs, loss-of-function studies were conducted to understand how chromatin-bound lncRNAs control the accessibility of chromatin for transcription.

References:
[1] Mondal T, Rasmussen M, Pandey GK, Isaksson A, Kanduri C (2010) Characterization of the RNA content of chromatin, Genome Res. 20: 899-907.
[2] Caudron-Herger M, Müller-Ott K, Mallm J, Marth C, Schmidt U, Fejes-Tóth K, Rippe K (2011) Coding RNAs with a non-coding function: maintenance of open chromatin structure, Nucleus 2:5, 410-424.
[3] Dinger ME, Amaral PP, Mercer TR, Pang KC, Bruce SJ, Gardiner BB, AskarianAmiri ME, Ru K, Solda G, Simons C (2008) Long noncoding RNAs in mouse embryonic stem cell pluripotency and differentiation, Genome Res.18: 1433–1445.

Abstract No. P090

The immunoproteasome complex – a superior gatekeeper of protein homeostasis in human pluripotent stem cells.

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Quality control is one of the most important processes in a cell. An instrumental part of a control center which orchestrates critical aspects of basic cellular functions (e.g. cell cycle regulation, differentiation, cellular response to stress, protein homeostasis) is the ubiquitin-proteasome system (UPS). The proteolytic machinery is responsible for intra-cellular protein destruction and is active either in all eukaryotic cells by the constitutive proteasome complex (cProt) or in cells with hematopoietic origin by the immunoproteasome complex (iProt). Interestingly, human pluripotent stem cells possess the catalytically active subunits of the immunoproteasome.

First, the existence of the inducible subunits LMP2 and LMP7, which anchor the proteolytic active sites of the iProt, in human embryonic (H1) and fetal foreskin fibroblast-derived induced pluripotent (B4) stem cells is demonstrated. Second, blocking the distinct proteasomal substrates with MG132 resulted in similar results as the specific differentiation towards ectodermal lineage: down-regulated expression of (i) iProt subunits (LMP2, LMP7), (ii) pluripotency-associated transcription factors (e.g. OCT4) and (iii) activation of cProt subunits (beta1/2/5) and de-ubiquitinating enzyme PSMD14/Rpn11 - propel the loss of self-renewal. Additionally, our data supports the recently published observation of the existence of the proteasome non-ATPase subunit PSMD11/Rpn6 in pluripotent stem cells and its loss upon differentiation. The transcriptome analysis of both ‘events’ (proteasome inhibition and ectodermal differentiation) revealed a broad spectrum of molecular cascades involving cellular processes like cell differentiation, nuclear division, cell-cell adhesion and stress response. Interestingly the expression levels of several genes involved in neural development and regulation were modulated upon proteasome inhibition.

We are currently attempting to understand the function of the UPS in pluripotency. Thus our data illuminates the pivotal role of the inducible proteolytic machinery in the maintenance of self-renewal. So far, it emerges that pluripotent stem cells are inclined to choose the immunoproteasome as the gatekeeper of protein homeostasis.

Abstract No. P091

Loss of Myc Activity Induces Cellular Dormancy in Embryonic Stem Cells Mimicking the Status of Diapause Embryos

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Mouse embryonic stem cells (ESCs) can be maintained in a naïve state of pluripotency by culture in the presence of LIF as well as MAPK and GSK3b inhibitors (2i). To genetically address the role of c-Myc and N-Myc in naïve ESCs, both genes were deleted by using Cre-mediated recombination. Myc double knockout (dKO) cells stop proliferating without signs of apoptosis. Upon deletion, dKO ESCs form small, undifferentiated colonies maintaining the expression of Oct4, Nanog and Sox2. Whole transcriptome analysis (RNA-seq) confirmed the expression of the core pluripotency network in dKO ESCs, but revealed down-regulation of most metabolic and biosynthetic aspects of cellular physiology, as 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 “biosynthetic dormancy”. Strikingly, we find that the signature of dKO ESCs is remarkably similar to the expression signature of diapauses arrested pre-implantation embryos. In mice, reversible diapause of early embryos is observed in mothers still feeding a litter, as a strategy to improve the reproductive fitness. Very low expression of networks such as DNA synthesis, cell division, metabolic activity is observed in both diapause embryos and Myc dKO ESCs. In summary, we show that in the context of naïve ESCs Myc is a master regulator of cellular biosynthesis. During the self-renewal process Myc controls cellular proliferation while the maintenance of stem cell fate is independent on Myc activity. Moreover, our data raise the possibility that Myc activity controls the reversible arrest of diapause embryos.

Abstract No. P092

Implementation of a pluripotent stem cell nomenclature in the Human Pluripotent Stem Cell Registry hPSCreg

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hPSCreg (http://hpscreg.eu) is a freely accessible global registry for human pluripotent stem cell (PSC) lines. In the last decade, the number of human embryonic stem cells (ESC) has steadily increased to over 2000 and new human induced pluripotent stem cell (iPSC) lines are generated in numerous laboratories. Large pluripotent stem cell (PSC) banks are currently under development to make many of the human ESC and iPSC lines widely accessible.

It is essential for applications of these lines to have comprehensive and standardized scientific information available, such as evidence for pluripotency, genetic background, donor phenotype and disease status. In addition, it is important to know which ethical standards were applied for their sourcing and establishment, and what standards apply for data protection. hPSCreg provides unambiguous provision of scientific as well as ethical information on each registered cell line. Moreover, hPSCreg is the only registry, which centrally delivers information on lines from multiple providers and banks facilitating effective use of the available resources.

The lack of a standardized nomenclature for PSC-lines and the use of random identifiers leads to chance duplication of names, confusion and errors in using names in the literature, uncertainty with regards to cell lines used in specific studies and creates difficulties in comparative analysis. Additionally, semantic errors, for example by inaccurate spelling or variable use of special characters or spaces, may lead to errors in literature searches, which are further inherited in references, registries and other databanks. In an attempt to solve these issues hPSCreg developed a standardized and informative nomenclature for PSC-lines. This nomenclature has been adopted by the European bank for induced pluripotent stem cells (EBiSC, www.ebisc.org) and WiCell (wicell.gov). A graphical interface is provided to automatically generate a name for a line. Furthermore, hPSCreg provides an API to assign names for large numbers of cells.

Abstract No. P093

Development of a Two Part System Providing Enhanced Post-Thaw Recovery of PSCs & Reducing Cellular Stress Associated with Broad Range of Applications

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Pluripotent stem cells (PSCs) and primary cells are foundational tools for basic research and applied applications including regenerative therapy, drug discovery, and toxicological assessment.

While stem cells have a tremendous proliferative capacity, long term culture of these cells has been shown to cause an accumulation of mutations that result in genetic instability, increasing tumorigenicity and thus limiting their usefulness in research and clinical applications. Improved solutions to reduce stress on these cells would provide multiple benefits including enhanced cryopreservation and post-thaw recovery, as well as providing support during manipulation of cells in diverse workflows including high throughput screening and gene editing. By using a combination of small molecule library screens, Design of Experiments (DOE) and mathematical modeling methods we developed optimized solutions for cryopreservation and recovery of PSCs passaged as clumps or single cells. Here we describe the utility of a two part system including a xeno-free cryomedium and animal-origin free recovery supplement that is composed of a pro-survival small molecule coupled with antioxidants and free radical scavengers.

SelectScreen® Kinase Profiling of the pro-survival small molecule contained within this cocktail indicates a higher degree of specificity of this ROCK inhibitor over traditional ROCK inhibitors, Y-27632 and Thiazovivin. This system provides >80% direct post-thaw viability of PSCs with >70% cell survival following 24 hours post-plating. The recovery supplement also enables single cell passaging, maintaining normal morphology, pluripotency, and karyotype for up to 30 passages. Additional utility of the recovery supplement includes improved recovery over Y-27632 for PSC cryopreserved in alternative cryomedia, recovery of cells post-electroporation, and recovery of cryopreserved normal primary cells. Taken together, these data demonstrate the robustness and versatility of this system in minimizing cellular stress and maintaining cell health and viability in a broad range of applications.

P094 – P107 Programing and reprograming

P094 miR-1/133a suppress cardiomyocyte dedifferentiation and proliferation in the adult mammalian heart
Johannes Besser

P095 Identification and characterization of Oct4 inducing compounds discovered by cell-based HTS
Xinlai Cheng

P096 Development of a microscope-based in-process control for the automated propagation of hiPSCs on the StemCellFactory
Andreas Elanzew

P097 Generation of clinically relevant iPS cell lines from human peripheral and cord blood derived endothelial progenitor cells using self-replicative RNA.
Brad Hamilton

P098 A xeno- and serum-free media formulation that supports rapid and efficient mRNA reprogramming of human fibroblasts into induced pluripotent stem cells
Hannah Johannsen

P099 Patient-specific iPSC-derived hepatocyte-like cells generated from archived PBMC samples
Mandy Kleinsorge

P100 Backward and forward programming of cardiomyocytes is an oncostatin M mediated survival mechanism
Thomas Kubin

P101 Reprogramming somatic cells – Evaluation of two different methods for the production of human induced pluripotent stem cells
Nina Kühn

P102 Novel tools improve the workflow for generation, purification & analysis of pluripotent stem cell-derived cardiomyocytes
Kristin Noack

P103 Efficient reprogramming of human fibroblasts and blood-derived endothelial progenitor cells with non-modified RNA for reprogramming and immune evasion
Marco A. Poleganov

P104 Stepwise Clearance of Repressive Roadblocks Drives Cardiac Induction in Human ES Cells
Boris Greber

T40/P105 Reprogramming triggers endogenous LINE-1, Alu and SVA retrotransposition in human induced pluripotent stem cells affecting host gene expression
Gerald Schumann

P106 *Ex vivo* and *in vivo* Pdx-1 mediated transprogramming of liver cells into insulin producing cells
Viola Stückemann

P107 Characterization of ABC transporters in human induced pluripotent stem cells derived from blood
Ágota Apáti

Abstract No. P094

miR-1/133a suppress cardiomyocyte dedifferentiation and proliferation in the adult mammalian heart

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Adult mammalian cardiomyocytes are characterized by a limited proliferative potential contributing to the low regenerative capacity of the heart. This is in contrast to the high capability of embryonic and fetal cardiomyocytes to proliferate that diminishes shortly after birth. On the other hand it is known that there is a low turnover of adult cardiomyocytes that may result from proliferation and differentiation of progenitor cells or by proliferation of existing cardiomyocytes with or without prior dedifferentiation. Here, we investigate posttranscriptional mechanisms modulating the differentiation state of cardiomyocytes including re-expression of known stem cell markers and cell cycle re-entry in adult cardiomyocytes.

The FGFR and OSMR pathways are known to control the differentiation state of cardiomyocytes. Interestingly, both regulatory pathways are posttranscriptionally repressed by the miRNAs miR-1/133a in postnatal cardiomyocytes. Release of this repression by deletion of miR-1/133a allows not only dedifferentiation of adult cardiomyocytes but also cell cycle re-entry. In addition, these miRNAs directly control the expression of stem cell specific genes.

Cell cycle re-entry of dedifferentiated cardiomyocytes potentially enables a proliferative response to replace lost cardiomyocytes and regenerate the heart. On the contrary, downregulation of contractile proteins and disarrangement of sarcomeres will reduce contractility of the heart and induce adverse cardiac remodeling when dedifferentiation is widespread and temporarily unrestricted. Therefore, any potential therapeutic intervention to induce cardiomyocyte dedifferentiation and proliferation needs to be controlled tightly to avoid negative effects. Formation of new cardiomyocytes has to occur in a timely and spatially restricted manner to maintain tissue integrity and guarantee continued contractility of the heart.

This work raises the possibility that the miR-1/133a-OSMR-pathway can be manipulated in the adult heart to stimulate an efficient proliferative response of the myocardium.

Abstract No. P095

Identification and characterization of Oct4 inducing compounds discovered by cell-based HTS

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The seminar finding that somatic cells can be dedifferentiated into a pluripotent status, termed iPSCs, generally induced by viral overpression of defined transcriptional factors, namely Oct4, Sox2, Klf4 and c-Myc, provides a promising alternative to ethically controversial (human) ESCs in regeneration medicine. Transfection and transduction based protocols suffer from several limitation, like very low efficiency, genomic instability and result in genetically heterogeneous cell populations. A lot of effort has been put into developing improved approaches to produce clinic-grade iPSCs, so far with limited success. Small molecules-based chemical reprogramming may provide several advantages, including rapid cellular response, convenient concentration control and less genetic toxicity, and thus may be emerging as one of the most favorable methods for iPSCs generation. In a collaborative project with other research groups we performed cell-based high throughput screening (HTS) and identified several Oct4 inducing (O4I) compounds. Two lead structures, 2-(4-(4-methoxybenzyloxy)phenyl)acetonitril and ethyl 2-(4-chlorophenylamino)thiazole-4-carboxylate, were cautiously selected and re-synthesized independently on the screening library. Both compounds showed time- and concentration-dependent activity and were named respectively O4I1 (1) and O4I2 (2). Further structure-activity-relationship study and molecular biological analyses revealed that the lead structures and derivatives not only promoted Oct4 expression at transcriptional level, detected by qRT-PCR and Oct4-promoter driven reporter assay, but also at translational level in immunoblotting and Oct4-response element driven reporter assays. Both compounds clearly switched on the Oct4-associated pluripotentcy signaling network in fibroblasts. In combination with other published, reprogramming relevant small molecules, like TGF-ß, HDAC, and GSK3-ß inhibitors, significant morphological changes and alkaline phosphatase activation have been observed without any genetic manipulation, suggesting that intrinsic cellular reprogramming-associated signaling is at least transiently stimulated. Further optimization of chemical reprogramming is still required before successfully chemical reprogramming can be achieved in the near future.

Cheng, X. et al. Identification of 2-[4-[(4-Methoxyphenyl)methoxy]-phenyl]acetonitrile and Derivatives as Potent Oct3/4 Inducers. J. Med. Chem. 2015, 58, 4976-83
Cheng, X. et al. Ethyl 2-((4-chlorophenyl)amino)thiazole-4-carboxylate and derivatives are potent inducers of Oct3/4. J. Med. Chem. Just accepted 2015.

Abstract No. P096

Development of a microscope-based in-process control for the automated propagation of hiPSCs on the StemCellFactory

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The biomedical prospects of human induced pluripotent stem cells (hiPSCs) have created an urgent need for standardized and automated processes for reprogramming and expansion of hiPSC lines from large patient cohorts. This need can be met by the StemCellFactory (www.stemcellfactory.de), a large system integration that automates all required cell culture steps, ranging from adult human dermal fibroblast (HF) expansion via feeder-free, Sendai virus-based reprogramming to clonal selection and enzyme-free expansion of the obtained hiPSC clones and lines. In this context, microscopic analysis of cells is vital for cell culture process control and decision-making.

In order to develop a fully automated sub-cultivation process for hiPSC we used an automated imaging platform (CellaVista) to determine cell confluence and schedule automated cell passaging. For hiPSC generation and cultivation well-based and plate-based splitting methods were developed to enable clonal expansion and scaled production of hiPSCs in 24- and 6-well plates, respectively. For clonal expansion of hiPSCs in 24-well plates individual splitting ratios were determined per well and implemented in a fully automated liquid handling procedure. Biological validation revealed that 1.5-times more hiPSC clones are preserved by the implementation of individual well-based splitting ratios in comparison to uniform plate–based splitting ratios during fully automated serial passaging (10 passages). In order to further expand quality controlled individual hiPSC clones liquid handling methods for the propagation and passaging of hiPSC lines in 6-well plates were established. Harvesting efficiencies (95.6% ± 2.9%) and cell viability (92.0% ± 2.5%) were similar to manual controls, and whole-plate confluence measurement was implemented to automatically determine splitting ratios per plate. In summary we showed that dynamic feedback through generation and analysis of in-process data is key to improve the stability of the system and to automate highly dynamic cell culture processes.

Abstract No. P097

Generation of clinically relevant iPS cell lines from human peripheral and cord blood derived endothelial progenitor cells using self-replicative RNA.

Sarah Eminli-Meissner[1], *Jung-Il Moon*[1], *Kevin Yi*[1], *Fedir Kiskin*[2], *Baraa Kwieder*[2], *C-Hong Chang*[2], *Amer Rana*[2], and *Brad Hamilton*[1,*]

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In 2010, it was first published that repeated transfection of fibroblasts with a cocktail of reprogramming mRNAs resulted in the generation of stable, integration-free human iPS cells. While many advancements have been made to refine this process on fibroblasts, to date no group has been able to apply this same technology to reprogram a blood derived cell type. Peripheral blood provides easy access to adult human cell types for reprogramming purposes. Notably, blood-outgrowth endothelial progenitor cells (EPCs) can be clonally isolated from both fresh and frozen human peripheral blood and cord blood. The EPCs adherent nature and high proliferative capacity while maintaining their cell identity makes them highly desirable for transfection with RNA.

In 2013, published results demonstrated the reprogramming of human neonatal fibroblasts into iPS cells using self-replicative RNA (srRNA), with as few as one transfection. Subsequently, we have extended the application of srRNA for cellular reprogramming to peripheral and cord blood derived EPCs as well as adult fibroblasts. The generation of RNA-EPC-iPS cell lines required optimization of mRNA delivery, culture media composition and transitions, as well as incorporation of reprogramming associated microRNAs. These improvements resulted in a simple and robust two transfection, no-split protocol on extracellular matrix without the need for conditioned medium. These integration-free EPC-derived iPS cell line exhibit unique genetic stability, making them an exceptional choice for applications requiring clinical grade cells. Lastly, those clinical grade EPC-iPS cells generated using this novel srRNA reprogramming technology present a therapeutic opportunity to treat myeloproliferative disorders in which the disease-causing somatic mutations are restricted to cells in the hematopoietic lineage.

Here we present data demonstrating the unique combined application of microRNA and srRNA for the cellular reprogramming of human EPC lines derived from human blood as well as adult fibroblasts into stable, pluripotent and clinically relevant iPS cells.

Abstract No. P098

A xeno- and serum-free media formulation that supports rapid and efficient mRNA reprogramming of human fibroblasts into induced pluripotent stem cells

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Human induced pluripotent stem cells (iPSC) play an important role in disease modelling and drug discovery. Moreover, they hold great promise for future clinical applications, especially due to their potential usage in autologous treatments. Nonetheless, there are still some obstacles to overcome when generating clinical grade iPSC. Cellular reprogramming with mRNA is considered to be the most straightforward method for generation of high quality iPSC, since the procedure eliminates the risk of genomic integration as observed for DNA-based methods or extended reprogramming factor replication as reported for the Sendai-virus system. Furthermore, it is one of the fastest and most productive reprogramming technologies available today. Importantly, the future clinical use calls for protocols and cultivation media that are compliant with GMP cell production. To meet this demand, we have developed a xeno- and serum-free media formulation for mRNA-based reprogramming. Our media supports rapid and efficient reprogramming of human fibroblasts into iPSC within 12-16 days using a 6 factor mRNA reprogramming cocktail (Oct4, Sox2, Klf4, c-Myc, Nanog, Lin28). Reprogramming is done under non-hypoxic, feeder-free conditions without the need of conditioned medium, and compatible with various cultivation matrices thereby enabling a safe and convenient way for generating iPSC. Human fibroblasts reprogrammed in this medium acquire typical stem cell morphology and marker expression as assessed by flow cytometry analyses. Moreover, resulting iPSC readily differentiate into the three germ layers endoderm, mesoderm and ectoderm using directed monolayer differentiation protocols.

In summary, our new media formulation supports mRNA based reprogramming of human fibroblasts under feeder- and xeno-free conditions. Notably, early results indicate that the media also supports reprogramming of renal epithelial cells (REC).

Abstract No. P099

Patient-specific iPSC-derived hepatocyte-like cells generated from archived PBMC samples

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The reprogramming of somatic human cells to induced pluripotent stem cells offers great possibilities for regenerative medicine as well as for disease modeling and drug screening purposes. This is due to the capability of iPS cells to be both expanded virtually indefinitely and differentiated towards any cell type possible, including cell types relevant for certain diseases. Directed differentiation towards hepatocyte-like cells might be of particular importance for drug screenings irrespective of the drug's purpose, since the liver serves as the major drug-metabolising organ in the body.

In this study, we intended to establish a facilitated protocol for generating patient-specific hepatocyte-like cells for use in disease modeling and drug screening. Peripheral blood mononuclear cells represent one of the easiest accessible cell sources and can be readily stored. We used small numbers of archived PBMCs as a starting cell source and successfully generated iPS cells by transduction of polycistronic lentiviral vectors encoding codon-optimized cDNAs of *OCT4, SOX2, KLF4,* and *C-MYC*. Here, 500,000 long-term stored PBMCs and as little as 100,000 short-term stored PBMCs proved to be sufficient cell numbers to obtain high quality iPS cell lines. In the derived iPS cell clones, pluripotency characteristics, like expression of SSEA4, TRA1-60, OCT4, SOX2 and NANOG, could be observed. The differentiation potential of the iPS cells towards all three embryonic germ layers was examined *in vitro* using the ScorecardTM assay. Also, their amenability to hepatic *in vitro* differentiation was confirmed by analysing the expression of hepatic marker genes, such as *ALB, HNF4α, AFP* and *TTR*.

With this, we established a protocol for obtaining patient-specific iPS cells from minimal numbers of easy-access blood cells by making use of frozen PBMC libraries. Upon directed differentiation towards hepatocyte-like cells, these iPSCs can now be used for modeling of liver diseases as well as drug screening.

Abstract No. P100

Backward and forward programming of cardiomyocytes is an oncostatin M mediated survival mechanism

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Oncostatin M (OSM) is a major mediator of cardiac protection through the regulation of evolutionarily conserved programs. This includes the degradation of the oxygen consuming contractile machinery and the reexpression of “immature” cardiomyocyte-specific genes which enables the heart to cope with stress (backward program). Stem cell marker such as c-Kit, Runx1 as well as Dab2 are rapidly re-expressed and sarcomeric proteins such as titin and α/β-myosin heavy chain are degraded. In culture, O2 consumption is massively reduced by OSM through the shutdown of mitochondrial activity and the metabolic performance is maintained by the glycolytic/pentose phosphate pathway even in the presence of sufficient oxygen reminding of the Warburg effect in cancer cells. In addition, cardiomyocytes rapidly reestablish live-saving cell-cell contacts by the formation of cell extensions. However, despite the substantial protective effect of OSM *in vivo* and *in vitro* prolonged backward programming drives cardiomyocytes to a “point of no return” when cells do not respond to environmental hypertrophic clues which finally causes heart failure. Thus, OSM activity has to cease in a certain time frame of cardiac regeneration in order to allow forward programming. Then, hypertrophic factors such as FGFs and IGFs induce the formation of mature sarcomeres and cardiomyocytes regain their contractile properties. In conclusion, short-term application of OSM accelerates backward programming after cardiac damage but prolonged treatment delays beneficial forward programming. Furthermore, the ratio of forward and backward programming factors in the damaged area might determine the therapeutic effectivity of stem cells.

Abstract No. P101

Reprogramming somatic cells – Evaluation of two different methods for the production of human induced pluripotent stem cells

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Human pluripotent stem cells hold great promise in regenerative medicine, cell replacement therapies and “disease in the dish” approaches. Somatic cells can be reprogrammed into induced pluripotent stem cells (iPS) cells by the ectopic expression of transcription factors such as Oct4, Klf4, Sox2 and c-Myc. Standard reprogramming procedure is the use of genome-integrating viruses. Since this approach can cause genetic modifications up to tumor formation alternative reprogramming methods are needed. We compared a non-viral and non-integrating method using minicircle DNA with the standard retroviral transduction.

Human fibroblasts and urine cells were reprogrammed by retroviral transduction with the four factors Oct4, Sox2, cMyc and Klf4 and cultivated with infection medium for 24h. While culturing the infected cells, the specific hiPS-medium was replaced daily. On days 5-10 after retroviral transduction we observed cell clusters with morphological similarities to hiPS cell colonies. After 20 days of culturing, hiPS cell colonies were picked based on their characteristic ES cell-like morphology.

When we used minicircle DNA, which contains the four factors Lin28, Nanog, Sox2 and Oct4, and a GFP reporter for reprogramming, the cells initially expressed GFP, indicating that the cells contained the vectors. However, the GFP level quickly decreased within the following first days. Around 20 days after electroporation, no hiPS cell-like colonies were detectable.

With our cells the retroviral transduction is still the preferable method for reprogramming.

Abstract No. P102

Novel tools improve the workflow for generation, purification & analysis of pluripotent stem cell-derived cardiomyocytes

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Pure and well-characterized cardiac cells derived from human pluripotent stem cells (hPSCs) are of high interest for cardiovascular disease modelling, drug safety studies and regenerative medicine. Although several protocols for cardiac differentiation of hPSCs have been developed, major limitations are clone-to-clone variations in differentiation efficacy and heterogeneity of generated cardiomyocyte (CM) populations. Therefore, we have developed novel tools for cardiomyocyte differentiation, magnetic cell sorting-based purification and cell analysis using new antibody-conjugates enabling for flow cytometry- and immunofluorescence-based identification of cardiomyocytes and subtypes.

hPSCs were maintained under xeno- and feeder-free conditions in StemMACS iPS-Brew XF medium to keep pluripotency for >20 passages. These cells were differentiated to cardiomyocytes with efficacies of up to 80% using a monolayer protocol with consecutive activation (CHIR99021) and inhibition (IWR-1) of Wnt signalling. In this setting, first contractions were observed at day 9 of differentiation. In order to identify antibodies suitable for cardiomyocyte enrichment or depletion of non-myocytes, we performed a surface marker screen with >400 antibodies. Besides identification of new surface markers, our screen confirmed expression of published markers like Sirpα (CD172a) and VCAM-1 (CD106). Their expression and correlation with intracellular markers of cardiomyocytes was analysed on low and high efficiency differentiations using flow cytometry. Our data indicate a dynamic expression pattern for both Sirpα and VCAM-1, either completely or partially overlapping with intracellular cardiomyocyte marker expression. Based on these data we developed magnetic cell separation procedures for the isolation of cardiomyocytes. Magnetically enriched cardiomyocytes showed a typical morphology and sarcomeric structure. Moreover they initiated contractions after replating and could be stably maintained in culture.

Taken together, we have developed novel tools supporting the workflow for efficient generation, purification and antibody-based characterization of PSC-derived cardiomyocytes.

Lian, X. et al. (2013) Nat. Protoc. 8:162-172

Abstract No. P103

Efficient reprogramming of human fibroblasts and blood-derived endothelial progenitor cells with non-modified RNA for reprogramming and immune evasion

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mRNA-based reprogramming has the potential to overcome many limitations concerning the use of induced pluripotent stem (iPS) cells for clinical applications by circumventing the risk of possible genomic alterations. Further, previously published mRNA reprogramming protocols have proven to be inconsistent, time consuming, and mainly restricted to fibroblasts thereby demonstrating the need for a simple, but reproducible protocol applicable to various cell types. Especially peripheral blood provides easy access to adult human cell types for reprogramming purposes. Nevertheless, so far there have been no published reports using mRNA to reprogram any human blood-derived cell type. Non-modified synthetic mRNAs are immunogenic and activate cellular defense mechanisms, which can lead to cell death and inhibit mRNA translation upon repetitive transfection. Hence to overcome RNA related toxicity we combined non-modified reprogramming mRNAs [OCT4, SOX2, KLF4, cMYC, NANOG and LIN28 (OSKMNL)] with immune evasion mRNAs [E3, K3 and B18R (EKB)] from Vaccinia virus (VACV). Additionally we included mature, double stranded microRNAs (miRNAs) from the 302/367 cluster, which are known to enhance the reprogramming process, to develop a robust reprogramming protocol for the generation of stable iPS cell lines from both human neonatal and adult fibroblasts and human endothelial progenitor cells (EPCs). EPCs are a rare, circulating population of cells that can be clonally isolated from both fresh and frozen human peripheral blood and cord blood samples. Our novel combination of RNAs enables the cell to tolerate repetitive transfections for the generation of stable iPS cell lines from human fibroblasts within 11 days while requiring only four transfections. Moreover, our method resulted in the first known mRNA-vectored reprogramming of human blood-derived EPCs within 10 days while requiring only eight daily transfections.

Abstract No. P104

Stepwise Clearance of Repressive Roadblocks Drives Cardiac Induction in Human ES Cells

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Human embryonic stem cells can be used to investigate early stages of human development. Here, we employ this system to reveal key principles underlying the formation of heart muscle cells. A stepwise manipulation of two signaling cascades, the BMP and WNT pathways, was necessary and sufficient to promote homogeneous differentiation into cardiomyocytes. As a first step, BMP and WNT activities collaborated closely, in a dose-dependent manner, to promote the induction of mesoderm (proposed cardiac corridor model). This cooperativity was based on repressing the mesodermal antagonist SOX2 through complementary mechanisms. At the mesoderm stage, WNT signaling inhibition was required for promoting subsequent cardiac induction. Unexpectedly, this pro-cardiac effect was based on restricting the expression of anti-cardiac regulators such as MSX1 and CDX2. We propose that a two-step derepression of cell state transitions drives human cardiac induction, which may have evolutionary conserved validity.

Abstract No. T40/P105

Reprogramming triggers endogenous LINE-1, Alu and SVA retrotransposition in human induced pluripotent stem cells affecting host gene expression

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Human induced pluripotent stem cells (hiPSCs) are capable of unlimited proliferation, can differentiate into every cell type of the adult body, and their use holds great therapeutic promise for regenerative medicine. However, reprogramming and subsequent cultivation of hiPSCs can induce genetic and epigenetic abnormalities that can result in tumorigenic iPSCs. Thus, it is unclear if iPSCs or their derivatives are safe for administration. Genomic mutations may undermine their use in regenerative medicine. Activation of the human endogenous mobile retrotransposons LINE-1 (Long Interspersed Element-1, L1), Alu and SVA can cause such mutations. In differentiated cells, L1 is primarily suppressed by methylation of its CpG-rich promoter, but reprogramming triggers L1 transcription.

To investigate if the observed L1 activation in pluripotent stem cells leads to L1-mediated mobilization, we applied retrotransposon capture-sequencing (RC-seq) to multiple hiPSC lines, their parental cells and human embryonic stem cell (hESC) lines. We identified, mapped and validated individual L1, Alu and SVA de novo retrotransposition events that occurred during reprogramming into iPSCs and cultivation of hiPSCs and hESCs. We calculated that each hiPSC carried ~4 L1 de novo insertions that occurred during reprogramming or hiPSC cultivation. As ~50% of all de novo retrotransposition events occurred in protein-coding genes actively transcribed in hiPSCs, including genes playing roles in oncogenesis or development, we investigated effects of these intronic de novo insertions on host gene expression in hiPSCs. To exemplify consequences of even short intronic L1 de novo insertions for host gene expression, we analyzed the effects of a 390-bp L1 de novo insertion in CADPS2 intron 23 on CADPS2 transcription in hiPSCs. We specified the period during hiPSC cultivation when this de novo insertion occurred. Our experiments demonstrate incidence and potential functional impact of reprogramming-activated endogenous retrotransposition in hiPSCs and imply consequences for the biosafety of hiPSC-derived cell therapies.

Abstract No. P106

Ex vivo and *in vivo* Pdx-1 mediated transprogramming of liver cells into insulin producing cells

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Background: In type 1 diabetes mellitus the insulin producing pancreatic beta cells are destroyed resulting in hyperglycemia, which then causes various long-term complications. Replacement with autologous insulin producing cells would be the ideal therapy. It has been shown that liver cells can be transdifferentiated into beta-like cells, but efficiency of transdifferentiation was low and regulation of blood glucose was limited.

Aims: Improve transdifferentiation of human liver cells into beta-like cells by identification of more efficient combinations of transcription factors and their application *in vitro* and *in vivo*.

Methods: *In vitro* transdifferentiation: Primary human liver cells were transduced with lentiviral vectors carrying the transcription factors Pdx1, Ngn3 and MafA in different combinations. After 9 days the cells were analyzed for insulin secretion and expression of beta cell markers.

In vivo transdifferentiation: NSG mice with streptozotocin-induced diabetes were injected with an adenoviral vector carrying Pdx1. The blood glucose level of mice was monitored for several weeks, and finally insulin levels in the blood were determined and liver tissue was analyzed for expression of insulin and other beta cell markers.

Results and Conclusions*: In vitro* transdifferentiation: The cells did not express insulin, though activation of rat insulin promotor was observed. Possibly, acetylation of genomic DNA prevents transcription. Acetylation can be prevented by application of histone-deacetylase inhibitors. It is also possible that complete transdifferentiation only happens *in vivo* after transplantation.

In vivo transdifferentiation: Animals showed expression of insulin and c-peptide in the liver but no insulin secretion, indicating incomplete transdifferentiation leading to expression but no or incomplete processing of preproinsulin/ proinsulin. A lack of prohormone convertases could be a reason for this. Therefore the expression of prohormone convertases and other factors will be analyzed. Additionally adenoviral vectors with other factor combinations will be applied.

Abstract No. P107

Characterization of ABC transporters in human induced pluripotent stem cells derived from blood

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Human ATP-binding Cassette (ABC) transporters have key physiological functions and provide chemical defense in differentiated tissues as well as in various stem cell types. As we have demonstrated earlier, the activity of ATP-binding Cassette (ABC) transporters in human embryonic stem cells may significantly contribute to stem cell defense mechanisms (1). Moreover, we found that the mRNA expression levels of the ABC proteins showed characteristic patterns in the pluripotent and the respective, differentiated cell types (2). In the present work we investigated the expression of several important ABC proteins in human blood-derived induced pluripotent stem cells (hiPSC) and in their differentiated offspring. hiPSCs were generated from mononuclear cells, using a Cytotune 2.0 reprogramming kit, then we established several iPCS clones, and the pluripotency of the clones was analyzed by alkaline phosphatase staining, qPCR, and immunofluorescence staining of specific markers. The ABC transporter expression profiles were studied by flow cytometry, fluorescence microscopy, and qPCR. The cellular localization of certain ABC transporters has been established in the pluripotent cell samples and differentiated cell types by using well-characterized specific antibodies in flow cytometry and confocal microscopy. These studies provide further information regarding tissue-specific ABC protein expression and localization, and the results may help to obtain further information concerning the specialized functions of selected ABC transporters in human pluripotent stem cells. This work has been supported by the Hungarian Brain Research Program [KTIA VKSZ_12, NAP-A-1.10].

1. Erdei, Z, Sarkadi, B, Brozik, A, Szebenyi, K, Varady, G, Mako, V, Pentek, A, Orban, TI, and Apati, A. Dynamic ABCG2 expression in human embryonic stem cells provides the basis for stress response. Eur Biophys J 42: 169-79 (2012).

2. Erdei Z et al. Expression pattern of the human ABC transporters in pluripotent embryonic stem cells and in their derivatives. Cytometry B Clin Cytom. 86(5):299-310 (2014)

P108 – P113 Somatic stem cells

P108 An injury to spinal cord activates neurogenesis related stress buffering
Sascha Dehler

P109 The ageing subventricular zone
Georgios Kalamakis

P110 Single cell transcriptomics reveals dormant neural stem cells that respond to brain injury
Enric Llorens Bobadilla

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Giuseppe Militello

P112 Characterization and banking of human third trimester amniotic fluid-derived mesenchymal stem cells obtained from cesarean sections
Lucas-Sebastian Spitzhorn

P113 Teads function During skeletal muscle regenration.
Shuichi Watanabe

Abstract No. P108

An injury to spinal cord activates neurogenesis related stress buffering

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Adult neurogenesis describes the process of neural birth, differentiation, migration, maturation and finally the correct integration into the existing network of neurons during adulthood. The process of adult neurogenesis is based on the existence of neural stem cells (NSCs) and is restricted to mainly two brain regions, the subgranular zone (SGZ) within the dentate gyrus of the hippocampus and the subventricular zone (SVZ) of the lateral ventricles. Decades after the discovery of adult hippocampal neurogenesis, many regulatory mechanisms at distinct stages have been resolved. Hippocampal neurogenesis is influenced in various conditions like an enriched environment, running, learning, and injury within the central nervous system (CNS). In the past, studies were predominantly focused on the partial recovery from brain injuries due to trauma, hypoxia or stroke. Here we set off to explore if the process of hippocampal neurogenesis changes within the brain upon spinal cord injury (SCI). Our results suggest that there is an acute increase in the rate of neurogenesis upon SCI, resulting in an enhanced production of new born neurons. Furthermore, we provide evidence that the acute increase of neurogenesis upon SCI results in a beneficial behavior for spinal cord injured animals, namely in a better working memory and in an elevated level of buffering acute stress situations.

Abstract No. P109

The ageing subventricular zone

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The adult rodent brain consists of two predominant neurogenic niches, the subgranular zone of the dentate gyrus of the hippocampus and the subventricular zone (SVZ) of the lateral ventricles (Ihrie and Alvarez-Buylla, 2011). Adult neurogenesis is regulated by a plethora of signals that are not only multidimensional – as they involve multiple cues – but they also vary dynamically with age. In the adult SVZ, neurogenesis is known to decline with aging (Shook et al., 2012) however the age-dependent mechanisms remain largely unexplored. Here, we studied the transcriptome of different cell types of the aged SVZ niche, namely neural stem cells (NSCs), neuroblasts, endothelial and microglial cells. Through detailed cell-specific gene expression analysis in the SVZ niche, our study provides evidence for an age-dependent increase in inflammation, which might subsequently affect adult neurogenesis.

Ihrie, R.A., and A. Alvarez-Buylla. 2011. Lake-Front Property: A Unique Germinal Niche by the Lateral Ventricles of the Adult Brain. Neuron. 70:674–686. doi:10.1016/j.neuron.2011.05.004.
Shook, B.A., D.H. Manz, J.J. Peters, S. Kang, and J.C. Conover. 2012. Spatiotemporal Changes to the Subventricular Zone Stem Cell Pool through Aging. Journal of Neuroscience. 32:6947–6956. doi:10.1523/JNEUROSCI.5987-11.2012.

Abstract No. P110

Single cell transcriptomics reveals dormant neural stem cells that respond to brain injury

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Neural stem cells (NSCs) in the adult SVZ contribute to olfactory homeostasis and regeneration after injury. It is assumed that the balance between quiescence and activation of NSCs and the induction of specific transcriptional programs regulate their diverse output. However, the molecular hallmarks of NSC activation and lineage specification have remained unclear. Here, we sequenced the transcriptome of single NSCs isolated from their *in vivo* niche.

During homeostasis, we identify a previously unrecognized subpopulation of dormant NSCs that already express lineage-priming factors. Transition from the dormant state via a primed-quiescent to an activated state occurs by downregulation of glycolytic metabolism, Notch and BMP signaling and a concomitant upregulation of differentiation factors and the protein synthesis machinery. Following ischemic injury, interferon gamma induces the exit of dormancy into the primed-quiescent state. This study provides general molecular principles of neural stem cell activation and may open new opportunities for regenerative approaches.

Abstract No. P111

An Evolutionary-Conserved Novel Long Noncoding RNA Myolinc Regulates Muscle Differentiation

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Transcriptome of mammalian genomes is very complex, which involves far more number of non-coding RNAs than protein-coding genes. Although the presence of long non-coding RNAs (lncRNAs) has been recognized, their functions are only elucidated for some. Given that several lncRNAs are expressed in the skeletal muscle, it is speculated that lncRNAs might be a missing link to the complete understanding of muscle regeneration. Indeed, it has been shown that an intergenic lncRNA called “linc-MD1” regulates expressions of muscle specific markers and differentiation of myoblasts. In this study, we identified an evolutionary-conserved, myocyte-enriched lincRNA named “Myolinc”. Utilizing gain- and loss-of-function experiments, we provide the evidences that Myolinc is important for the proper differentiation of muscle precursor cells into myotubes *in vitro* and *in vivo*. Through biochemical assays, a DNA/RNA-binding protein was found to bind to Myolinc to control the muscle differentiation originated from MyoD. These findings add the knowledge to the existing understanding of gene regulatory network of skeletal muscle differentiation.

Abstract No. P112

Characterization and banking of human third trimester amniotic fluid-derived mesenchymal stem cells obtained from cesarean sections

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Amniotic fluid (AF) which can be routinely obtained during cesarean sections is a source of stem cells with unique characteristics. First described by De Coppi et al. (2007), these amniotic fluid stem cells are immune-privileged, with low immunogenicity and anti-inflammatory properties thus amenable for transplants. AFMSCs are able to self-renew, have higher proliferation and differentiation potential than adult bone marrow-derived MSCs and cord blood-derived stem cells. Typical of MSCs, they express CD73, CD90 and CD105 and not the haematopoietic markers CD14, CD20, CD34, and CD45. They can differentiate into osteoblasts, chondrocytes and adipocytes. They secrete C5/C5a, GM-CSF, GROα, sICAM-1, IL-6, IL-8, MCP-1, MIF and Serpin E1. A sub-population of bulk cells express typical stem cell markers CD133, C-KIT and pluripotency associated markers OCT4, NANOG, SOX2 and SSEA4. AFMSCs are at an intermediate stage between pluripotent stem cells and multipotent adult stem cells. Our earlier studies demonstrated that AFMSCs can be reprogrammed into induced pluripotent stem cells at high efficiencies with (Wolfrum et al. 2010) and without exogenous Yamanaka factors OCT4, SOX2, KLF4 and C-MYC (Moschidou et al. 2012). Furthermore AFMSCs possess the ability to form sphere-like aggregates under non-adherent conditions. Banking of HLA-matched AFMSCs is an attractive cell source for research and individualized regenerative therapies in the long term.

De Coppi P, Bartsch G Jr, Siddiqui MM, Xu T, Santos CC, Perin L et al. (2007). Nat Biotechnol 25:100–106

Wolfrum K, Wang Y, Prigione A, Sperling K, Lehrach H, Adjaye J (2010). PLoS One.5(10):e13703.

Moschidou D, Mukherjee S, Blundell MP, Drews K, Jones GN, Abdulrazzak H, Nowakowska B, Phoolchund A, Lay K, Ramasamy TS, Cananzi M, Nettersheim D, Sullivan M, Frost J, Moore G, Vermeesch JR, Fisk NM, Thrasher AJ, Atala A, Adjaye J, Schorle H, De Coppi P, Guillot PV (2012). Mol Ther.(10):1953-67.

Abstract No. P113

Teads function during skeletal muscle regeneration.

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The homeostasis of tissue specific stem cell is regulated by certain sets of transcription factors and signaling molecules. Hippo signaling pathway is well known to regulate organ size through the regulation of cell proliferation and apoptosis. A member of the TEA/ATTS domain family, termed Tead1-4 (Teads), is core transcription factor of Hippo signaling and binds to specific DNA sequence known as M-CAT sequences. They are often found in the regulatory region of smooth, skeletal and cardiac muscle specific gene, whereas hippo signaling generally regulates cell proliferation and apoptosis. These facts suggest that different Teads may acquire multiple function and selectively target proliferation, apoptosis or muscle gene regulatory regions through the interaction with their co-regulators. We firstly aim to study individual Teads function during adult muscle regeneration and understand how each Teads acquire their functional specificity in tissue, time and cell type dependent manner. Analysis of myogenic cell specific Teads conditional ablation in mice revealed individual Teads function during muscle regeneration. Tead1 and 3 double mutant muscle precursor cells hardly contributed to self-renew but tended to be differentiated resulting in the exhaustion of myogenic stem cell population. On other hand, skeletal muscle terminal differentiation was inhibited by Tead1 and 4 double mutations. These results support our hypothesis that each individual Teads function is diverse and essential during skeletal muscle regeneration.

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Abstract No. P114

Isolation and scalable culture of Cardiovascular Progenitor Cells (CVPCs) from human Pluripotent Stem Cells

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Cell-based regenerative therapies hold great promise for treatment of heart diseases.

Cardiovascular progenitor cells (CVPCs) may represent a favourable cell type for heart repair as compared to differentiated cardiomyocytes. CVPCs maintain proliferative and might be expandable *in vitro* whilst maintaining their multi-lineage differentiation potential, comprising cardiomyocytes, endothelial cells and smooth muscle cells *in vitro* or following transplantation in situ.

Starting from human pluripotent stem cells we aim to characterize markers and pathways of early stages of cardiovascular commitment to isolate and expand CVPCs.

We have directed cardiomyogenic differentiation in monolayer and suspension culture using Wnt pathway chemical modulators1. To monitor mesendodermal induction, a human embryonic reporter cell line is utilized expressing eGFP under control of the endogenous MIXL1 locus. Following expression of the cardiac mesoderm marker MESP1, the transcription factor ISL1 is upregulated: a well described marker of a cardiac progenitor cell stage, and it is expressed shortly before NKX2.5, which marks the development of committed cardiomyocytes2.

A close interplay between ISL1 and Wnt pathway was reported in the mouse embryo, in which Wnt pathway activation is known to promote expansion of cardiac progenitor cells and ISL1 is necessary for further differentiation of CVPCs.

We hypothesize that the balance between Wnt pathway activation and ISL1 expression could allow us to identify an expandable stage of properly committed CVPCs.

Using transgenic human embryonic reporter line carrying eGFP in the endogenous NKX2.5 we screened for small molecular inhibitors which will eventually allow us to constantly maintain ISL1 expression, avoiding further progression into NKX2.5 expressing cells. Our results provide an important step towards a better characterization of *in vitro*-derived human CVPCs, support the understanding of pathways involved in their commitment, and might enable the long term *in vitro* expansion of CVPCs in larger scale.

Kempf H., Olmer R., Kropp C., Rückert M., Jara-Avaca M., Robles-Diaz D., Zweigerdt R. Controlling expansion and cardiomyogenic differentiation of human pluripotent stem cells in scalable suspension culture (2014). Stem Cell Reports 3, 6: 1132-1146.
Qyang Y, Martin-Puig S, Chiravuri M, Chen S, Xu H, Bu L, Jiang X, Chien KR (2007). The renewal and differentiation of Isl1+ cardiovascular progenitors are controlled by a Wnt/beta-catenin pathway (2007). Cell Stem Cell 1(2):165-179.

Abstract No. P115

The role of SRF in skeletal muscle stem cell

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The serum response factor (SRF) regulates growth factor-inducible genes and genes controlling cytoskeleton structures through its interactions with 2 families of signal-regulated cofactors: the MAP-kinase–regulated ternary complex factors and the G-actin–regulated myocardin-related transcription factors. While the role of SRF in regulating adult stem cell with high turn over rate such as HSC is known, its role in regulating the homeostasis of adult stem cell with low turn over rate but with high regenerative potential for example skeletal muscle stem cell is still elusive. Here we focus on elucidation of the function of SRF in skeletal muscle stem cells by using mouse genetics. We could show ablation of SRF in adult muscle stem cells results in accelerated stem cell differentiation leading to loss of satellite cell pool. Consistently inactivation of SRF impairs muscle regeneration upon acute muscle injury. In the future we are aiming to elucidate the molecular mechanisms underlying SRF mediated satellite cell maintenance and muscle regeneration.

Abstract No. P116

Hepatocyte or cholangiocyte like cells: The fate of human pluripotent stem cell derived hepatoblasts is determined by cell density

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The liver is responsible for metabolic co-ordination and for detoxification of a wide variety of drugs. Cholangiocytes, epithelial cells which line the intra-hepatic bile ducts, are of major importance for the structure and function of the liver. Up to now it is not known exactly how liver regeneration takes place. Residual stem/progenitor cells could differentiate to replace the lost cells, normally quiescent hepatocytes could start proliferating or cholangiocytes could transdifferentiate in order to give rise to functional hepatocytes. Therefore, we decided to investigate the differentiation potential of hepatic progenitor cells in more detail. While there exist several elaborate protocols for the *in vitro* differentiation of human pluripotent stem cells (hPSCs) into hepatocytes, the differentiation into cholangiocytes has been only marginally investigated and the existing protocols are rather complicated. Here we present an easy and straightforward method for the differentiation of hPSCs into bipotential hepatoblasts that have the capability to differentiate into hepatocyte (HLCs) or cholangiocyte (CLCs) like cells. In our protocol the cell fate decision between HLCs and CLCs is made at the stage of the last common progenitor which closely mirrors the *in vivo* situation. It depends highly on cell density and is influenced by NOTCH signalling. Immunostainings and global transcription analyses revealed gene expression patterns characteristic for the two different cell types. HLCs express for example Albumin and HNF4a while CLCs are positive for Osteopontin and CK19. Additionally, GO-analyses revealed that in CLCs pathways connected to channel activity are over-represented, while HLCs show a characteristic enrichment of metabolism and lipid transport associated pathways. Our new protocol enables us to study the differentiation of hepatic cells in a scenario close to the *in vivo* situation.

Abstract No. P117

Impact of early retinoid signalling on morphological and functional characteristics of human induced pluripotent stem cell-derived neurons

Sandra Horschitz[1,*], *Friederike Matthaeus*[1], *Anja Groß*[1], *Jan Rosner*[2], *Marta Galach*[3], *Wolfgang Greffrath*[2], *Rolf-Detlef Treede*[2], *Jochen Utikal*[3], *Patrick Schloss*[1], and *Andreas Meyer-Lindenberg*[1]

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Human induced pluripotent stem cells (hiPSCs) are a suitable tool to study basic molecular and cellular neuronal mechanisms of neurodevelopment. The directed differentiation of hiPSCs via the generation of a self-renewable neuronal precursor cell line supports the standardization of defined differentiation protocols. Here, we have investigated whether retinoid signalling during early neural induction impacts on morphological and functional characteristics of the neuronal culture after terminal differentiation. Therefore we have analyzed neuronal outgrowth, soma size and specific marker protein expression during terminal differentiation. With respect to functional aspects we measured depolarization-induced distal shifts of the axon initial segment as well as glutamate-evoked calcium influx. Our results show that the treatment of neuroectodermal cells with retinoic acid during early development, i.e. during the neurulation phase, influences morphological aspects of the neuronal culture in a later phase of neurodevelopment, i.e. during terminal differentiation, however, does not impact on the functionality of terminally differentiated neuronal cells.

Abstract No. P118

Elucidation of the role of Airn in murine cardiomyocytes

Mohammed Rabiul Hosen[*], *Giuseppe Militello*, *Yuliya Ponomareva*, *Stefanie Dimmeler*, and *Shizuka Uchida*

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The current understanding in the field of transcriptomics is that a majority of the human genome is transcribed, which only a few percentages of them encode for proteins. Those RNAs that do not encode for proteins are collectively called as “non-coding RNAs (ncRNAs)”, which includes well-known ribosomal and transfer RNAs as well as microRNAs (miRs). For those ncRNAs whose lengths are longer than 200 nucleotides (nt) are currently classified under a general category called “long non-coding RNAs (lncRNAs)”.Airn is one of the first lncRNAs to be characterized to date. Airn (antisense Igf2r RNA) is an imprinted gene transcribed from the paternal chromosome and is in antisense orientation to the imprinted but maternally expressed Igf2r gene, which Airn exerts its regulation in cis. Although both Airn and its isoforms are expressed highly in the heart, their functions are not known besides imprinting. Here, we aimed to elucidate the functions of Airn isoforms in the heart using murine cardiomyocytes cell line HL-1 by employing loss-of-function experiments followed by molecular profiling. To understand the mechanism of action, we performed RNA pull down experiments to identify proteins that bind to Airn isoforms. In this conference, we would like to share our preliminary data regarding the functions of Airn isoforms besides imprinting.

Abstract No. P119

Human amniotic membrane – unrealized source of pulmonary surfactant?

Angela Lemke[1,*], *Anton Klotz*[1], *Andrea Lindenmair*[1], *Sylvia Nürnberger*[2], *Simone Hennerbichler*[3], *Heinz Redl*[1], and *Susanne Wolbank*[1]

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Pulmonary surfactant reduces surface tension which prevents the collapse of alveoli and is therefore essential for lung function. It is composed of a lipid-protein complex, amongst others of pulmonary proteins A, B, C and D (SP-A to –D) and is produced by alveolar epithelial type 2 cells (AEC2). All SPs have been found in amniotic fluid, and are an indication for fetal pulmonary maturity. So far the origin of SPs was not clearly determined, though they were supposed to be secreted by the fetal developing lung.

The human amniotic membrane (hAM) is the innermost layer of the fetal membranes and the cells thereof exhibit stem cell characteristics and have been successfully differentiated towards all three germ layers. In our study we discovered that both, isolated epithelial cells from human amniotic membrane (hAECs) as well as the membrane itself, produce pulmonary surfactant. We have detected intracellular surfactant protein expression via Flow Cytometry and Immunohistochemistry/Immunofluorescence staining not only directly after isolation but over several passages (hAECs) or weeks (hAM) of incubation. Moreover we could show with ELISA that SP-D is actively secreted into cell culture medium over a longer period in culture.

All in all we could show for the first time that human amniotic membrane is likely to be at least one of the producers of pulmonary surfactant found in the amniotic fluid.

As accumulation of surfactant in the lung is crucial for the fetus, this could mean that surfactant secreted by hAM might be important for the pulmonary development. Moreover, since hAM is an alternative source of stem cells for regenerative medicine, our results could imply a new therapeutical approach, such as for respiratory distress syndrome (RDS), which needs to be further investigated.

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Abstract No. P120

Comparative induction of pluripotency in human umbilical vein endothelial cells and dermal fibroblasts and further differentiation into Hepatocytes

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Generating induced pluripotent stem cells (iPSCs) is a standardized technique. To date iPS cells have been generated from HUVECs using viral-based approaches. We employ an episomal plasmid-based approach to generate integration-free iPSCs (E-iPSCs) from human somatic cells. In this study, we compared the generation of E-iPSC lines from human umbilical vein endothelial cells (HUVEC) and fetal foreskin derived fibroblast (HFF1). The efficiency of inducing pluripotency in HFF1 was 0.03% compared to 2.5% in HUVEC. This implies that the efficiency of reprogramming HUVECs is 83-fold higher than in HFF1 cells. Additionally, the kinetics of reprogramming was much faster using HUVECs, i.e. three weeks for the stabilization of E-iPSC colonies compared to HFF1 cells which needed four months. The E-iPSCs from both somatic cell types were fully characterized and are comparable to human embryonic stem cells (hESCs). Both E-iPSC lines express pluripotency associated transcription factors as OCT4, NANOG, SOX2 and also the surface markers SSEA-4, TRA-l-60, TRA-1-81 and TRA-2-49 but not SSEA-1. Additionally, they have the ability to differentiate to all cell types representative of the three germ layers endoderm, ectoderm and mesoderm *in vitro* (by formation of embryoid bodies) and *in vivo* (teratoma formation in immunodeficient mice).

We have already shown that E-iPSCs generated from HFF1 cells could be used to study hepatogenesis and represent a tool for studying human gastrulation at the molecular and cellular levels *in vitro*. We now demonstrate that HUVEC-derived E-iPSCs can be used to study hepatogenesis. A careful comparative analysis to determine which cell type would be better for generating hepatocyte-like cells with a more mature phenotype is in progress.

Abstract No. P121

BSA-free differentiation of hPSCs into cardiomyocytes

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Cardiovascular diseases represent a high number of deaths in the world. Every year around 17 million people are affected. The heart has a very low regeneration capacity and donor organs for transplantation are scarce. Therefore regeneration of lost myocardium with stem cell derived cardiomyocytes is an attractive strategy for regenerative medicine. hPSCs can be differentiated into cardiomyocytes but the molecular mechanism behind that differentiation are poorly understood. It is likely that differentiating cells secrete paracrine factors into the supernatant that function as important regulatory determinants. A recent and very effective protocol leading to high proportions of cardiomyocytes from hPSCs depends on modulation of Wnt signaling at early stages of differentiation. The differentiation medium according to Lian et al. contains 2.5 mg/ml BSA, which originates from the used B27 supplement. Considering that secreted paracrine factors are found at concentrations in the range of ng/ml, the enormous excess of BSA in cell culture supernatants hampers analytics to identify little amounts of secreted proteins. Therefore the development of a BSA-free cardiomyocyte differentiation protocol is pivotal. According to Roth et al., we generated a custom made B27 and omitted the BSA. hESCs and hiPSCs could be successfully differentiated under BSA-free conditions into cardiomyocytes. Measurement of the cardiac marker cTNT by flow cytometry revealed differentiation efficiencies of up to 75 % cardiomyocytes. The differentiation process was further analyzed and approved at different days (d0, d3, d7, d12 and d15) via real time PCR for the stem cell marker OCT3/4, the mesodermal markers Brachyury and MESP1 and markers of the cardiac linage (Nkx2.5, αMHC and MYL4). Our results are in line with a recent publication describing the suitability of BSA-free conditions for cardiomyogenic differentiation. Applying the novel medium, analysis of secreted proteins during cardiomyogenic differentiation is currently under process.

Abstract No. P122

Improving the efficiency of pancreatic *in vitro* differentiation of human pluripotent stem cells via microRNA and glucose modulation

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Human embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC) are a valuable source for the generation of insulin-producing cells, but current protocols for pancreatic *in vitro* differentiation show low efficiency and derivatives display virtually no glucose-dependent insulin secretion. 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. This could be one reason why to this day *in vitro* formation of fully functional beta cells is not possible. Therefore, we analyzed the expression profile of a variety of pancreas-associated miRs (e.g. miR-375, -124a, -335, -30d, -200c, -9 and -182) during *in vitro*-differentiation of two different human ESC lines (WA09 (H9), HUES-8) and the human iPSC line IMR90-4. Assessing our data obtained from the different lines we show that changes in the microRNA expression profiles are not cell line specific, but demonstrate general mechanisms during the differentiation process. Furthermore, we compared our results with known *in vivo* expression profiles. The forced induction of miR-375 at later differentiation stages led to an increased expression of endogenous pre-miR-375, more efficient formation of endocrine pancreatic progenitors expressing typical marker genes like PDX1 and NGN3, as well as an increased INSULIN production. In addition to microRNAs, nutrients like glucose are important for a successful pancreatic development. We show that *in vitro* differentiation under high glucose conditions in a specified time frame also leads to an increased expression of crucial pancreatic markers such as PDX1 and the combination of both strategies resulted in a synergistic impact improving current differentiation strategies.

Abstract No. P123

Gene targeting in human embryonic stem cells to study the primary and secondary heart fields *in vitro*

Daniel Ortmann[1,*], *Sasha Mendjan*[1], *Andreia Bernardo*[2], *Mariaestela Ortiz*[1], *Steven Harmer*[3], *Muriel Nobles*[3], *David Elliott*[4], *Victoria Mascetti*[1], *Barry Rosen*[5], *Andrew Tinker*[3], *Edouard Stanley*[4], *Andrew Elefanty*[4], *Ludovic Vallier*[1], and *Roger Pedersen*[1]

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Objective: Human pluripotent stem cells offer unprecedented opportunities to generate a variety of cell types that can be used for toxicity testing, cell therapies or developmental studies. Cardiomyocytes especially those with a left ventricular identity, are amongst the most sought after, because of their therapeutic relevance.

We focused our study on the characterisation of cardiomyocyte progenitor cells, in particular those that constitute the primary heart field (PHF), which gives rise to the left ventricle. The lack of suitable surface markers has so far prevented the isolation of a primary heart field (PHF) population.

Material and methods: Using gene targeting in human embryonic stem cells, we have generated a double fluorescent reporter cell line for NKX2.5 (a pan cardiac marker) and TBX5 (a transcription factor widely used for the identification of the PHF). We utilised this line to isolate and study TBX5 positive and negative cardiac progenitor cells, which we generated by employing a chemically defined, monolayer based cardiac differentiation protocol. It yields high percentages of NKX2.5- and TBX5- expressing cardiac progenitors in under 10 days.

Results and conclusion: The isolated TBX5 positive and negative populations were characterised by transcriptional profiling and functional studies of developmental potency. These experiments revealed similarities between these populations and previous characterisations of primary and secondary heart fields in model organisms such as the mouse. Furthermore, genes previously unknown in this context were also identified and may offer new insights into human cardiac specification.

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Abstract No. P124

Epigenetic control of cardiopulmonary development and congenital heart disease

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Development of the cardiovascular and respiratory systems is tightly interconnected, which is illustrated by the recent identification of a population of Isl1+ cardiopulmonary mesoderm progenitor cells giving rise to the mesoderm lineages within the cardiac inflow tract and the lung. Deregulation of cardiopulmonary mesoderm progenitor cells has been implicated for many cases of patients suffering from congenital heart disease (CHD) with malformations of the right ventricle or development of pulmonary hypertension (PH). Interestingly genome wide association sequencing in human CHDs suggested that sporadic mutations within epigenetic modifiers leading to dysregulation of H3K4me3 and H3K27me3 of bivalent chromatin structure being the main mutations in human CHDs. In this study we have investigated the role of one of CHD related epigenetic modifiers Suv4-20h1, an enzyme catalyzing histone H4 lysine 20 (H4K20) dimethylation, in cardiopulmonary development *in vivo* as well as the biochemical feature of mutated Suv4-20h1 *in vitro*. Our future work aims to decipher Suv4-20h1 mediated epigenetic network that alters bivalent chromatin structure and directs co-regulation of the right ventricle and pulmonary vasculature using molecular, cellular and genetic methods, which might give mechanistic insight in pathology of CHDs.

Abstract No. P125

Reciprocal feedback between microRNA-9/9*and Notch calibrates proliferation and differentiation of human neural stem cells

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The balance between self-renewal and differentiation of neural stem cells requires tight regulation to assure proper neural development. In this context, Notch signaling has been shown to be an important promoter of stemness. In contrast, the brain-enriched microRNA miR-9/9*has been implicated in promoting neuronal differentiation. We set out to explore a potential interplay between Notch signaling and miR-9/9*using a homogeneous population of human embryonic stem cell-derived neuroepithelial-like stem cells generated in our lab (lt-NES cells, Koch et al., PNAS 2009). Our miR-9/9*gain-of-function analyses indicate that both miRNAs contribute to the shift of lt-NES cells from self-renewal to neuronal differentiation. In line with this effect, miR 9/9*decrease Notch activity by targeting NOTCH2 and HES1, two members of the Notch signaling cascade. Vice versa, expression levels of miR 9/9*were found to depend on the activation status of Notch signaling. While gain of Notch function inhibits differentiation of neural stem cells, we observed that it also induces miR-9/9*expression via direct binding of the NICD/RBPj transcriptional complex to miR-9/9*genomic loci. Taken together, these data point to an intricate feedback interaction between bifunctional miRNA-9/9*and the Notch pathway controlling the delicate balance between self-renewal and differentiation of human neural stem cells.

Abstract No. P126

Functional phenotyping of human induced pluripotent stem cell-derived neurons

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Introduction: Human induced pluripotent stem cells (hiPSCs) have become a popular *in vitro* model in the study of diseases affecting the CNS. A neural induction protocol using triple-SMAD (TGFβ receptor signalling) inhibition was used to generate a stable, self-renewable neuronal precursor cell (NPC) line. Terminal differentiation by means of basic fibroblast growth factor (bFGF) withdrawal led to the generation of glutamatergic neurons.

Methods: FURA-2-AM calcium microfluorimetry was performed around day 50 of terminal differentiation. High potassium (140 mM) solution, glutamate, AMPA, and NMDA were used as agonists. APV and CNQX were used at different concentrations as competitive inhibitors of NMDA and AMPA/Kainate receptors. As superfusion either normal Tyrode´s solution, or Tyrode's solution substituted with 10 µM glycine but devoid of Mg2+ was used. A sodium free superfusion was achieved by equimolar replacement with NMDG.

Results: About 50% of the cells within the cellular networks responded with a significant rise in intracellular calcium upon stimulation with 100 µM glutamate independent of the superfusion media used. The number of cells responding to 100 µM NMDA was significantly higher when superfused with a Mg2+-free and glycine substituted solution. Using CNQX as an inhibitor, the glutamate response could be almost completely abolished (IC50 = - 5.78 ± 0.04).

A subset of the cells responding to 100 µM glutamate also responded to NMDA and AMPA. The number of cells responding to 100 µM NMDA is significantly dependent on the superfusion used, with a higher number of responders after exclusion of Mg2+. Under sodium free conditions, AMPA stimulation still led to an increase in intracellular calcium within a subset of cells.

Abstract No. P127

Sphingosine-1-phosphate promotes survival and differentiation of pancreas progenitors through YAP stabilisation and Notch attenuation

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Identifying the signals that regulate the survival and lineage allocation of pancreas progenitors will help elucidate the embryonic origins of pancreas dysfunction and provide important cues for the efficient conversion of PS cells into fully functional β cells.

Levels of Notch signaling play a key role in segregating the pancreatic lineages but long-range signals that may regulate lineage selection remain elusive. Using a combination of genetic approaches and air to liquid interface (ALI) cultures of embryonic pancreata we first show that GPCR signaling is necessary for full endocrine specification. S1p receptor 2 (S1pr2) was a candidate because it mediates endocrine cell clustering to form islets and its expression peaks in the developing epithelium around the secondary transition and declines as differentiation proceeds. S1pr2 null mice showed early defects in endocrine and acinar specification that were subsequently compensated for by upregulation of the closely related receptors S1pr3 and LPAR.

Acute disruption of S1pr2 signaling in ALI organotypic cultures resulted in the loss of both acinar and endocrine lineages, cell death and excessive ductal specification. CTGF rescued the cell death, but not endocrine or acinar differentiation, by maintaining cells in the progenitor state. Notch activation phenocopied the disruption of S1pr2 signaling, whereas disruption of both S1pr2 and Notch signaling restored the number of Ngn3+ endocrine progenitors but only transiently restore terminal differentiation. Finally, we found that S1P2 signaling acts through YAP activation to promote CTGF expression and activation of the acinar and endocrine differentiation programs.

Therefore, YAP-mediated S1pr2 signaling mediates survival of pancreas progenitors through upregulation of CTGF, activates the acinar and endocrine terminal differentiation programs and promotes Ngn3 expression.

Abstract No. P128

Defining the embryonic precursors for adult intestinal stem cells

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The internal surface of the intestine, the gut epithelium, is constantly renewed throughout adult life. Adult intestinal stem cells (ISCs) are essential for this regeneration process. How these stem cells arise during embryonic development was so far unknown. Using lineage tracing analyses we found that two temporally distinct progenitor cell populations give rise to the adult ISCs. These embryonic progenitor populations are molecularly and phenotypically distinct, and also differ from adult ISCs. These novel findings will provide new directions for investigating the biology of intestinal tissue formation and regeneration.

Abstract No. P129

Motor neuron generation from hESCs by combination of small molecules

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Introduction: Motor neuron differentiation from human embryonic stem cells (hESC) is a goal in regenerative medicine for cell therapy in diseases that loss these cells. Most protocols have low efficiency in generate functional motoneurons, therefor small molecules are new idea for dominance this challenge.

Methods: According to signaling pathways, we used small molecules Dorsomorphin, A8301, XAV, Retinoic acid and Purmorphamine in the protocol of motor neuron differentiation.

Results: Immunofluorescent staining and flow cytometry analysis of hESC-derived neural ectoderm (NE) showed these structures are 92.68%±6.33% NESTIN+, 82.11% ± 3.84% SOX1+, 64.40%±3.46% PAX6+ in a chemically defined adherent culture. We verified expression of neural markers such as TUJ1 and MAP2, also motor neuron markers such as HB9 and ISL1 and estimated efficiency of neural cells and motor neuron generation. After replating of hESC-derived NE, the differentiated cells are 61% ±11% Tuj1+, 62% ± 1.55% MAP2+, 37% ± 1.48% MAP2+/HB9+, 39%±0.52 HB9+ /ISL1+. Efficiency of motor neuron generation was 39% according to HB9/ISL1 expression. We studied electrophysiology properties of hESC-derived neurons to determinate their functional maturation. We evaluated the cells in 11 days and 20 days after plating of hESC-derived NTs. Differentiated cells demonstrated neuron like currents including voltage gated delay rectifier K+ currents as well as Ca2+/Na+ inward currents.

Conclusion: Our results suggest that small molecules have important effects on signaling pathways and lead to the expression of specific markers. Small molecules can replace expensive growth factors, however, there is still a need for improvement of differentiation protocols that accelerate maturation and improve cell function. According to our cellular and molecular consequences, cellular properties of differentiated neurons don't consistent with physiological properties. Furthermore, it seems that the cells should be more developed for electrophysiological properties on basis of this protocol.

Shahbazi E, Kiani S, Gourabi H, Baharvand H. Electrospun nanofibrillar surfaces promote neuronal differentiation and function from human embryonic stem cells. Tissue Eng Part A. 2011;17(23-24):3021-31.
Li XJ, Hu BY, Jones SA, Zhang YS, Lavaute T, Du ZW, et al. Directed differentiation of ventral spinal progenitors and motor neurons from human embryonic stem cells by small molecules. Stem Cells. 2008;26(4):886-93

Abstract No. P130

Epigenetic control of muscle progenitor and adult stem cell homeostasis

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Epigenetic mechanisms have been suggested to distinctly control homeostasis of progenitor cells during development and their derivative stem cells in adulthood. We used muscle progenitors and stem cells as a paradigmatic model, and identified Prmt5 by functional proteomics and mouse genetics as a crucial epigenetic modifier that generates a poised state of adult muscle stem cells thus allowing them to re-enter cell cycle and undergo rapid amplification by restricting p21 expression via direct epigenetic silencing. Importantly ablation of Prmt5 in embryonic muscle progenitor cells showed no inhibition of cell amplification, supporting distinct genetic and epigenetic requirements for proliferation of progenitor cells and stem cells . Meanwhile we uncovered an epigenetic pathway consisting of Suv4-20h1-MyoD-Ezh2 in regulating high order chromatin organization of adult muscle stem cell, deregulation of which led to loss of stem cell quiescence and long-term muscle regeneration but without influencing muscle progenitor cell homeostasis as well. Further study will focus on elucidating the epigenetic crosstalk between Prmt5 and Suv4-20h1 in control of adult muscle stem cell homeostasis, and identifying epigenetic regulatory pathway regulating progenitor cell amplification during development.

Zhang et al., Prmt5 is a regulator of muscle stem cell expansion in adult mice. Nat Commun. 2015

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Abstract No. P131

Aldehyde dehydrogenase is regulated by beta-catenin/TCF and promotes radioresistance in prostate cancer progenitor cells

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Radiotherapy is a curative treatment option in prostate cancer. Nevertheless, patients with high-risk prostate cancer are prone to relapse. Identification of the predictive biomarkers and molecular mechanisms of radioresistance bears promise to improve cancer therapies. In this study, we show that aldehyde dehydrogenase (ALDH) activity is indicative of radioresistant prostate progenitor cells with an enhanced reactive oxygen species (ROS) scavenging and DNA repair capacity and activation of epithelial-mesenchymal transition (EMT). Gene expression profiling of prostate cancer cells, their radioresistant derivatives as well as purified ALDH+ and ALDH- cell populations revealed the mechanisms, which link tumor progenitors to radioresistance, including activation of WNT/β-catenin signaling pathway. We found that expression of ALDH1A1 gene is regulated by WNT signaling pathway and co-occurs with expression of β-catenin in prostate tumor specimens. Inhibition of WNT pathway led to a decrease in ALDH+ tumor progenitor population and to radiosensitization of cancer cells. Our results indicated that irradiation drives histone modification changes on the promoter sequence of ALDH1A1 which is specific for the β-catenin/TCF binding sites and led to the activation of gene transcription. We found that inhibition of histone methylation may be useful in epigenetic therapy leading to the prostate cancer cell radiosensitization. Our study revealed that progenitor cell population undergoes a phenotypic switching during the course of radiotherapy, and phenotypic plasticity of tumor initiating cells might be associated not only with certain genetic but also with epigenetic changes induced by irradiation. Taken together, our results indicate that ALDH+ tumor initiating cells can contribute to prostate tumor radioresistance, and therapies that specifically target this cell population or inhibit tumor cell reprogramming might be a biologically-driven strategy to enhance the efficacy of radiotherapy.

Cojoc M et al. Aldehyde Dehydrogenase Is Regulated by β-Catenin/TCF and Promotes Radioresistance in Prostate Cancer Progenitor Cells. Cancer Res. 2015 Apr 1;75(7):1482-94.
Castro Nava A et al. Development of novel radio-chemotherapy approaches targeting prostate tumor progenitor cells using nanohybrids. Int. J. Cancer 06/2015; DOI:10.1002/ijc.29614

Abstract No. P132

Expression pattern of stemness-related and EMT genes in breast cancer cells, breast epithelial cells exhibiting stem-like properties and their hybrids

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It is well recognized that cell fusion plays a pivotal role in several physiological events, such as fertilization and wound healing, as well as pathophysiological processes including cancer. Here it is assumed that hybrid cells, derived from fusion events between tumor cells and normal cells, like macrophages or stem-like cells, will foster tumor progression due to possessing novel properties, such as an increased metastatogenic capacity. However, in order to metastasize and in accordance to the cancer stem cell (or tumor initiating) hypothesis hybrid cells should possess stem cell properties enabling them to successfully seed secondary lesions at distant organ site. We thus investigated hybrid cells, which originated from spontaneous cell fusion events between human breast cancer cells and human breast stem-like cells, for the expression of stemness-related genes as well as EMT genes. A panel of stemness-related and EMT marker genes were analyzed by microarray and validated by Western Blot analysis including the four Yamanaka factors as well as breast (cancer) stem cell factors like SOX9, SLUG, SNAIL, and Vimentin. Additionally, EMT markers such as N-Cadherin, Twist, ZEB1 and ZEB2 were analyzed. A co-expression of OCT4, KLF4, and c-MYC was observed in all cell lines, whereby MDA-MB-435-Hyg breast cancer cells were also positive for SOX2 expression. Interestingly, all investigated hybrid cell lines were positive for SOX9 and SLUG, whereas the human breast cancer cells were solely positive for SLUG. Co-expression of SOX9 and SLUG has been associated with a reprogramming of mammary cancer cells towards a cancer stem cell like phenotype. In fact, hybrid cells exhibited both an increased clonogenic as well as an increased mammosphere formation capacity. In summary, cell fusion between human breast cancer cells and human breast epithelial cells having stem-like properties could lead to the origin of hybrid cells possessing a cancer stem-like phenotype.

Abstract No. T19/P133

Triple-negative inflammatory breast cancer: Syndecan-1 is a biomarker and regulator of cancer stem cell phenotype via IL-6/STAT-3 and Notch-3

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Syndecan-1 (Sdc-1) is a heparan sulfate proteoglycan that is highly expressed on the surface of epithelial cells and a corecetor for growth factors and chemokines modulating tumor adhesion, invasion, and metastasis. Recently, we addressed the potential role of Syndecan-1 in regulating breast cancer stem cells via regulation of LRP-6 and IL-6-mediated STAT3 signaling. Inflammatory breast cancer (IBC) is a particularly aggressive form of breast cancer and posses a chemo- and radio-resistant stem cell phenotype. Herein, we characterized the expression level of Sdc-1 and cancer stem cell marker CD44 in triple negative IBC (n=15) and non-IBC (n=15) patients. Moreover, we deciphered the functional impact of Syndecan-1 knockdown *in vitro* using RNA interference on the breast cancer stem cell phenotype of the triple negative IBC Sum-149 cells and HER-overexpressing non-IBC SKBR-3 cells employing real time PCR, flow cytometry and western blot. Our data indicate upregulation of Sdc-1 and CD44 in triple negative IBC compared to those of non-IBC patients. Aldehyde dehydrogenase-1 activity revealed that Syndecan-1 knockdown Sum-149 and SkBR-3 cells exhibited significantly reduced putative cancer stem cell pools by 5% compared to controls. In Sum149 cells, the CD44(+)CD24(-/low) phenotype decreased significantly by 29% upon siRNA-mediated Syndecan-1 depletion. Intriguingly, qPCR results indicate downregulation of IL-6, CCL20, gp-130, Notch-1, -3 and -4 by 20-30% in Sdc-1-silenced Sum-149 cells compared to controls. Furthermore, activation of STAT-3 and NFkB transcription factors was reduced by >30% in Syndecan-1-depleted cells compared to controls. At the functional level, Syndecan-1-silenced Sum-149 and SKBR-3 cells exhibited reduced colony formation by 40-50% compared to controls. In conclusion, Syndecan-1 acts as a biomarker and a modulator of cancer stem cell phenotype for triple negative IBC via IL-6/STAT3 and Notch-3 signaling pathway, thus it emerges as a promising novel target for therapeutic approaches in IBC patients.

Abstract No. P134

Primary gallbladder organoid culture as a model to study the role of Salmonella Typhi infection in development of gallbladder cancer

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Gallbladder cancer (GBC) is the most common malignancy of the biliary tract. It is the sixth most common gastrointestinal cancer. The malignancy progresses silently and is incidentally diagnosed during cholecystectomy. The dismal prognosis results in a high mortality, with a five year survival rate in 5% of the patients. Some factors that significantly increase the risk of GBC development are advanced age, female gender and presence of gallstones. Epidemiological studies have confirmed a strong link between chronic infection with Salmonella Typhi (S. Typhi) and the development of GBC. The molecular mechanism of pathogen-host cell interaction is currently unknown. The study of the mechanism by which S. typhi is involved in malignant transformation is complicated by the lack of adequate animal models, since S. Typhi exclusively infects human cells. Existing gallbladder cell lines are derived from cancer tissue and are therefore already transformed. To overcome this problem we are currently establishing organoid long term 3D primary epithelial cell culture from adult stem cells of the gallbladder. Starting with mouse gallbladder tissue, these conditions will later be used to optimize the culture conditions for cells derived from human gall bladder tissue. In the organoid culture model we aim to investigate the cellular and genetic changes that arise in the gallbladder epithelial cells post infection with S. Typhi. We will verify potential disruption of downstream signaling pathways that lead to malignant transformations. This infection model will thus contribute to a better understanding of the role of S. Typhi in GBC development.

Abstract No. P135

Osteopontin in Breast Cancer Metastasis and Therapy Resistance

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Although many cancer patients respond well to initial use of chemotherapy, tumors frequently relapse and grow again massively, losing the sensitivity to these therapeutic agents. In addition, they metastasize more efficiently, indicating that the nature of the disease has become more aggressive and thus life-threatening. Increasing evidence suggests that metastasis and chemoresistance occurring in late-stage cancer patients are linked phenomena. Osteopontin (or secreted phosphoprotein 1 - SPP1) is a highly conserved matricellular protein that contains several functional domains, including binding sites for integrins and CD44. SPP1 is also susceptible to protease-mediated cleavage, which modulates SPP1 activity. For example, the serine protease thrombin cleaves SPP1 into two fragments of about equal size. Upon cleavage, the N-terminal fragment exposes the cryptic SVVYGLR sequence, which can bind to integrins. Our *in vivo* studies indicate that SPP1 mediates breast cancer metastasis to the lungs and chemoresistance. SPP1 deficiency abrogated lung metastatic burden in a mouse xenograft model for breast cancer. In addition, SPP1 deficiency sensitized tumors to chemotherapy. Further molecular studies indicate that SPP1 promote stemness activity in breast cancer cells; upon knockdown of SPP1, the pluripotency markers nanog homeobox (NANOG), POU class 5 homeobox 1 (POU5F1), also known as OCT4, and SRY box 2 (SOX2) were downregulated. We have also data indicating that thrombin promotes stemness activity, possibly by cleavage of SPP1. Thrombin knockdown also reduced the expression levels of the pluripotency markers. Inhibition of thrombin by the small molecule inhibitor argatroban significantly reduced lung metastases in a breast cancer xenograft model. These findings highlight the relevance of SPP1 as a metastatic mediator and a molecule that protects cancer cells from cytotoxic stress. We also propose thrombin inhibition as a putative therapeutic option targeting SPP1 activity in metastatic cells.

Abstract No. P136

CD133 expression is not synonymous to immunoreactivity for the CD133 epitope AC133 and fluctuates throughout the cell cycle in glioma stem-like cells.

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A transmembrane protein CD133 has been implicated as a marker of stem-like glioma cells and predictor for therapeutic response in malignant brain tumours. CD133 expression is commonly evaluated by using antibodies specific for the AC133 epitope located in one of the extracellular domains of membrane-bound CD133. There is conflicting evidence regarding the significance of the AC133 epitope as a marker for identifying stem-like glioma cells and predicting the degree of malignancy in glioma cells. The reasons for discrepant results between different studies addressing the role of CD133/AC133 in gliomas are unclear. A possible source for controversies about CD133/AC133 is the widespread assumption that expression patterns of the AC133 epitope reflect linearly those of the CD133 protein. Consequently, the readouts from AC133 assessments are often interpreted in terms of the CD133 protein. The purpose of this study is to determine whether and to what extent do the readouts obtained with anti-AC133 antibody correspond to the level of CD133 protein expressed in stem-like glioma cells. Our study reveals for the first time that CD133 expressed on the surface of glioma cells is poorly immunoreactive for AC133. Furthermore, we provide evidence that the level of CD133 occupancy on the surface of glioma cells fluctuates during the cell cycle. Our finding that does not adequately reflect the level of CD133 protein offers a unifying explanation for existing controversies regarding the roles of CD133 in human gliomas and necessitate re-consideration of previous interpretations based on the assumption that AC133 immunoreactivity is synonymous to CD133 abundance in GSCs. Our results offer a new explanation for numerous inconsistencies regarding the biological and clinical significance of CD133/AC133 in human gliomas and call for caution in interpreting the lack or presence of AC133 epitope in glioma cells.

Abstract No. P137

Cancer stem cells and their role in radioresistance of head and neck squamous carcinoma

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Due to their stem cell characteristics, as the ability to self-renew and to differentiate, cancer stem cells (CSC) are proposed to be tumorigenic and drive tumor formation, metastasize, and potentially persist therapy. Around 60% of head and neck squamous carcinoma (HNSCC) patients are diagnosed with loco-regionally advanced disease (stage III-IV), which is associated with a poor prognosis and an unsatisfactory overall 5-year survival rate of approximately 50% of patients. Several markers have been proposed to identify CSCs in HNSCC, including CD133, CD44, stemness-related transcription factors Nanog, Oct4, Sox2, and aldehyde dehydrogenase (ALDH) activity. In this study, we investigated the irradiation dependent expression of those CSC markers and their contribution to radioresistance. We found that ALDH activity in HNSCC cells is attributed to ALDH1A3 isoform, and its expression in xenografts correlated with the occurrence of the CSC marker Oct4 and phosphorylated pro-survival Protein kinase B (pAkt). In contrast to ALDH negative cell populations, ALDH positive cells maintained their tumorigenic properties *in vivo* after irradiation, and therefore may contribute to tumor relapse. Additionally, we found the expression of CSC markers to be dependent on the number of irradiation fractions given to the HNSCC cells as well as on the time point after irradiation, underlining the importance to observe CSC dynamics during treatment. To establish potentially prognostic and predictive CSC related markers to monitor radioresistance, we performed comparative microarray analysis of radioresistant, ALDH positive and non-irradiated HNSCC cell populations. Further we have established color coded HNSCC non-irradiated and radioresistant cell lines to trace the dynamic changes of CSC characteristics upon treatment within a distinct cell population. Taken together, our data suggest that a positive ALDH activity contributes to CSC survival and tumor formation after irradiation and that HNSCC cells change the expression pattern of CSC markers upon treatment.

Abstract No. P138

Karyotypic abnormalities in EP iPSCs are mainly observed in cells derived from elderly donors and acquired during cell culture prior to reprogramming

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Recent studies observed alarming levels of genetic abnormalities in human induced pluripotent stem cells (iPSCs) (1). In a prospective study we derived 84 iPSC lines under comparable conditions from neonatal, adult (20-28y) and aged (39-88y) individuals and investigated to what extent the frequency of different karyotypic abnormalities in early passage (EP) iPSCs depends on primary cell expansion and donor age.

In contrast to EP iPSCs from EP neonatal or young adult cell sources, aberrations were observed in >40% of EP iPSC clones derived from of elderly individuals, with many of them detectable in corresponding EP source cells accompanied with significant telomere shortening. Prolonged passaging, however, led to complex karyotypic aberrations even in neonatal cells.

As previously reported, telomere dysfunction in cultured somatic cells leads to initiation of repeated break-fusion-bridge cycles, with incidental inactivation of p53 enabling continued chromosome fusion, missegregation of chromosomes and abortive cytokinesis instead of preventing apoptosis and senescence. During iPSC generation, the reprogramming process itself may specifically select for abnormal cells with p53 inactivation, and finally restores telomere activity in the respective cell clones.

Thus, extended source cell culture and the use of parental cells from elderly donors support the generation of EP iPSC clones with of translocations, aneuploidies and polyploidies.

1. Taapken S. et al 2011 Karyotypic abnormalities in human induced pluripotent stem cells and embryonic stem cells Nature Biotechnology, 29, 313-314

Abstract No. P139

Isolation and Characterisation of Colon Cancer Stem Cells

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The organization of solid cancer parallels normal tissue in terms of a hierarchical structure of cells showing different degrees of maturity with cancer stem cells (CSCs) as the most undifferentiated cells. CSCs not only build the bulk fraction of higher differentiated tumour cells, they also possess the ability to self-renew and to initiate and maintain tumours. For a better understanding of their genetic composition and signalling pathways associated with self-renewal and stemness, a reliable identification and isolation of CSCs is essential.

Some colon cancer cell lines show stem-like features as they react to growth factor treatment by sphere formation as a read-out for stemness. Microarray based transcriptome analysis of induced spheres in comparison to the corresponding adherent cells have shown regulation of many embryonic and cancer associated pathways. We found cell cycle promoting genes like CDC7 and CK1 upregulated whereas differentiating factors like SMAD3 and BMP4 were downregulated. We are also focussing on the family of OCT/POU domain containing transcription factors of which we could show regulation of POU2F2 during sphere formation.

As an *ex vivo* approach, we were able to culture dissected human colorectal cancer tissue. Primary, unsorted cells cultured under stem cell conditions formed three-dimensional tumour organoids including crypt-like structures showing a hierarchy of maturation. However, we have observed that organoid formation can be easily induced in tumour cells obtained from liver metastasis whereas cells of the primary tumour grow mainly as single cells. To induce dedifferentiation and increase organoid formation, we are evaluating the effect of the Wnt-pathway agonist R-spondin 3 as well as hypoxic culture conditions.

In addition, we are investigating the heterogeneity of putative cancer stem cell by analysing EpCAM+, CD44+ and ALDH1+ sub-populations at the single cell level after whole genome amplification as well as clonality and tumour initiating capacity.

Abstract No. P140

Adipose-derived stem cells: function in oncology

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The stem cell-based therapies for the repair and regeneration of various tissue and organs have raised an increasing interest worldwide. Especially adipose-derived stem cells (ASCs) have recently attracted much attention because of their availability, ability in suppressing inflammation and promoting regeneration, and less ethical consideration. We are interested in their potential influence on the surrounding cells and cancer cells. Our data demonstrate that ASCs derived from subcutaneous and visceral adipose tissues display a characteristic expression of cell surface markers such as CD90, CD73, CD106 and CD146. Isolated ASCs are capable of differentiating into mesenchymal and non-mesenchymal cells *in vitro*. Interestingly, they affect proliferation rate, invasion behavior and induce the epithelial-mesenchymal transition (EMT) of mammary cancer cells. Moreover, we show that this transition is triggered by multiple pathways including PI3K, STAT and MAPK signaling cascades. More investigations are on the way to elucidate the molecular communications of ASCs with environmental cells in more depth.

Abstract No. P141

Genetic expressions of EPCAM, Syntaxin and VEGF as Cancer Stem Cells bio-markers in retinoblastoma progress and their clinico-pathological correlation

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Cancer stem cells (CSC), are cancer cells that are highly undifferentiated and drive cancer growth *in vivo*. CSCs represent important targets for developing novel anti-cancer therapies, because they are highly tumorigenic and resistant to treatments.

Study's aimed to identify differences in gene expression profile of cancer stem markers: Epcam, Syntaxin and VEGF, which potentially relate to development of retinoblastoma. Differential expression of these candidate genes were analyzed and compared with clinico-pathological profiles characterizing tumor invasion and chemo resistance.

A prospective nonrandomized study, where 53 patients with a clinical diagnosis of retinoblastoma were enrolled after informed consent. They were grouped namely: Primary enucleation, chemo résistance failure and extra ocular cases. These patients were analyzed for their history of symptoms like laterality, palpitation of lymph nodes and other risks features of tumor by Immuno-histochemistry.

Gene expression of Epcam, Syntaxin and VEGF were analyzed in fresh unfixed retinoblastoma tumor tissue through qRT-PCR and compared among primary, chemo resistant and extraocular retinoblastoma along clinico- pathological features. Statistical analysis was computed using STATA VERSION 9.0

Among the 3 genes, distribution of primary(36), chemo-resistant(6) and extra ocular cases (7) respectively, VEGF showed marked significantly higher expression in primary enucleated patients. All the 3 genes showed higher expressions in chemoresistant tumours as compared to chemosensitive tumours. Variable expression of Epcam, Syntaxin and VEGF were observed with high risk features of retinoblastoma and data were comparable. Syntaxin expression was significantly noted low in features depicting absence of anterior chamber seeding, iris infiltration, ciliary body infiltration, scleral and extrascleral invasion.

Exploring quantitative molecular expression at RNA level was the first attempt by our group showing marked expression of VEGF> Epcam> Syntaxin specific to CSCs in retinoblastoma, could serve as potential biomarkers which may lead to progress in therapy, interventions and early prognosis to combat disease progression in clinical settings.

Seigel GM, Campbell LM, Narayan M, Gonzalez-Fernandez F. Cancer stem cell characteristics in retinoblastoma. Mol Vision 2005; 11:729–37.
Kim M, Kim JH, Kim JH, Kim DH, Yu YS. Differential Expression of Stem Cell Markers and Vascular Endothelial Growth Factor in Human Retinoblastoma Tissue. Korean J Ophthalmol 2010; 24(1):35-39.

Abstract No. P142

Investigation of TLR-4 as well as -9 signaling in breast cancer cells, breast epithelial cells and their hybrids

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Background
Cell fusion plays an important role in several physiological events and is also involved in tumor progression. The fusion of tumor cells between themselves or with other cells results in hybrid cells, which could exhibit new properties such a higher proliferation rate. Tumor progression might also be triggered by expression of Toll like receptors (TLRs). Activation of TLRs leads to secretion of various cytokines for immune responses. In particular, TLR-4 is associated with tumor growth and TLR-9 can mediate metastasis. The aim of this study was to analyze TLR signaling to show if they were up-regulated or not after CpG and LPS application.

Results
TLR-4 and -9 expression was analyzed in breast cancer cells MDA-MB-231, MDAMB435Hyg and MCF-7, breast epithelial cells M13SV1eGFP-Neo as well as in hybrids M13MDA435-1 and M13MDA435-3. The migratory activity was examined by using 3D collagen matrix migration assay. Only in hybrid and parental cells a CpG dependent decrease in migration potential was observed. Cell proliferation analysis showed a significant inhibition of proliferation rate by LPS in hybrids, due to induction of apoptosis. Western blot analysis were made for proteins from TLR signaling. The treatment with CpG and LPS seems to induce translocation of NF-κB to the nucleus only in MDA-MB-231 and hybrid clones.

Conclusions
These data show a differential expression pattern of TLRs. Moreover the tested cells responded differently to the TLR ligands. The obtained data could provide important information about the mechanism of metastasis and proliferation rate in breast cancer and how this results could be positively to use for new therapy approaches.

Abstract No. P143

MAN1 regulates nuclear TGF signaling and DNA damage response in muscle stem cells

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Muscular dystrophies and aged muscle exhibit increased TGF-beta signaling while TGF-beta inhibition improves the physiological response of muscle stem cells (satellite cells) following injury. Increased DNA damage and genomic integrity is also observed in aged satellite cells. As such, tight control of TGF-beta signaling and maintenance of DNA integrity is vital to avoid muscle stem cell dysfunction, muscle aging and disease. MAN1 is a member of the LEM-domain family of nuclear envelope proteins. Mutations in genes encoding these proteins leads to several human diseases termed laminopathies. MAN1 antagonizes TGF-beta signaling by interacting with Smads while recent work has uncovered a novel role for nuclear envelope proteins as protectors of genomic stability. To explore the role of MAN1 in muscle stem cells and muscle regeneration we generated conditional MAN1 KO mice. Deletion of MAN1 in muscle stem cells using Pax7 Cre results in significantly impaired regeneration of muscle following cardiotoxin (CTX)-induced muscle injury. Double MAN1/emerin cKO mice display a more severe phenotype demonstrating functional overlap between these 2 LEM-domain family members. We show that emerin can additionally antagonize TGF-beta activity. shRNA-mediated knockdown of emerin and MAN1 in myoblasts leads to delayed G2 exit and impaired myogenic differentiation concomitant with a massive increase in both the amplitude and duration of TGF-beta mediated Smad2 phosphorylation as well as Smad target genes. Treatment with a TGF-beta inhibitor only partially rescues differentiation of cells lacking emerin and MAN1. We show that the loss of emerin and MAN1 results in increased basal DNA damage and an impaired response to DNA damage that is rescued by wild type emerin but not mutants that cause X-EDMD. Our findings contribute to understanding how the loss of LEM-domain proteins leads to a cell-autologous dysfunction of muscle stem cells and could have therapeutic implications for human disease.

P144 – P147 Computational stem cell biology

P144 Mathematical modeling of bone marrow failure and accelerated aging of hematopoietic stem cells in Fanconi anemia
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P145 Identification of human bone marrow specific mesenchymal stem/progenitor cell (MSPC) gene expression signature through meta-analysis
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Wasco Wruck

P147 Analysing the impact of errors in single cell tracking experiments
Thomas Zerjatke

Abstract No. P144

Mathematical modeling of bone marrow failure and accelerated aging of hematopoietic stem cells in Fanconi anemia

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Fanconi anemia (FA) is a rare, inheritable disease which is characterized by early bone marrow failure and increased susceptibility to cancer, especially leukemia. FA patients exhibit a specific mutation of so-called FA genes, which inactivate specific DNA repair pathways. The resulting accelerated accumulation of DNA damages typically leads to a hematopoietic phenotype that mimics premature aging. Therefore, model systems like mouse models of FA allow investigating the molecular mechanism of FA as well as the association with aging of the hematopoietic system.

We developed a compartment-based modeling approach to mathematically describe progressive bone marrow failure on a cellular level. In particular, we use a ODE based model of hematopoietic stem cell (HSC) organization to investigate, which cellular mechanisms appear suitable to account for an accelerated aging process. We have identified progressively increasing proliferation as a potential process that mimics the experimental phenotype and leads to more pronounced loss of stem cell quality on the level of individual cells. We use experimental findings on repopulation ability and DNA damage accumulation in FA mice to qualitatively adapt and validate our models. Based on these findings, we are able to simulate early HSC exhaustion and competitive repopulation scenarios for different aged HSC populations. Our model provides suggestions how the progression of FA is systemically linked to the normal aging process in HSCs.

We demonstrate the general ability of our modeling approach to reflect the phenotype of FA, to support experimental design, to study the increased susceptibility for the acquisition of secondary diseases, and to speculate about the development of FA under different medicative therapies. Furthermore, we consider similar diseases of the hematopoietic system to which our model approach can be applied.

Abstract No. P145

Identification of human bone marrow specific mesenchymal stem/progenitor cell (MSPC) gene expression signature through meta-analysis

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We have recently shown that MSPCs from different organs can be distinguished based on their epigenomic and transcriptomic signatures and this unique transcriptional profile corresponds to distinct *in vivo* differentiation particularly of human bone marrow (BM)-MSPC (Reinisch A et al; www.ncbi.nlm.nih.gov/pubmed/25406351). Taking cues from this study, we aim to identify a transcriptomic signature that is specific for BM-MSPCs irrespective of culture conditions and donor variation. Further, we examined biological processes and pathways that are enriched in this distinct gene signature.

Microarray data for human MSPCs from different organs (BM; cord blood; cord; adipose tissue; skin; olfactory membrane; amnion; placenta), different donors and different culture conditions were obtained from Gene Expression Ominbus (GSE6029, GSE18698, GSE24598, GSE57151 and E-TABM-880). Raw data was downloaded and pre-processed in R using several Bioconductor packages and genes showing a p-value<0.05 and log2-fold change ≥ +0.58/ ≤ -0.58 were considered significantly up or down regulated in BM-derived MSPCs when compared to MSPCs from other organ sources.

Through our analysis we identified gene signature containing 178 genes that are differentially regulated in 80% of the data sets including 39 genes which are differentially regulated in all five data sets. Significantly up-regulated genes in the core signature include DLX5, EYA2, BRINP1 and LRRC15. Through functional enrichment analysis of the combined gene signature using KEGG, Reactome and Gene ontology databases we identified TGF-beta, Wnt receptor signalling pathway and collagen formation (n=8; p-value≤5.86e-11). Further, biological processes enriched in BM-MSPCs include neurogenesis, skeletal development, cell adhesion and MHCII-mediated immunity.

In conclusion we report a signature dataset of 178 genes specific to BM-MSPCs irrespective of donor variation and culture conditions. Functional enrichment analysis identified TGF-beta and Wnt receptor signalling pathways and processes related to neuron development to be enriched in BM-MSPC gene signature.

Abstract No. P146

An in-silico-based analysis of transcription factor-driven germ layer differentiation in pluripotent cells

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Pioneer transcription factors provide other transcription factors access to binding sites or occupy binding sites in the pluripotent state[1]. In the context of reprogramming, *POU5F1*, *SOX2* and *KLF4* act as pioneer transcription factors at distal enhancer sites[2] The rationale behind this study was that analysis of commonalities and differences within transcription factor families might aid identify key drivers of germ layer-specific specification. We analyzed gene expression data from pluripotent cells differentiated into endoderm, ectoderm and mesoderm cell derivatives with emphasis on these transcription factor gene families bearing POU, T-box, SOX and Forkhead domains. Microarray-based transcriptome datasets included: cardiomyocytes for mesoderm, neurons for ectoderm, and hepatocytes for endoderm. We characterized the lineage specific differentiation potential of transcription factors and found lineage markers via PCA. Interestingly, all gene families investigated revealed distinct germ layer differentiation characteristics. For example, the POU domain seems to promote differentiation into ectoderm and a common mesoderm/endoderm lineage. Specifically, *POU5F1* was a driver for maintaining the undifferentiated state whilst *POU3F2* directed ectoderm. The T-box family maintained the pluripotent state in addition to driving endoderm (*TBX3*), ectoderm and mesoderm (*TBX2*) into distinct clusters. FOX clusters mesoderm (*FOXK1*) separately from ectoderm -, endoderm and undifferentiated pluripotent cells. SOX clusters iPS cells with early endoderm stages and ectoderm separately. We found lineage markers, e.g. ectoderm markers *POU3F2*, *SOX21* and *PAX6* which are in line with already published findings. Additionally, our method enables the generation of new hypotheses, e.g. we hypothesize, (i) *BMP5* could be a mesoderm marker, (ii) mesoderm markers *SOX7* and *SOX18* might also have endoderm potential and (iii) *SOX11* is down-regulated in endoderm.

1. Zaret, K. S. & Carroll, J. S. Pioneer transcription factors: establishing competence for gene expression. Genes Dev. 25, 2227–2241 (2011).
2. Soufi, A., Donahue, G. & Zaret, K. S. Facilitators and Impediments of the Pluripotency Reprogramming Factors' Initial Engagement with the Genome. Cell 151, 994–1004 (2012).

Abstract No. P147

Analysing the impact of errors in single cell tracking experiments

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Time-lapse video microscopy is an increasingly popular method to study the temporal and spatial behaviour of single cells. It can be used for a broad range of applications, e.g. the analysis of cell motility and migration, proliferation properties, clonal composition, or the reconstruction of the complete divisional history of cells, represented as cellular genealogies.

A large number of automated methods has been developed for segmenting and tracking single cells. Although these methods are increasingly sophisticated to cope with a broad spectrum of situations they inevitably produce errors in the reconstruction of cellular tracks. The number of errors can be reduced by using post-processing tools for the manual correction of automatically created tracks. However, ambiguous situations can occur that lead to different subjective decisions of individual raters in the assignment of cellular objects. The number of these ambiguous situations and hence the number of differences in the reconstructed cellular tracks depends on cell type specific properties like migration speed or proliferation rate, as well as on specific properties of the experimental setting like the spatial and temporal resolution of the image sequence or the density of seeded cells.

Here we study this inter-rater variability exemplarily for *in vitro* cultures of haematopoietic stem and progenitor cells and analyse its impact on the reconstruction of cellular genealogies and statistical measures of e.g. migration properties. Furthermore, we use computer simulations of *in vitro* cell cultures that allow to comprehensively analyse a broad range of cell type specific and experimental properties. Specifically, we aim to quantify maximum error rates that are admissible to reliably measure a particular statistical outcome. These maximum admissible error rates can then be accounted for in the design of the experimental set-up and the choice of the cell tracking procedure.

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